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STUDIES ON GROWTH AND GRAVITROPISM
IN ROOTS OF *ZEA MAYS*
USING A COMPUTER-BASED VIDEO DIGITIZER

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

Amy Jane Nelson, B.A.

********

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1986

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LIST OF ABBREVIATIONS

RGR : Relative Growth Rate
NBD : Bicyclo [2.2.1] hepta-2,5-diene (or 2,5-norbornadiene)
AVG : L-α-(2-aminoethoxyvinyl)-glycine
SAM : S-adenosylmethionine
ACC : 1-aminocyclopropane-1-carboxylic acid
MES : 2 [N-morpholino] ethanesulfonic acid
INTRODUCTION
HISTORY OF THE STUDY OF GROWTH

Growth Monitoring Techniques

Techniques for measuring growth have been developing for over a century. The first methods generally required direct contact with the plant material. One of the earliest growth-measuring devices was a direct auxanometric method developed by Pfeffer (1881). It consisted of a long needle with one weighted end tied to the plant by a thread. The other end of the needle crossed a graduated arc, and, as the plant grew, the arc of fall was measured. Bose (1928), Bose and Das (1931) and Wyneken (1939) improved on this method by applying amplifying gears and a writing stylus to produce a hard-copy read-out. In the first automated technique for recording plant growth, Koningsberger (1922) positioned a coleoptile under a light platinum strip. When the coleoptile grew, the strip made contact with a metal screw above, and an electrical circuit was completed, and point recorded. The contact point was then raised 20 μM. Ubisch and Zachmann (1931) attached a thread to the
plant, which crossed a pulley and was attached to an arm fastened to a round wire. The wire had a mirror mounted on it which reflected a beam of light, and consequently produced a light spot moving across a scale. Jaffe (1970, 1976) replaced these devices with an electric circuit through an amplifier with a chart recorder.

The problem with direct methods for growth measurement is that the unavoidable perturbations or forces applied to the growing plant may affect the rate of growth. Consequently, indirect methods for detecting growth were readily adopted. Meissner (1929) and Laibach (1932) developed a complex auxanographic interferometer which utilized light interference for the precise determination of very small linear displacements in plant tissue. Lundegårds (1947), working with root hairs, devised an automated technique for recording growth every 3 minutes, using a clock-work mechanism that triggered a camera focused on the root. Audus and Brownbridge (1957) employed a technique similar to Lundegårds to determine growth rate distributions on the upper and lower sides of pea roots during gravitropic response. Records of root growth and curvature were taken by photographing on plates. A series of records was made on one negative by arranging for the camera to rotate in a horizontal plane through prescribed angles with its lens at the center of rotation.
Still regarded as one of the more elegant methods for recording relative growth rates along a plant surface is that of Erickson and Sax (1956). They marked a root of maize by brushing it with a suspension of lamp black in water. The seedling was then placed in a moist Plexiglas chamber and photographed during growth with a photomicrographic camera. In the back of the camera, a film carrier was fitted which moved at a constant speed by a synchronous motor. In front of the film carrier was a vertical slit, through which the central portion of the marked root was imaged on the moving film. The method was in essence a kinematograph; it employed a narrow unilateral beam of light, which cast a shadow on a moving photographic paper. A streak photograph was produced which recorded the movement of the markers over time. From this, the relative elemental growth rates along the plant surface could be estimated.

To determine growth rates of isolated coleoptile segments, Köhler (1956) used a microscope with an ocular micrometer. Ray (1961) and Ray and Ruesink (1962) used a modified version of this apparatus to examine growth of coleoptile cylinders. The basal end of the cylinder was fixed in position and the free end was observed with a 4-mm objective lens, and a 10X ocular containing an ocular micrometer with 100 divisions. One unit on the ocular
scale was equal to 1.5 μm. Nissl and Zenk (1969), Penny (1969) and Rowan et al. (1972) also employed this method in studies of growth responses in stem tissue. An apparatus for measurement of stem elongation utilizing electric field interruption or induction was developed by Hunter (1923) and later employed by Heathcote (1966) in studies of micronutation.

Elongation of a series of hollow coleoptile segments strung on a thread was measured shadowgraphically by Evans and Ray (1969a) and Evans and Hokanson (1969b). A weight was placed on the uppermost segment and the entire series was positioned within a glass chamber containing a growth medium. A baffle with a slit was placed 1 m from the growth chamber, and behind, a roll of polycontrast photographic paper was pulled past the slit by a kymograph drum. A zirconium arc lamp was positioned within the body tube of a horizontal microscope in place of the objective lens. Light from the lamp fell on the weight, but not on the coleoptile segments, producing a sharp shadow upon the slit. The light exposed the portion of the photographic paper above the edge of the shadow, which moved as growth occurred. This technique was utilized later in studies of the timing and effect of growth substances on coleoptile extension (Evans and Rayle 1970; Evans et al. 1971; Barkley and Evans 1970;
List (1969) improved upon Erickson's streak photography method by employing seven parallel slit cameras concurrently. Each camera was focused on a single root and projected a 5X image of that root on the vertical slit within. Sheet film was driven behind each slit, and a family of curves was produced on it. The curves were of sufficient sharpness and spacing to represent the approximate size of the cells in the region of elongation. Any nonuniformities of growth were readily detected. Transverse motion of the root was also detected on the film because the angle of reflection changed. Following growth recording, the streak photographs were placed in a photographic enlarger and projected so that the final magnification was 53.8-fold.

In the last fifteen years, growth-measuring devices have become even more sophisticated. De la Fuente and Leopold (1970) developed a method employing a position-sensing transducer. The sensing axis was attached to a 2-cm needle, the tip of which rested on a piece of capillary tubing at the top of a stack of coleoptile sections. Rotation of the axis of the transducer altered the balance of current output by two opposing coils and converted a degree of rotation into a voltage change in
electrical output. Hence, displacement of the needle by the elongating section could be traced on a recorder which was calibrated to the displacement of the sensing needle with a traveling microscope. Growth was measured with a sensitivity of 1 μm/min, read at 2 min intervals. This method was employed extensively in studies of growth regulator responses in stem tissue (Warner and Leopold 1971; Barkley and Leopold 1973; Rehm and Cline 1973a, 1973b).

Rayle et al. (1970b) and Rayle and Cleland (1972) used an Instron extensometer to measure relaxation of coleoptile segments under applied stress. The specimen was placed between clamps, and immersed in an incubation medium. An automatic control caused the cross-head carrying the lower clamp to move downward whenever the tension in the specimen fell below 20 grams. The extension was read directly from a dial which showed the position of the crosshead. Rayle and Cleland (1972) and Cleland and Rayle (1972) developed another system for monitoring coleoptile extension, similar to that of Warner and Leopold. A sample was inserted between a lower immobile clamp and an upper clamp attached to a pivoting beam. Extension of the sample caused rotation of the beam. This displacement was detected by a linear displacement transducer. The output from the transducer
was rectified by a demodulator unit, and the rectified signal was passed to a recorder which recorded the average value of the signal every 30 seconds. The method was employed by Cleland et al. (1972b).

Gordon and Dobra (1972) followed elongation responses of oat shoots to blue light by capacitance auxanometry. Their method was based on the following two considerations: (1) the dielectric constants of air and tissue are sufficiently different so that incremental intrusion of a shoot between two plates of an air capacitor would change the capacitance, and (2) the magnitude of the electrical charge is large enough to detect and follow with a precision sufficient to characterize the kinetics of the photoresponse. Alternate measurements were made by switching between an upper (test) capacitor and a lower (monitor) capacitor. The net capacitance changes were due entirely to changes in the geometry of the plant, with the significant variable being the distance from the tip of the shoot to the bottom of the plate. A change in shoot length of 1 μm was normally within the range of sensitivity, and the entire measuring, switching and monitoring cycle could be performed every 3 min.

Murayma and Ueda (1973) employed a differential transformer in growth studies of stem segments. An iron
rod was attached to the top of a stem segment. Initially, the transformer was positioned such that the iron rod was just at the center of the transformer, and hence no electrical potential was induced between the terminals of the two secondary coils. As the stem segment grew, the iron rod was pushed upward inducing an electrical potential between the terminals of the secondary coils. This electrical potential was amplified and recorded continuously to obtain a growth curve.

Short-term measurement of elongation of Nitella was performed with a laser optical lever auxanometer by Métraux and Taiz (1977). This method consisted of an optical lever in conjunction with a laser, and was capable of resolving increments as small as 0.1 μm. The optical lever arm was simply a small glass tube attached perpendicularly to a razor blade. A front surface mirror was cemented to one side of the razor blade and the edge of the blade positioned in a groove in a horizontal axis beam. Cells were anchored to the base of the chamber and to the optical lever arm. A small counterweight induced a slight tension in the system without stretching the cell.

During the 1980's, with an increasing interest in gravi- and phototropism, a strong desire to measure surface extension rates of curving plant organs evolved.
Also, with the advent of significant utilization of microcomputers in laboratory research, digitization became a useful monitoring device. Kuzmanoff and Evans (1981) digitized analog information to determine short term kinetics of various types of roots. Employing an IMSAI PCS-42 microcomputer, the digitization process required about 128,000 measurements to achieve precision of 4 μm over a 1 mm range.

Gordon et al. (1982) studied the growth patterns of hypocotyls, by time-lapse photography with a cine-camera and a timing unit. The developed film was projected using a data analyzer to give a 25X magnification. Marker beads which were placed on the hypocotyl could be tracked, and the distances between the beads as a function of time measured, to allow the growth rates of individual zones of the hypocotyl to be calculated. Growth patterns in inverted hypocotyls were studied with another technique, namely image analysis (Gordon et al. 1984). Growth measurements of marked seedlings were made by digitization of analog video signals. A video camera was used to transmit signals from an experiment or from a screened projection of a filmed experiment. The image was transmitted directly to a monitor, or recorded for subsequent display. Horizontal and vertical cursor lines activated by a video analyzer were manipulated by a
joystick controller to intersect on each marker bead, and
the coordinates of its position fed into an IBM model
8032 Pet microcomputer via an analog-to-digital
convertor. Lengths of individual 1-mm zones were measured
with an accuracy of 10%.

A system for measurement of growth by video image
analysis was also devised by Wakefield and coworkers
(Wakefield et al. 1983; Telewski et al. 1983; Jaffe
et al. 1985). An analytical video signal from a video
camera was fed into a video digitizer in an Apple II+
microcomputer, which could analyze a maximum of 65,000
matrix coordinates. (This was later increased to 245,760
coordinates). A program scans the plant, locates the tip
of the plant and reconstructs the outline of the plant.
It then calculates the midline of the curving organ, and
determines its length and curvature. It does the same
for the upper and lower sides. Recent improvements of
the system allow for the analog output to be fed into a
video cassette recorder, modified for time-lapse
operation. With this feature, images of the plant can be
captured by a series of 30 s recordings made every 6 min.

Although the last few years have brought promising
developments in growth measuring techniques for use on
curving organs, the quality of those techniques (in terms
of accuracy as well as frequency of measurement) is still
far from that achieved with methods for straight growth. In addition, many of the methods described above fail to elucidate the distribution of growth along the curving organ, as was possible for straight-growing organs using various techniques. Obviously, research to date is in need of a highly accurate, nearly continuous means of monitoring growth extension patterns along curving surfaces, and along any surfaces with more complex growth patterns than those observed during straight, unidirectional growth.

Growth Analysis Methodology

"Geotropic curvatures have served far more frequently as a means of demonstrating theories than as the subject-matter of exact observation." Such were the words of L. Jost (1907) early in the century, and such was the impetus for development of quantitative means for analyzing gravitropic responses. It is true that all changes of form, inasmuch as they necessarily involve changes of actual and relative magnitude, may in sense be looked upon as a phenomena of growth; and it is also true, since the movement of matter must always involve an element of time, that in all cases the rate of growth is a phenomenon to be considered (Thompson 1917). In short,
it is obvious that the form of an organism is determined by its rate of growth in various directions; hence the rate of growth deserves to be studied as a necessary preliminary to the theoretical study of form, and organic form itself is found, mathematically speaking, to be a function of time.

Blackman (1919) was one of the first to analyze closely plant growth and in so doing he employed the compound interest law. Briefly, in many phenomena of nature we find processes in which the rate of change of some quantity is proportional to the quantity itself. Since money put out at compound interest increases in this way—the rate of increase being clearly proportional to the amount of capital at any time—Lord Kelvin called the law which such processes follow "the compound interest law." According to Blackman, the rate of interest ($r$) is clearly a very important physiological constraint. It represents the efficiency of the plant as a producer of new material, and gives a measure of the plant's economy of working. The rate of interest ($r$) may hence be termed the efficiency index of dry weight production, since it indicates the plant's growth efficiency as measured by increase of dry material, and appears as an exponential term in the equation which expresses the relation between the initial dry weight,
the final dry weight, and the period of growth. It may also be termed the "economy constant" of the plant; it is of course comparable to the velocity constant of a chemical reaction. Although Blackman studied mostly dry weight increases in plants, he applied the concept to elongation processes as well.

Briggs et al. (1920a, 1920b) attempted to fit Blackman's law, as well as several other empirical laws, to actual growth data in order to determine their applicability. Among the other laws they attempted to fit were Robertson's autocatalytic equation (generally applied to chemical reactions) and another more complex logarithmic equation developed by Mitscherlich. In accordance with Blackman, they found that, if it is assumed that growth increases at a uniform rate, the relative rate of plant growth can be determined by the following equation:

\[ w = w_0e^{rt} \]

where \( w \) = dry weight of the plant at time \( t \), \( w_0 \) = initial dry weight, and \( r \) = rate of interest or "efficiency index."

This equation, of course, also assumes that growth is exponential for any given time interval. This same formula was employed by Williams (1946), who recognized
that the calculated relative growth rate \( (r) \) for any given time interval is simply the average value for that interval, and is independent of any changes in \( r \) during that interval. Williams also developed the concept of the "net assimilation rate," which is the rate of increase in a growth parameter actually attributable to a permanent increase in dry weight.

A major advancement in methods of analyzing growth patterns came with the work of Richards and Kavanagh (1943). They studied the growth of tobacco leaves and attempted to analyze the gradients in relative growth contributing to the changing form of the leaves. They defined the absolute rate of increase of a segment as \( \frac{d\Delta s}{dt} \), with \( \Delta s \)=length of the segment, and \( t \)=time. The specific growth rate, or change in growth per unit of growth per unit of time, was defined as \( \frac{d\Delta s}{\Delta s dt} \). As \( \Delta s \) becomes infinitesimally small, the specific growth rate approaches a limit, which they termed the elemental specific growth rate. Working with such complex organs as leaves, they observed that as growth proceeds, each point moves with a velocity which may be expressed (as in mechanics) in terms of its components, \( \frac{dx}{dt}, \frac{dy}{dt}, \frac{dz}{dt} \), in the directions of the axes. With this in mind, they derived a complex formula for description of the elemental growth rate at any point on the leaf surface.
based on its component expansion in the X, Y and Z directions.

Application of these concepts to the mathematically simpler growth of roots was begun by Goodwin and Stepka (1945). Using roots of *Phleum pratense*, they found that it was possible to measure the amount of growth which occurred during short time intervals, in the region between the root apex and characteristically-shaped epidermal marker cells. When such growth increments per unit time (r) were plotted against the initial distance of the marker cells from the root apex (x), sigmoid curves, representing the rates of displacement of x from the root apex, were obtained. The first derivatives of these sigmoid curves, \( \frac{dr}{dx} \) (the slope of the tangent to the curve at any point), gave the rates of elongation of the roots as a function of distance from the tip.

Hejnowicz (1956), also working with *Phleum*, observed with a microscope the rate of displacement of various points on the surface of the root. He interpolated this data with the least squares method and computed the derivative from the interpolated curve to define the average conditions of the distribution of growth in *Phleum* roots.

Erickson and Goddard (1951) and Erickson and Sax (1956) developed a protocol for analyzing growth which is still widely accepted today. Calling x the distance of a
point from the root tip, then \( \frac{dx}{dt} \) is the rate at which this point is displaced from the tip. This rate might be expected to change with distance from the tip and with time. Using their streak photography method, they were able to obtain records of \( x \) vs \( t \). They could calculate slopes \( (\frac{dx}{dt}) \) along these records, and plot these slopes against distance from the tip \( (\frac{dx}{dt} \text{ vs } x) \). Since the curves all looked similar, it could be assumed that at a given distance from the tip, the rate of displacement does not change with time, and hence \( \frac{dx}{dt} \) is only a function of \( x \). It is more valuable to know the rate at which each small portion of a root is growing, rather than the growth rates of entire apical segments. Therefore, they defined the length of a small portion of root as \( \Delta x \), and its rate of change as \( \frac{d\Delta x}{dt} \). Its relative rate of growth would then be \( \frac{(d\Delta x/dt)}{d\Delta x} \). As \( \Delta x \) approaches 0, it is replaced by \( dx \), and the expression becomes \( d(dx/dt)/dx \). Analytically \( dx \) is an element of root length, and its relative rate of change may be termed its "relative elemental growth rate" (REGR). These rates were evaluated at each \( x \) by finding slopes of the curve of \( dx/dt \) vs \( x \).

Goodwin and Avers (1956) used these same mathematical methods in analyzing growth of roots of *Phleum pratense*. They emphasized the limitations of
experimental techniques for measuring growth by differentiating between $dx/dt$, the instantaneous rate of displacement of a point from the apex, and $x/t$, the experimental approximation of that rate. They also differentiated between $d(dx/dt)/dx$, the relative elemental rate of elongation as defined by Erickson and Sax, and $d(x/t)/dx$, the experimental approximation of the relative elemental rate of elongation.

List and coworkers (List 1969, Salamon et al. 1973) employed Erickson's methods to examine growth of primary roots of *Zea mays*. They eliminated Erickson's assumption that growth rate depends only on distance from the apex. Hence, they plotted velocity against time, as well as against distance from the tip. They calculated the values of displacement and velocity by employing a least squares fitting of straight lines to 5 points at a time. The derivative was found by taking the slope of the fitted line, which is a coefficient in the least squares fitting process. They used equally spaced time intervals to obtain a set of velocities along the root axis at given time instants. By interpolating these velocities to equally spaced values of distance along the root axis, they were then able to use orthogonal polynomials for both further smoothing and differentiation, to obtain relative elemental growth rates.
Green (1976) defined surface extension as basically the ability to separate two marks along an axis. Like all biological processes, it is carried out by cells, but can occur in strong gradients within a single cell and hence is subcellular. The process is continuous at least down to the level of a micrometer, and is, in fact, continuous over significant periods of time, as is indicated by the smoothness of the trajectories of marks in continuous-marking experiments. Extension, for short periods of time at least, is exponential and follows the law established by Blackman:

\[ L_1 = L_0 e^{rt} \]

However, according to Green, measurement by studying the behavior of a pair of marks on a surface suffers from the fact that if the marks are far apart, they may span changing values of the rate gradient on the organ. Also, if a long period of time is taken for the measurement, not only will the point pair span more of the gradient than initially, they both will be displaced away from the tip due to the concurrent expansion of surface between the points and the tip. Green claims that in spite of these inaccuracies, it is possible to estimate the rate of relative extension at a point in space and at an
instant in time. This relative elemental rate of elongation is conceptually identical to the relative extension rate \( r \) defined above, but is measured by studying the velocity by which successive marks on the axis are being displaced from the tip. The rates are obtained by calculating the change in velocity as a function of position.

In a qualitative study of leaf expansion, Wolf et al. (1986) assessed the theoretical and numerical distinctions between the two expressions for relative growth (i.e. the relative elemental growth rate and the exponential growth rate). Although they were primarily concerned with growth in area, an analogous relationship can be understood for unidirectional growth. Terming the relative elemental rate \( k_1 \) and the exponential rate \( k_e \), they defined for any time interval, \( h \), that \( A_T/A_T-n = e^{k_1 h} \). Of course, the \( A \) used here represents area, but can easily be replaced by \( L \) to represent length. Since \( k_1 \) is defined as \( (A_T-A_T-n)/(A_T-n)(h)^{-1} \), if \( h=1 \), then \( k_1 \) and \( k_e \) can be related by the Taylor Series expansion for \( e^{k_e} \):

\[
k_1 = e^{k_e} - 1
= k_e + (k_e^2/2!) + (k_e^3/3!) + (k_e^4/4!) + \ldots
\]
From this equation they claimed that it is clear that only if the time interval between observations is short enough that an element increases 15% or less, will magnitudes of \( k_1 \) and \( k_2 \) be approximately equal. As the amount of growth in the observation period increases, \( k_1 \) will become progressively larger than \( k_2 \), as was indicated by their data. They pointed out the importance of remembering, however, that \( k_1 \) and \( k_2 \) provide different measurements of growth although the two may be numerically equal at low rates of growth. The \( k_1 \) is equal to the finite relative change in size (area and length) over a discrete period, while \( k_2 \) is the rate constant of exponential growth.

In his review article on plant growth modeling, Erickson (1976) discussed the various traditional growth curves and equations (linear, exponential, autocatalytic, monomolecular, Gompertz, Bertalanffy, etc.) which had been used to help in characterizing growth. Although such equations have little theoretical value, they can be useful in estimation or prediction of certain growth processes. But, for fitting of empirical growth data, he recommended polynomials. They lend themselves to the derivation of orthogonal polynomials, smoothing formulas and differentiation formulas, which have the advantages of ease of calculation and statistical evaluation. Using
his streak photographs as automatic plots of distance from the tip (x) and time (t), he was able to determine the rate of displacement of points, and then estimate relative elemental growth rates, by numerical smoothing and differentiation formulas (based on least squares fitting of polynomials to the empirical data). Erickson analyzed the spatial and temporal components of root growth and found that time invariance could be assumed true for a considerable period of root growth, but not for the initial stages of growth, for long roots approaching the end of their growth, or for experimentally-treated roots.

Silk and Erickson (1978) used the concepts of fluid dynamics to relate relative elemental growth rates to curvature changes in the plumular hook of lettuce seedlings. They defined curvature, as the rate of change of the tangent angle with respect to the arc length of a curve. Hence:

\[ m(s,o) - m(s,i) = \frac{d}{dt} [ \ln(1 + Kw) ] + u(s) \frac{d}{ds} [ \ln(1 + Kw) ] \]

where \( m(s,o) \) and \( m(s,i) \) are the relative elemental growth rates at the outer and inner edges, respectively, \( w \) is the width of the hook element (s), \( t \) is time, \( u(s) \) is the velocity of departure of s from the apex, and \( K \) is curvature. As
streak photography was inappropriate for tracing growth of a curving organ, they used time lapse photographs, and employed additional smoothing and differentiation formulas to obtain the above parameters. By analogy to fluid systems, they maintained that an implication of steady growth is that one should be able to distinguish between the spatial and material specifications of variables (Erickson and Silk 1980). For example, in roots, the local growth rates may be invariant with time, whereas each material element expands more rapidly and then less rapidly with time as it is displaced from the root tip. Fluid dynamicists term these two specifications Eulerian (or spatial) and Lagrangian (or material). Silk (1984) studied these two aspects of growth and curvature, and correlated the local derivative (site specific) with the material derivative (particle specific) by another term, the convective rate of change. The convective term represents the rate of growth or curvature change due to displacement of a tissue element to a new location. This work laid a foundation for the beginning of growth dynamics.

Gandar (1983) improved upon the notational conventions for spatial (Eulerian) and referential (Lagrangian) descriptions of growth in the root apex. In a spatial description, the growth variables (such as
spatial velocity, field or cell density) are expressed as functions of position on the axis or of time. In a referential description, growth variables are associated with marked particles of root material, and are expressed as functions of the position that a particle occupied at a reference time, or that it occupies at the current time. Gandar devised mathematical formulae for describing spatial and referential velocity and acceleration, and linked them together with a convective component (since individual particles move through a spatially varying velocity field).

In a lengthy review of plant growth curves, Hunt (1982) describes the advantages and drawbacks of the various equations which have been employed in attempts to fit empirical plant growth data. Although most are still useful for many purposes, he recognizes the spline function as one of the most objective methods for fitting curves to lengthy and/or complicated series of data on plant growth (Parsons and Hunt 1981). The method employing this fairly flexible function is free from many of the problems which beset earlier work in plant growth analysis. The number of splines, and the position of the knots joining them, may be determined by objective statistical tests or by the experimenter. The only problem with this method is that it is quite involved,
and it poses an extensive programming task for those wishing to use it regularly.

It is clear that the history of plant growth analysis has been long, and that methods have been expanding continuously. This is not to say that the older methods are not still applicable. Some less sophisticated methods may be effectively employed where high accuracy is not necessary. In addition, some growth equations may be good approximations of certain regions of the growing zone but not of others, or during certain times of the growing period but not during others. Whatever method is employed, it must be recognized that none is perfect, each has its limitations, and that the method chosen should minimize these limitations to the degree desirable.

Conclusion

The methods currently available for measurement of surface extension during development of relatively complex plant forms are still of much poorer quality than those which have been used for measurement of straight, unidirectional growth. The video digitizer which I have developed should offer significant improvement on these methods, and help to better understand growth patterns
during gravitropism in roots of *Zea mays*. Quantitative methods for describing growth patterns range from the relatively simplistic and predictive to the more sophisticated and unbiased. Therefore, I have exercised great discretion in selecting the most appropriate method for estimating relative growth rates in graviresponding roots. I have also attempted to improve on the quantitative methods for describing curvature patterns during gravitropism.
"Tropism" is a term of widespread use among plant physiologists, and a subject of active study recently. Interest in tropisms has grown with the increasing interest in growing plants in space, an environment lacking one important tropic stimulus, gravity. Exactly how a plant perceives gravity, or any tropic stimulus, and responds to it is a topic of great controversy. Endless radioactive tracer and inhibitor experiments have been carried out in attempt to understand the mechanism of tropic response, but perhaps lost in all of this biochemical confusion is one important issue: the essence of the tropism itself. A tropism is defined, after all, as a growth response to a stimulus, in which the direction of the growth response is determined by the direction of the stimulus. Thus tropisms involve changes in cell extension, which bring about changes in growth pattern, and subsequently changes in tissue form. It therefore seems essential that we first establish the
nature of such a growth response before proceeding further.

Firn and Digby (1980) have outlined four criteria which need to be considered when analyzing a tropic curvature. First, and probably most important, the form of differential growth responsible for the curvature must be established. Obviously a differential in growth rate between the two sides of an organ is necessary for the organ to curve. However, that growth differential could result from an acceleration on one side, a deceleration on one side, a differential acceleration of the two sides, a differential deceleration of the two sides, or an acceleration of one side in combination with a deceleration of the other side. Elucidation of the type of growth differential involved in any tropic response could prove valuable in analyzing the involvement of chemical substances in prompting the response. Second, it is helpful to determine where the growth differential is induced. A simple angle measurement of curvature cannot localize the growth differential to a specific region of the curving organ, nor can it distinguish between curvature of a small radius versus curvature of a large radius. Third, the timing of the onset of differential growth is useful. By knowing the latent period prior to alterations in growth rates brought about
by tropic stimulation, and assessing changes during this time, it may be possible to narrow the list of stimulus-induced biochemical/biophysical changes to be considered as likely causes of the differential growth ultimately responsible for curvature. Finally, the last criterion for tropic curvature is the independence of the responding cells. It is important to know whether the cells showing the growth response are dependent on other cells for the perception of the tropic stimulus. If it is not possible to distinguish between site of perception and site of response, the necessity for a message, and hence the whole idea of hormonal control, must be questioned. Of particular interest to me, and what has spurred the development of my surface extension analysis system, are the first two criteria, i.e. the nature of the differential growth causing tropic curvature and where this growth differential is induced. However, the other two criteria will also be considered.

Although inconclusive, many attempts have been made to decipher the growth pattern leading to tropic curvatures. Gravity responses have been studied repeatedly in both roots and shoots, yet the subject remains controversial. As my research has been restricted to curvature in response to that type of stimulus in roots, I will review only the literature on root gravitropism.
Early work on graviresponding roots failed to distinguish between extension on the upper and lower surfaces. Instead, overall growth of the root during curvature was simply compared to overall growth prior to gravistimulation. Cholodny (1932) and Navez (1933) both failed to observe a "geo-growth" reaction in horizontally-positioned roots. Keeble et al. (1931), on the other hand, found that roots placed horizontally exhibited an acceleration of growth. However, in most cases, workers discovered an overall decrease in growth rate in horizontally-oriented roots (Brain 1935; Larsen 1953; Bennet-Clark et al. 1959).

The first comparisons of the upper and lower surfaces of graviresponding roots were not made until the 1950's. Rufelt (1957) discovered that in wheat roots, the graviresponse consists of a positive reaction which is followed by two negative reactions (curvature away from gravity). The positive reaction is due mainly to growth inhibition on the lower side. Audus and Brownbridge (1957), studying roots of pea, also observed a sequence of gravireactions. During the first period, however, positive gravicurvature results from an inhibition on the lower side along with a slight acceleration on the upper side. Positive curvature
continues during the second period by an inhibition of growth on both the upper and lower sides, with a greater inhibition on the lower side. Following this period, a recovery, or negative gravireactive, phase occurs. Konings (1964), also working with pea roots, found that the first positive response results from alternating periods of slight acceleration on the upper side with strong inhibition on the lower side, and strong acceleration on the upper side with slight inhibition on the lower side. This was followed by a period of extreme inhibition on the lower side with growth on the upper side occurring at a rate comparable to that of vertically-oriented control roots. Veen (1964) observed, in roots of *Vicia faba*, that gravicurvature resulted at first from simply an acceleration of growth on the upper side. This was followed by the onset of a marked inhibition of growth on the lower side. Obviously, from these results, no consensus could be reached as to the timing of the various reactions encompassing the total graviresponse, nor could the growth differentials yielding such gravireactions be elucidated. Unfortunately, the next two decades of research did not prove much more fruitful.

In a review article by Jackson and Barlow (1981), the overall complexity of the bending process in roots
was again emphasized, and the curvature was at least in part attributed to a stimulation of growth on the convex half of the root. They described studies by Shen-Miller et al. (1978) of cell division in graviresponding roots, in which fewer mitotic figures were detected in the upper half as opposed to the lower half of the roots. However, they failed to see an instrumental role for the mitotic differences in promoting curvature, since bending normally commences before newly-dividing cells can progress to the zone of rapid elongation. In addition to comparison of mitotic events, Shen-Miller and coworkers observed differences in cell elongation between the upper and lower surfaces of graviresponding maize roots, especially in the 1.5 - 2.5 mm region. Jackson and Barlow were unable to determine whether this was due to an increase in growth in the upper cells, a decrease in growth in the lower cells, or some combination of the two, and they could not rule out the possibility that gravitropic curvature occurred simply from a change in the boundaries of the cell extension zones of the two sides, without a change in the rates of cell extension. In roots of lentil, Darbelley and Perbal (1984) reported that in the region just distal to the meristem, gravitropic curvature occurs because of greater elongation of the upper cells. In the major portion of
the extending region, however, curvature occurs because of an inhibition of growth of the lower cells.

Pilet and Nougarède (1974) confirmed the overall decrease in growth during gravireponse in studies on maize roots. When primary vertical roots were repositioned horizontally, a decrease in growth was observed, with a greater decrease on the lower than on the upper side. In a further study (Pilet and Ney 1981), they again noted the overall reduction in growth during the initial phases of gravitropic curvature of maize roots. The growth of the lower side was found almost to stop during the maximum rate of gravicurvature. In contrast, later stages of curvature resulted from a slight enhancement of growth on the upper side of the roots. A more recent study by Versel and Pilet (1986) compared the distribution of growth and proton efflux in gravireactive roots of maize. They found that when roots were kept horizontal, the growth of the lower side was strongly inhibited and that of the upper side slightly stimulated as compared with vertical roots. The maximum on the upper side occurred between 3 and 4 mm from the tip. The maximum on the lower side occurred between 4 and 5 mm. For vertical roots, the maximum rate was around 4 mm from the tip.
Barlow et al. (1984) made an elaborate study of the temperature effect on the gravitropic response of roots of maize. At 20-25 C, a stimulation of growth along the upper side and a reduction in growth along the lower side both contributed to curvature. Over the temperature range of 30-35 C, curvature resulted simply from a reduction in growth along the lower side. They also noted that curvature first appears just distal to the region of maximal growth rate, and that the proximal limits of convexity and concavity move back along the root, i.e. differential growth propagates basipetally. However, the extent of the growing zone did not change on either side during the course of gravitropic response.

In a more recent study (Barlow and Rathfelder 1985), maize roots positioned horizontally at 21-25 C were found to respond to gravity with a stimulation of elongation on the upper side and a reduction in elongation on the lower side. Elongation rates could be related to the development of root curvature, and the magnitude of differential growth between upper and lower sides required for a particular rate of bending could be estimated.

Although growth studies during the course of graviresponse have become more accurate, they still fail to yield an noncontroversial description of the growth pattern leading to curvature in roots. Many of the
discrepancies in the reports may in fact be due to variations in growing conditions, seedling age, or the species or variety of plant chosen. However, it is also possible that the differences may be due to insufficient precision in growth measurement. The computer-based system described below was employed in the hope of resolving some of the conflicting observations on the type, location, and time course of growth patterns involved in graviresponse in roots.

Materials and Methods

Plant Material

Maize grains (Zea mays L., B73 x Missouri 17, Mike Brayton Seeds, Ames, Iowa) were soaked in distilled water overnight and then placed on wet paper towels on brown plastic trays (30 x 40 cm). The embryos of the grains were placed against the back surface of the trays and aligned along the vertical axis. The trays were stacked together vertically in a plastic tub, with about 5 cm of water on the bottom. Although the trays were kept under laboratory lighting at 26 C, the developing seedlings received little light and were etiolated. The seedlings were used 2 days after planting, when the primary roots were about 2.5 cm long.
The Apple Computer System

In order to measure surface extension along a root, glass beads (approximately 0.25 mm in diameter) (Arthur H. Thomas Co., Philadelphia, PA) were placed at 1 mm increments along one side of the organ (for vertical orientation) or along both sides of the organ (for horizontal orientation). The seedling was attached (with Mortite caulking compound) to the inside of a vertically-oriented Petri dish, the root being parallel to the back of the dish. The back of the dish was covered with black felt, to provide maximum contrast with the white tissue and glass marker beads.

The Petri dish, containing the seedling, was mounted vertically inside a reinforced Plexiglas chamber (30 x 30 x 30 cm) housed in a humidified growth chamber maintained at 28 C, with a light intensity (Sylvania Lifeline cool white lights) of 60 μEm⁻²s⁻¹ at seedling level (Plate I). The relative humidity inside the Plexiglas chamber was maintained at 100 %, as measured with a hygrometer (G. Lufft GmbH and Co., Stuttgart, West Germany), by wet paper towels lining each wall of the chamber, except the portion of the front wall used as a viewing port for the video camera. The towels were in contact with standing water at the bottom of the chamber. Air was bubbled into
Plate I.

Preparation of Plant Material for the Apple System

The seedling was marked with glass beads and then mounted with putty on a Petri dish containing a black felt background. The dish was placed inside a Plexiglas chamber where the relative humidity was kept at 100% as measured with a hygrometer.
Plate I.
the water through an air stone at a flow rate of 5000 ml min$^{-1}$.

The displacement of the marker beads during growth and gravitropic curvature of the roots was monitored using a Panasonic model WV-1500 TV camera fitted with a 16 mm lens. Video output went first to a CRT monitor (Koyo model TMC-9M) (Plate II) and then to a video digitizer (Dithertizer II, Computer Stations, Inc., Granite City, Illinois) in an Apple II+ microcomputer (64K memory), located in a laboratory adjacent to the room containing the growth chamber. The digitizer reproduced the video image with an array of 280 x 192 pixels (53,760 total pixels), each with one of the 64 apparent light levels ranging from white to black. The white/black mode was most useful for measurement of glass bead displacement against the black background. The resolution of the digitized image was about 86 μm/pixel, i.e. a marker had to be displaced at least 0.086 mm for it to be detected as a growth increment by the computer.

A program was written for semiautomatic tracking of the beads during displacement by growth (Appendix A). The program operated by superimposing two movable cross hairs over the digitized image displayed on the monitor (Amdek Color I, Amdek Corp., Arlington Heights, IL). The
Plate II.

The Apple System for Growth Measurement

A video camera was focused on the seedling growing within the Plexiglas chamber. Output went to a CRT monitor, which reproduced the image of the white marker beads on the white plant surface against the black background. Video output then went to a video digitizer in an adjacent laboratory, for growth analysis.
Plate II.
cross hair being traced by the computer was keyboard selectable, and flashed on and off repeatedly. It could be moved to any point on the screen by touching a light pen (Symtec, Inc., Farmington, MI) to the screen at the desired location, or by keyboard control. At the bottom of the monitor a table was displayed with the sample number, X and Y coordinates of the flashing cross hair (in screen units), and running real time (Thunderclock Plus, Thunderware, Inc., Oakland, CA). Upon command, the computer stored the position and time data in an array, and periodically it moved these stored data from a filled array to a disk as a sequential file. A print-out of a stored data file could be made, displaying X- and Y-coordinates, distance between markers, and real time (Table 1). The computer could also store whole digitized images to a floppy or hard disk for later retrieval and analysis by the same software.

As growth occurred in a root, the glass beads became displaced from each other (Fig. 1 and 2). These displacements were used to monitor growth patterns on the surfaces of vertically- or horizontally-oriented organs. With the cross hairs, the locations of markers and distances between adjacent markers were monitored through time. These data were stored as previously described. Such a data file was used to approximate relative growth
Table 1.

Sample Data from the Apple System

A print-out of a stored data file reveals the X- and Y-coordinates of a given marker, the distance between it and the next adjacent marker, and the real time of measurement.
Table 1.

ROOT-INVENTORY GROWTH DATA FILE

FILE: GEO1UPPER

2 ROOTS

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Figure 1.

**Sequential Digitized Images of Growth of a Vertically-Oriented Root of Maize**

Digitized images of a vertically-oriented root of maize are presented, demonstrating the displacement of the glass marker beads over time. Images are for every 60-90 min over a period of 5 h. The real times are displayed below each image in hours: minutes: seconds.
Figure 1.
Figure 2.

Sequential Digitized Images of Growth of a Horizontally-Oriented Root of Maize

Digitized images of a horizontally-oriented root of maize are presented, demonstrating the displacement of glass marker beads over time. Images are for every 30-60 min over a period of 4 h. The real times are displayed below each image in hours: minutes: seconds.
HORIZONTAL

1.49.85

2.47.15

3.33.31

4.6.12

5.14.07

Figure 2.
rates along the surface of the organ (Appendix B). By analogy with bacterial colony growth, extension of small surface regions over short periods of time was assumed to be exponential and hence approximated by the formula:

\[ r = \frac{(\ln L_2 - \ln L_1)}{(t_2 - t_1)} \]

where \( r \) = relative growth rate (mm mm\(^{-1}\) h\(^{-1}\)), \( L_1 \) = length at time 1 (\( t_1 \)), \( L_2 \) = length at time 2 (\( t_2 \)) (Green 1976). The change in length of a segment over time was used to calculate the relative growth rate for that segment. The location corresponding to the rate was taken to be in the middle of the segment used for the rate calculation, and was expressed in terms of the total distance from the organ tip (mm). These calculated values were stored in a new sequential file as an array of distance from tip and relative growth rate. A print-out of such data could also be made. The time transpired from the onset of the experiment (h) was included in this print-out.

In addition, the marker bead locations could be used to calculate curvature in horizontally-oriented roots (Appendix C) (Fig. 3). First, slopes were calculated for lines passing through the first 2 markers on either end of the organ. The angle of curvature (\( \theta \)) was calculated from these slopes (\( m_1 \) and \( m_2 \)) by the simple equation:
Description of Curvature with the Apple System

A diagram of the method of curvature description with the Apple System is presented. The angle of curvature ($\theta$) (in degrees) was calculated from the slopes ($m_1$ and $m_2$) of the lines passing through the first two markers on either end of the organ.
Figure 3.
\[
\tan \theta = \frac{(m_a - m_1)}{1 + m_a m_1} \quad 0 < \theta < 180
\]

The angle of curvature (in degrees) was determined at each sample time, and was stored along with the time transpired in a separate sequential file. Print-outs of such stored files could also be made.

These data files, stored as described with either relative growth rates or angle of curvature, could be compiled from several experiments and fed into a Curve Fitter Program (Interactive Microware, Inc., State College, PA). This program allowed averaging and smoothing of the data, as well as fitting of a curve to the data. The polynomial least squares fit attempted to fit the data by an equation of the form:

\[
Y = A + (B \cdot X) + (C \cdot X \cdot 2)...
\]

This was satisfactory for an estimation of the relationship between relative growth rate and distance from the tip, as well as for angle of curvature vs. time data. Both types of fitted curves, along with the averaged and smoothed data points, were printed out on graphs, and used to examine growth and curvature patterns.
Data were collected from 10 roots oriented vertically at approximately 15 min intervals for a total of 4 hours. Then roots were oriented horizontally and data were collected similarly for the upper and lower surfaces of these roots. Data analysis allowed comparison of growth patterns of normal and graviresponding roots. The time course of curvature was calculated from data for the upper surface of graviresponding roots.

The IBM Computer System

In order to measure surface extension, glass bead markers (approximately 0.15 mm in diameter) (Arthur H. Thomas Co., Philadelphia, PA), were stained black with India ink and placed at regular increments (approximately 0.5 mm) along one or both sides of the plant organ. The seedling was then mounted on the inside of a vertically-oriented Petri dish using putty to attach the grain to the dish. The back of the dish was covered with white felt to provide maximum contrast with the black glass beads (Plate III). This is different from the method adopted for the Apple System. Rather than allowing the root and the markers to both stand out, this system isolates the markers alone.

The Petri dish containing the seedling was mounted vertically or horizontally inside a reinforced Plexiglas
Plate III.

**Preparation of Plant Material for the IBM System**

Above: The seedling was marked with black glass beads and then mounted with putty on a Petri dish containing a white felt background.

Below: Video monitor display of the black markers as they appear distinct from both the plant surface and the background.
Plate III.
chamber, as described for the Apple system, and illuminated with Sylvania Lifeline cool white lights (60 \( \mu E m^{-2} s^{-1} \) at seedling level). The temperature inside of the chamber varied from 25-31 C depending on the outside room temperature. The relative humidity inside the chamber was maintained at nearly 100 %. Prior to each experiment, a beaker of boiling water was placed in the rear of the chamber. This helped to keep the temperature and humidity up during the course of the experiment.

The displacement of the marker beads during the growth and gravitropic curvature of the roots was monitored using a Panasonic model WV-1500 TV camera fitted with a 16 mm lens. Video output went both to a CRT monitor (Hitachi model VM-129U) (Plate IV), and to a video digitizer (Video Van Gogh, Tecmar, Inc., Cleveland, OH) in an IBM personal computer (256 K memory). The digitizer reproduced the video image with an array of 256 x 256 pixels (65,536 total pixels), a 22 % increase over the Apple System. Each pixel had a light level ranging from 0 (black) to 255 (white). With the 16 mm lens, the resolution of the digitized image could reach 50 \( \mu m/pixel \), i.e. a marker had to displaced at least 0.050 mm in order to be detected as a growth increment by the computer. This resolution is a 43 % improvement upon the Apple System.
Plate IV.

The IBM System for Growth Measurement

A video camera was focused on the seedling growing within the Plexiglas chamber. Output went to a CRT monitor, which reproduced the image of the black marker beads on the white plant surface against the white background. Video output also went to a video digitizer for growth analysis.
Plate IV.
A program was written for automatic tracking of the glass bead markers during displacement by growth (Appendices D and E). The program operated by first requiring cursor localization of all of the markers. It then continually checked their locations based on light levels (markers = 0; background and roots > 0). When a given marker moved between trackings, as a result of extension of the surface on which the marker was situated, the cursor automatically went into a search routine to determine its new location. In this way, the location of all of the markers was known almost continually. Experiment duration and the time interval for marker location recording were specified by keyboard input at the beginning of each experiment. The collected data (marker locations and elapsed time) were stored in an array (Table 2). Upon completion of each experiment, the stored data were transferred from the array to a floppy or hard disk (IBM) where they were stored as a sequential file.

As growth occurred, the distances between marker beads increased. The displacement of the beads was used to monitor growth patterns on the surfaces of vertically- and horizontally-oriented roots, as was done with the Apple System. The data files, stored as previously described, contained the X and Y coordinates of the
Table 2.

**Sample Data from the IBM System**

The X- and Y-coordinates for two adjacent markers in the region of maximum elongation of a maize root are shown for a period of 60 min. Individual 2 min readings are shown for the first 20 min and for 10 min intervals thereafter. The root was mounted vertically (extension along the Y axis). One unit on the Y axis = 0.062 mm. Initial spacing of the two markers was approximately 0.68 mm.
<table>
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<th>Y-coordinate</th>
<th>Time (min)</th>
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marker locations and the time elapsed since the onset of the experiment. These values were used to calculate relative growth rates as a function of position along the root (Appendix F). As for the Apple System, growth was assumed to be exponential and was approximated by the formula:

\[ r = \left(\frac{\ln L_2 - \ln L_1}{t_2 - t_1}\right) \times 100 \]

where \( r \) = relative growth rate (\% h\(^{-1}\)), \( L_1 \) = length at time 1 \( (t_1) \), \( L_2 \) = length at time 2 \( (t_2) \). Note that relative growth rates (RGR's) were expressed as percentages per hour, rather than as mm mm\(^{-1}\) hr\(^{-1}\). The change in length of a segment of root surface over time was used to calculate the relative growth rate for that segment. The location corresponding to the rate was taken to be the middle of the segment used for the rate calculation, and the time corresponding to the rate was taken as the middle of the time interval used for the rate calculation. The locations of calculated relative growth rates were expressed as distance from the root tip (mm), and were continually adjusted to account for the elongation occurring in more apical zones. These values along with their respective relative growth rates were stored in a new sequential file.
These relative growth rates (RGR's), obtained according to Blackman's protocol, are conceptually identical to those obtained by Erickson, which were termed relative elemental growth rates (REGR's) (Green 1976). Theoretically, discrepancies between rates calculated by either method and true instantaneous, elemental, relative growth rates can be eliminated by reducing the distance between markers and the time interval between measurements. To determine whether the length of the time interval chosen is sufficiently short to allow accurate estimates of instantaneous rates, the time interval between measurements can be repeatedly halved until no significant shift in the growth curve accompanies further shortening of the measurement interval (Silk 1984). Experiments were run on several roots with target locations stored every minute. Relative growth rates were calculated as described above from this position data, using data from every 8 min. These rates were plotted against distance from the tip to generate a growth curve. Then the time interval was halved to 4 min and another growth curve generated. This process was continued until the growth curve stabilized (see below, Fig. 7).

To assess the suitability of such a simplistic equation for estimating accurately true RGR's, a growth
curve generated from this method was compared to one generated by Erickson's method. His method is more complicated and perhaps less biased mathematically. It involves smoothing and differentiating of the position data plotted against time, to yield absolute growth rates. These rates are plotted against distance from the tip, and interpolated to evenly spaced values. Then they are smoothed and differentiated to yield relative growth rates, which can also be plotted against distance from the tip. The entire calculation procedure requires a substantial amount of computer time (Appendix G). Therefore, the growth curve generated by the method of Erickson was compared to one generated by the simpler method developed by Blackman, using the same data set with a 2 min time interval for measurement (see below, Fig. 8).

The glass bead marker locations were also used to calculate curvature in horizontally-oriented roots (Appendix H) (Fig. 4). First slopes were calculated for lines passing through the first two markers at the basal (non-growing) portion of the root (slope $m_1$) and for the two markers nearest the tip (excluding the tip mark to avoid error due to the tapering of the root surface at the tip) (slope $m_2$). The angle of curvature ($\theta$) was calculated from these slopes ($m_1$ and $m_2$) by the equation:
Description of Curvature with the IBM System

Diagrams of the method of description of curvature with the IBM system are presented.

Above: The angle of curvature (θ) (in degrees) was calculated from the slope of the line passing through the first two markers at the basal, nongrowing portion of the root (m₁) and the slope of the line passing through the two markers nearest the tip, excluding the tip mark (m₉).

Below: The radius of curvature (R) (in mm) was calculated by finding the intersection of two perpendiculars to the lines passing through markers on each end of the curving region. The average distance between this center point and the two segment midpoints (tangent points) was accepted as the radius of curvature.
Figure 4.

\[ R = \frac{1}{2} (R_1 + R_2) \]
\[
\tan \theta = \frac{(m_a - m_1)}{(1 + m_a m_1)} \quad 0 < \theta < 180
\]

The angle of curvature (in degrees) was determined at each sample time, and was stored along with the elapsed time in a separate sequential file. Although this method of curvature description provides an accurate and convenient estimation of the kinetics of curvature development, it fails to evaluate the extent of the curving zone. The radius of curvature (based on the fitting of a circle to the overall curving surface) was easily calculated by finding the point of intersection between two lines perpendicular to the segments on the perimeter of the curving zone (which are in essence tangent lines to the circle) (Fig. 4). That point was termed the center of the best fit circle, and the average distance between it and each of the two segment midpoints (i.e. tangent points) was calculated as the radius of curvature (R) (in mm). The radius of curvature was determined at each sample time, and was stored along with the elapsed time in a separate sequential file.

These files, containing either relative growth rate, angle of curvature or radius of curvature data, were compiled from several experiments, i.e. several roots. The data were sorted, averaged and smoothed by additional
software (Appendix I), and then sent to a Line Chart Program (Energraphics, Enertronics Research, Inc., St. Louis, MO), which could fit a variety of line types to the data. The high-order regression line type fits a curve with a polynomial equation of the form:

\[ Y = A + (B \cdot X) + (C \cdot X^2) + \ldots \]

Choice of an appropriate polynomial was based on an attempt to find a reasonable balance between good fit and smoothness, avoiding both the strong fine structure oscillations characteristic of higher degree polynomials and the reduced accuracy of lower degree polynomials.

Data concerning the time course of radius of curvature development were more appropriately fit by simply a line segment fit (which connects the data by straight lines), due to the sharpness of the curve. All fitted curves were printed as graphs and used to examine growth and curvature patterns.

Data were collected at 2 min intervals for 2 h from 5 roots oriented vertically and from 5 roots oriented horizontally. Then data were collected from 5 roots oriented vertically, and these same 5 roots were oriented horizontally for further data collection. Both experiments allowed comparison of growth patterns in
Results and Discussion

Growth Rate versus Position in Vertical and Gravistimulated Roots (Apple System)

Figure 5 illustrates the dependence of RGR on distance from the tip in roots of maize oriented vertically, as determined by the Apple System. The figure shows a polynomial curve fit by the Curve Fitter software to growth rates after compilation, averaging and smoothing of rates calculated for 10 roots, at 15 min intervals for a total of 4-5 h. Those averaged rates are also indicated on the figure.

Maximal rates of surface extension appeared to occur at approximately 3.5-4.0 mm from the root tip in vertically-oriented roots, with the rate decreasing on either side of the maximum to yield a bell-shaped curve of RGR vs distance from the tip. The same region of surface extension was apparently present in horizontally-oriented roots (Fig. 6), with the same location of the maximal rate. However, maximal rates were lower for
Growth of Vertically-Oriented Roots of Maize (Apple System)

The relative growth rates of vertical roots are indicated as they vary with distance from the root tip. The rates appear as (tenths of mm)/mm/hr. They were calculated from data for 10 roots, monitored for 4-5 h at 15 min intervals. The growth curve was plotted by Curve Fitter software as a polynomial fit to the compiled, averaged and smoothed rates. Maximal extension (0.28 mm/mm/hr) appears to occur at 3.5-4.0 mm from the root tip, and the rate decreases on either side of the maximum.
Figure 5.
The relative growth rates of horizontal roots are indicated for the upper side (above) and for the lower side (below) as they vary with distance from the root tip. The rates appear as (hundreths of mm)/mm/hr. They were calculated from data for 10 roots, monitored for 4-5 h at 15 min intervals. The growth curve was plotted by Curve Fitter software as a polynomial fit to the compiled, averaged and smoothed rates. Maximal extension on the upper side was 0.24 mm/mm/hr and on the lower side was 0.19 mm/mm/hr. Both maximal rates occurred at 3.5-4.0 mm from the root tip, as in vertical roots.
Figure 6.
roots oriented horizontally. The maximum relative growth rate for vertically-oriented roots was 0.28 mm/mm/hr, while that for the upper surface of horizontally-oriented roots was 0.24 mm/mm/hr and that for the lower surface of horizontally-oriented roots was 0.19 mm/mm/hr.

These results indicate that under normal conditions, gravitropic curvature in roots of maize occurs by a deceleration of growth on both the upper and lower surfaces, with the deceleration being greater on the lower surface. In other words, gravitropic curvature results from a differential inhibition of growth. These results are critical supporting evidence for any growth inhibitor model of root gravitropism. They are consistent with the Cholodny-Went hypothesis for tropisms, and are in agreement with earlier studies showing that a deceleration of growth along the lower side is a major factor in establishing positive gravitropic curvature in roots. However, it should be pointed out that the pattern of differential curvature observed for root gravitropism in this cultivar of maize may not apply to seedlings of other cultivars or species. In light of data suggesting that gravitropism in some roots may in part result from a slight acceleration of growth on the upper side, it was important to establish definitively the nature of the growth pattern leading to
gravitropic response in this cultivar of maize roots, before proceeding with additional growth inhibitor studies on these roots.

**Time Course of Gravitropic Curvature (Apple System)**

Curvature began after about 0.5 h in horizontally-oriented roots, with maximal (75°) being reached in about 3 h (Fig. 7). A slight autotrophic response did appear to occur at this time. The response time of 30 min must be kept in mind during further studies of graviperception and response. In addition, any acceptable mechanism of gravireponse must appropriately explain the reverse gravireaction of autotropism. The growth patterns leading to this response will be considered in Chapter 4.

**Determination of Appropriate Time Interval Between Position Measurements (IBM)**

To determine the measurement interval that provides an accurate approximation of instantaneous rates and total length of the extending region, growth curves of RGR versus position were plotted using time intervals between position measurements ranging from 1 to 8 min (Fig. 8). Increasing the frequency of measurements resulted in a shortening of the apparent zone of elongation at the basipetal end, and an increase in the
Figure 7.

Curvature of Horizontally-Oriented Roots of Maize (Apple System)

The time course of curvature development is indicated for graviresponding roots. Curvature was calculated as the angle (in degrees) between the tangent to a pair of marker beads at the base of the root and the tangent to a pair near the tip of the root. Curvature began after about 0.5 h and maximized at 75° after 3 h.
Figure 7.
Figure 8.

Dependence of Relative Growth Rate Distribution on Time Interval between Recording of Marker Positions

The four curves represent relative growth rates calculated from position data taken from the same 5 roots at different frequencies. Time (min) between data sampling is indicated by the number adjacent to each curve. It can be seen that no further change in the growth curve occurred when the measurement interval was reduced from 2 min to 1 min.
Figure 8.
apparent maximal rate of elongation. The maximum RGR appeared to increase from 25% h⁻¹ for 8 min intervals to 43% h⁻¹ for 2 min intervals, and the basipetal end of the elongation zone appeared to shift from 8 mm to 6 mm from the tip. The apparent shift in the basal limit of the elongation zone away from the apex with decreasing frequency of data collection most likely reflects the additional total root extension that occurs during long measurement intervals. This would be expected to cause an apparent shift in the basal boundary of the elongation zone away from the apex since the position assigned to the calculated rate for each measurement interval is based on the distance between the tip and the center of that measurement interval. The apparent decrease in relative growth rate with decreasing frequency of data collection is most likely indicative of a narrow zone of peak RGR. For short-term measurements, the data would be collected primarily or exclusively from that zone. For longer measurement intervals, the markers may be displaced to adjacent regions of reduced RGR resulting in a lower average for that measurement interval. No further change in the growth curve occurred when the measurement interval was reduced from 2 min to 1 min. Therefore, growth rate data obtained using 2 min measurement intervals were used to approximate instantaneous growth rates along the root surface.
Comparison of Blackman and Erickson Methods for Calculating RGR's

In order to establish the validity of the Blackman method for estimating relative growth rates, a program was written for RGR calculation based on another widely accepted method, i.e. that of Erickson (see Appendix G). This method is particularly appropriate when long time intervals between position measurements are employed, and hence the assumption that growth is exponential is invalid. However, with the computer system, position measurements are made every 2 min, and over such short time intervals the assumption of exponentiality would seem to be mathematically appropriate. This is stated in other terms by Wolf et al. (1986) in their demonstration, with a Taylor series expansion, that when the time interval between measurements is short enough to suitably reduce the amount of growth, the magnitudes of the relative elemental growth rate and the exponential growth rate are approximately equal (see Introduction). To determine whether this is the case, growth curves generated by Blackman's formula were compared with curves generated by Erickson's method (Fig. 9), which utilizes less biasing mathematical formulae for deriving growth velocities. Although the smoothing and differentiation
Comparison of the Blackman and the Erickson Method of Relative Growth Rate Calculation

The two curves represent relative growth rates calculated from position data taken from 5 roots at 2 min intervals. The rates were calculated either by the Blackman logarithmic equation, or by the Erickson method of double differentiation. The two curves are similar in maximal rate and differ only slightly in the extent of the elongation zone.
Figure 9.
formulas, as well as the least squares parabola interpolation method, are indeed means of fitting curves to a given data set (and hence require some degree of mathematical bias), they do not go so far as to replace the double derivative with a logarithmic function. So, for long measurement intervals, where growth is not fully exponential, Erickson's method is certainly more suitable. However, when the time interval is sufficiently reduced so as to closely estimate instantaneity, the error involved in the logarithmic approximation is also reduced. The growth curves generated by the two methods, therefore, appear similar in maximal rate, and differ only slightly in the boundaries of the extending zone. Unfortunately, the appropriateness of either method at estimating elemental growth rates cannot be determined until the distance between markers on the extending surface is adequately reduced so as to approach infinitesimal.

Growth Rate versus Position in Vertical and Gravistimulated Roots (IBM System)

Figure 10 indicates the dependence of RGR on distance from the tip in roots of maize oriented vertically and horizontally, as determined by the IBM system. The figure is redrawn from a computer print-out
Figure 10.

Growth of Vertically- and Horizontally-Oriented Roots of Maize (IBM System)

The relative growth rates ($\% \text{ h}^{-1}$) of vertical and graviresponding roots are indicated as they vary with the distance from the root tip. Each curve is the best fit through relative growth rate versus position data from 5 roots, with marker bead positions recorded every 2 min for a 2 h period. $V =$ vertical; $HU =$ horizontal, upper side; $HL =$ horizontal, lower side.
Figure 10.
obtained from the Energraphics software, after compilation, averaging and smoothing of growth rates measured in 5 roots, at intervals of 2 min for a total of 2 h.

The region of maximal extension occurred approximately 3 mm behind the tip in vertically-oriented roots, with the rate decreasing on either side of the maximum to yield a bell-shaped curve of RGR versus distance from the tip. This is similar to the growth curve generated by the Apple system, although the maximal extension rate is higher and the overall extension zone is shifted somewhat apically. This again demonstrates the improvement in accuracy with a shorter time interval between position measurements. In addition, the figure illustrates more reliably the actual growth curve of maize roots due to the higher resolution of the IBM digitization. Surface extension occurred from about 1 to about 6 mm from the root tip.

In horizontally-oriented roots the region of maximal elongation was displaced basipetally, as was the entire extending zone. Maximal surface extension occurred approximately 4.5 mm from the tip on the upper side, and 3.5 mm from the tip on the lower side. The basal boundary of the overall extending zone reached about 9 mm on the upper side and 8 mm on the lower side.
Experiments of greater overall duration indicate that this basipetal shift may be transient, and the zone may return to a more acropetal region after several hours. (see Chapter 4).

In vertically-oriented roots, the maximal relative rate of elongation was about 43% h\(^{-1}\). The maximal rate observed for horizontally-oriented roots was about 32% h\(^{-1}\) along the upper surface, and 20% h\(^{-1}\) along the lower surface.

Since maximal rates and the boundaries of the extending zone do vary a bit from root to root, or with changing temperature and humidity, these studies were repeated employing the same roots for both analyses. Growth curves were determined for vertically-oriented roots observed for 2 h; then the same roots were turned horizontally so as to provide a gravitropic stimulus, and growth curves were generated again. The results were comparable to those shown in Figure 10.

In accordance with the results from the Apple system, these data indicate that it is a differential inhibition of growth which is responsible for the gravitropic response in roots. They support the Cholodny-Went theory of tropisms, suggesting that a lateral gradient in a growth inhibitor such as auxin may facilitate the response. However, these results also
indicate that a shift in the growth zone may occur during gravitropism. Any interpretation of the gravitropic mechanism must take this into account, and provide an adequate means of explaining the reactivation of mature tissue to assume the role of active, elongating tissue, and possibly also the delay in meristematic tissue to begin the elongating process. Such results indicate that gravitropism is a finely tuned phenomenon involving not only changes in the degree of growth in certain cells tagged as growing cells, but also triggering of the on/off switch which controls the presence or absence of growth.

**Time Course of Gravitropic Curvature (IBM System)**

Curvature (in degrees) again appeared to begin about 0.5 h after gravistimulation, with maximal (50°) curvature occurring at about 2 h (Figure 11). Again, some autotropism is evident. The final angle of curvature achieved is somewhat reduced from that observed with the Apple System. This may in part be explained by the fact that the tip marker was not used in curvature calculation with the IBM system. Use of the tip marker added some error to the Apple system calculations, due to the noticeable tapering of the root surface at the tip.
Curvature of Horizontally-Oriented Roots of Maize (IBM System)

Above: The time course of curvature development is indicated for graviresponding roots. Curvature was calculated as the angle (in degrees) between the tangent to a pair of marker beads at the base of the root and the tangent to a pair near the tip of the root. Curvature began after 0.5 h and maximized at 50° after 2 h.

Below: The time course of radius of curvature establishment is indicated for graviresponding roots. The radius was calculated as the average distance between the point of intersection between two lines perpendicular to the perimeter root segments, and the midpoints of those two segments. The radius of curvature dropped below infinity after 0.5 h and stabilized at about 5 mm after 90 min.
The overall radius of the curving zone decreased consistently from infinity beginning after 30 min. It stabilized after about 120 min at approximately 5 mm. This value, in itself, is only as useful as the overall degree of curvature; it gives some indication of the sharpness of the curving zone. However, a combination of the degree of curvature and the radius of curvature yields an approximation of the extent of the curving region. Obviously, two roots with the same degree of curvature may differ in the radius of curvature, or two roots with the same radius of curvature may differ in the degree of curvature. Both sets of roots will differ in the extent of the curving region. Unfortunately, such a simplistic estimation of the curving region is only useful for comparative purposes. A more detailed description of the shape of the curving zone, especially as it varies through time, is needed to thoroughly understand the gravitropic response (see Chapter 4). The overall radius of curvature does, however, serve to improve on the simple degree of curvature measurements which have been used by most investigators of gravitropism to date.
CHAPTER II

ETHYLENE INVOLVEMENT IN GROWTH AND GRAVITROPISM

Introduction

Because ethylene and auxin have so many effects in common and since auxin has been found to stimulate ethylene evolution, some of the effects attributed to auxin might be due indirectly to ethylene produced in the tissues (Abeles and Rubenstein 1964). Before an auxin-ethylene relationship can be established, however, it is necessary to show that auxin-enhanced ethylene evolution occurs and that ethylene evolution can be controlled by endogenous levels of auxin. According to the Cholodny-Went theory, tropistic responses are due to an asymmetric distribution of auxin across stimulated tissue (Cholodny 1927, Went 1928). Since auxin controls ethylene production, the auxin gradient thought to be caused by a tropistic stimulus should cause a parallel gradient of ethylene production (Abeles 1973). Abeles and Rubenstein (1964) found that the synthetic auxin, NAA, stimulated ethylene evolution from roots, stems and leaves of 9 species of plants. In addition, they found that changes
in ethylene evolution could be correlated with endogenous auxin levels in bean hypocotyls. Thus, the asymmetric auxin distribution in geotropically and phototropically stimulated hypocotyls was paralleled by a similar asymmetric distribution of ethylene evolution.

Burg and Burg (1966) studied the interaction between auxin and ethylene and its role in plant growth. They found that IAA induced ethylene formation in stem sections from a variety of plants. Ethylene in turn caused both swelling and inhibition of extension growth in pea tissue, and inhibition of extension in sunflower sections. When pea seedlings were placed on their sides, the stems would start to curve upward within 2 h, and the roots downward within 3-4 h. However, when ethylene was present, both would continue to grow in a horizontal direction. Furthermore, when ethylene was present, the uptake and polar transport of auxin was not affected, but lateral transport was almost completely abolished. They proposed that the ability of ethylene to interfere with tropistic responses is due to an inhibition of lateral auxin transport.

Chadwick and Burg (1967) found that low concentrations of IAA inhibited the elongation of pea root sections by inducing the formation of ethylene. This ethylene was produced within 15-20 min after IAA
application, and the roots began to swell immediately after they were exposed to the gas. Carbon dioxide competitively inhibited ethylene action in roots, impeding their gravitropic response, and partially reinstating auxin-inhibited growth (at concentrations of 5-10%, although at higher concentrations growth was again inhibited). They concluded that ethylene participates in the gravitropic response of roots, and that the overall decrease in growth during gravitropic curvature of roots is unlikely to be due solely to auxin redistribution. The release or synthesis of a growth-inhibiting substance such as ethylene might be involved.

As discussed earlier, growth of the upper side of roots of pea and other plants has often been reported to decrease during gravitropic curvature. Burg and Burg (1968) proposed that a portion of the ethylene produced in the lower side of a gravitropically-responding root might be expected to diffuse to the upper side and there slightly retard elongation. They also suggested that ethylene formed in the lower side of the root during gravitropic response may act by feedback to limit the amount of IAA transported laterally. This is consistent with the evidence that the gas is a potent inhibitor of lateral auxin movement and that it renders many roots and shoots ageotropic (Burg and Burg 1967).
Chadwick and Burg (1970) demonstrated that a large portion of IAA-induced inhibition of the elongation of excised root tips and virtually all such inhibition of elongating intact roots was the result of IAA-dependent ethylene production. They also showed that the amount of ethylene produced in response to applied IAA was governed by the level of the applied IAA which moved into the root tissue. In pea roots, both auxin and ethylene caused substantial inhibition of elongation within 1 hr. Carbon dioxide, an ethylene antagonist, interfered with root gravitropism, indicating the participation of ethylene in the gravitropic response. (This was not the case in stems.) Applied ethylene immediately and completely prevented gravitropism in pea roots. It was suggested that this was due to swamping by the gas, i.e. the amount of ethylene applied was such that the upper and lower sides responded maximally to it, and hence could not discern endogenously produced ethylene gradients.

Radin and Loomis (1969) studied more carefully the effect of ethylene and carbon dioxide on the growth and development of cultured radish roots. They observed that when roots were grown in controlled atmosphere, ethylene was inhibitory to elongation. One percent CO₂ similarly affected roots not given ethylene. Elongation and
lateral root production was stimulated by 1% CO\textsubscript{2} in ethylene-treated roots. The results suggested that the often-observed stimulation of root growth by CO\textsubscript{2} was due to the ability of CO\textsubscript{2} to antagonize the inhibitory action of endogenous ethylene.

In striking contrast to the work of Burg and coworkers, Andreae et al. (1968) presented evidence against the involvement of ethylene in the mediation of root growth inhibition by IAA. In studies of pea roots, they found that the inhibitory action of IAA is fully reversible on transfer of the excised sections to IAA-free solutions. Inhibition by ethylene, however, is almost totally irreversible. Whereas IAA inhibited growth from zero time, ethylene was generally without effect during the first 3-6 h. Although ethylene production was enhanced by 100 \textmu M IAA, conditions which reduced the rate of ethylene evolution 2 to 3-fold at that same IAA concentration, failed to affect the inhibitory action of IAA on elongation. Finally, while the IAA effect was not dependent on tissue age or on solution composition, the ethylene effect was dependent on both of these factors. These results suggest that ethylene does not play a major role in mediating inhibition of pea root elongation by IAA. They also provide strong evidence against the theory of ethylene mediation of auxin-regulated gravitropic responses.
Studies on the ethylene biosynthetic pathway (Adams and Yang 1979; Yu and Yang 1979) have revealed that L-α-(2-aminoethoxyvinyl)glycine (AVG) is effective at blocking the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), and that cobalt interferes with the conversion of ACC to ethylene. IAA exerts its stimulatory effect on ethylene production by enhancing the conversion of SAM to ACC. Another ethylene inhibitor, silver (either as AgI or as AgNO₃), has been studied by Beyer (1976, 1979). He found that silver specifically blocks the action of applied ethylene in growth retardation. In a study on pea (Pisum sativum) seedlings, he found that silver effectively inhibited not only ethylene action, but also ethylene metabolism, suggesting that metabolism of ethylene may be related to its action. The ability of various butenes and cyclic olefins to induce an ethylene response or to counteract ethylene’s action in etiolated pea seedlings was investigated by Sisler and Yang (1984). The ethylene antagonist, 2,5-norbornadiene (NBD) was found to competitively inhibit ethylene binding with a Kᵢ=170 μl/l. It was also found to reduce ethylene-induced abscission of citrus leaf explants and to be effective in preventing the reduction of indole-3-acetic acid transport induced by ethylene (Sisler and Yang 1985).
Of interest in studying auxin-stimulated ethylene production and its interrelationship in the control of plant morphology and physiology is the diageotropic form of tomato (*Lycopersicon esculentum* Mill.) discovered as a spontaneous mutation of the cultivar VPN8. Zobel (1973) studied this mutant tomato, characterized by unsupported horizontal growth of shoots and roots, and found that the horizontal growth habit was not the result of a lack of response to gravity. Rather it was a preferential response whereby a vertically-oriented shoot would assume a horizontal posture as its apex elongated. In addition, experiments with growth regulators indicated that the mutant did not produce normal amounts of ethylene in response to auxin treatment. The mutant required ethylene for normal (geotropic) growth and development (Zobel 1974). This ethylene requirement was thought to result from a reduction in or elimination of auxin-stimulated production of ethylene. The characteristic response of the diageotropic mutant to exogenous ethylene suggested to Zobel that an endogenous balance between the two growth regulators was the most probable method of control of gravitropism in shoots and roots. Since ethylene appears to modify auxin-mediated responses and synthesis of ethylene is controlled by auxin, their
balance appears to be controlled by a feedback type mechanism. Jackson (1979), on the other hand, studied the same mutant and found no difference in the rate of ethylene production in response to wounding, as compared to non-mutant upright plants. He also observed that application of inhibitors of ethylene action to non-mutant plants did not induce diageotropism. He concluded that the diageotropic mutant was not deficient in its ability to synthesize ethylene. Instead, it was its response mechanism to ethylene and to gravity which had been radically altered. Likewise, Harrison and Pickard (1986) evaluated ethylene as a mediator of gravitropism in tomato hypocotyls. They found that gravitropism can occur without substantial change in ethylene production. Moreover, lowering or elevating ethylene levels, over a considerable range, as well as inhibiting ethylene action, failed to influence gravitropic bending. Although these data showed that ethylene does not mediate gravitropism of tomato hypocotyls, they did not address the concept elaborated by Zobel that a very low level of ethylene is required for the gravitropic response system to function.

An understanding of the effect of ethylene on root growth and its subsequent control of many physiological and morphological responses can only be complete when a
comparative study is made of the influence of ethylene concentrations below, equal to, and above physiological levels. While the effect of ethylene concentrations below physiological levels on root growth has been studied by application of CO₂ and other ethylene inhibitors, the effect of ethylene concentrations above physiological levels has been studied by exogenous application of ethylene. Smith and Robertson (1971) found that low concentrations (less than 1 ppm) of exogenous ethylene caused small increases in root extension in both rice and rye, while higher concentrations (up to 10 ppm) significantly reduced extension. Likewise, Konings and Jackson (1979) found that elongation was stimulated by 0.02 ppm ethylene in roots of rice, white mustard and tomato. Concentrations of 1.0 ppm and above inhibited elongation. The extent of the stimulation was greatest in roots with low rates of endogenous ethylene production and least in those which produced ethylene at high rates. The extent of inhibition was least in roots with low rates of endogenous ethylene production and greatest in roots which produced the gas at high rates. Although the study focused on the roots of plants grown under exceptionally wet conditions (where accumulation of ethylene is great) and their adaptation to such conditions by low rates of
endogenous ethylene production, it also revealed the complexity and sensitivity of ethylene responses in plant tissues, and the sharp differences in both endogenous ethylene production levels and dose response curves to ethylene, in different plant species.

Research in the 1980's has not yielded a clear understanding of ethylene involvement in root and shoot gravitropism, nor of the role of auxin-induced ethylene production in tissue growth. Wheeler and Salisbury (1980, 1981) worked with a number of inhibitors of ethylene synthesis or action (cobalt, AVG, silver and CO₂) and found that they all significantly delayed the gravitropic response of mature dicot stems positioned horizontally. The results were similar in cocklebur, tomato and castor bean stems. They also observed deflections in stems treated with a 1% ethephon solution on one side and then placed on a horizontal clinostat. The bending was directed toward the side of ethephon application, suggesting that direct inhibition of cell elongation on the upper side of horizontal stems by ethylene is a possible mode of graviresponse.

In studies on root tissue, Bucher and Pilet (1981) found no significant difference in the amount of ethylene production between vertically- and horizontally-growing root segments. This suggests that there is no direct
role for ethylene in control of root elongation and gravireaction. In contrast, Mulkey et al. (1982) studied the auxin dose/response relationship in maize roots treated with ethylene biosynthesis inhibitors. They found that in roots pretreated with cobalt and AVG, IAA in concentrations from $10^{-10}$ M to $10^{-8}$ M promoted growth. Higher concentrations (e.g. $10^{-8}$ M) inhibited growth of either pretreated or non-pretreated roots. This suggests that normal root growth and perhaps also root gravitropism is at least in part controlled by ethylene.

Studies of peduncles of dandelion (Clifford et al. 1983) also supported ethylene involvement in growth and gravitropism. Significant amounts of ethylene were evolved from gravistimulated peduncles as compared with vertical controls, but only after 2 h of gravistimulation. However, reduction of the amount of ethylene produced by gravistimulated peduncles by pretreatment with ethylene-biosynthesis inhibitors did not affect or enhance subsequent gravibending. It was proposed that ethylene may control the later stages of gravitropism and influence the straightening process (autotropism).

It is evident that a good understanding of the involvement of ethylene in various growth processes, including gravitropism, has not been attained. The variability in ethylene dose/growth response curves for
different tissues is marked. The wide range of factors which may influence ethylene biosynthesis in normal plant tissue is overwhelming, and the added complexity of many conceivable feedback loops, involving ethylene's influence on biosynthesis or transport of growth substances, makes the task even more difficult. Finally, the controversial results which have been obtained by different means of attempting to determine the necessity of ethylene in the graviresponse have led to only highly speculative models of this growth process. Therefore, utilization of the devised video digitizer system will hopefully lead to at least a partial answer to the question of ethylene's role in root gravitropism.

Materials and Methods

Plant Material

Maize seedlings (*Zea mays* L., B73 X Missouri 17, Mike Brayton Seeds, Ames, Iowa) were germinated as described in Chapter I.

Hypobaric Studies

Roots were marked and placed in the Plexiglas chamber, oriented either vertically or horizontally, as described in Chapter I. One-fifth atmosphere pressure,
as measured with a manometer (VWR Scientific, Inc., St. Louis, MO), was obtained with a vacuum pump (Precision Scientific, Inc., Chicago, IL) and a pressure regulator (Spectrum Medical Industries, Inc., New York, NY). The withdrawal of ethylene was made possible via an output port located at the top of the Plexiglas chamber next to an input port. Under hypobaric conditions, 100% oxygen was supplied to the growing tissue through the input port, in order to simulate the oxygen availability under normal atmospheric conditions (1/5 atm * (100% O₂ / 1 atm) = 20% O₂).

The hygrometer malfunctioned at low partial pressures. So, to ascertain that the water availability remained satisfactory for roots growing under hypobaric conditions, experiments were repeated with water dripping down one side of the root, at 1 second intervals.

In some experiments, low concentrations of ethylene (1.2 - 2.8 ppm at 1/5 atm; simulating 0.24 - 0.56 ppm at 1 atm) were supplied in combination with the oxygen. This was done to attempt to replace the vacuum-withdrawn ethylene, and hence test the reversibility of the response to ethylene removal.

All gases supplied to the growing tissue were humidified by bubbling, through a piece of plastic tubing fitted with an air stone, into the standing water at the
bottom of the chamber. Flow rates were monitored by flowmeters (Gilmont Instruments, Inc., Great Neck, NY), and total input always equalled 2000 ml/min.

Data were collected every 15 min for about 6 h, with the Apple System, and every 2 min for 2 h with the IBM System. Curves of growth rate distribution and of the time course of curvature were plotted as previously described (Chapter I).

Treatment with Norbornadiene (NBD)

Roots were marked and placed in the Plexiglas chamber in the appropriate orientation. Air was bubbled into the chamber (at 5000 ml/min) for approximately 10 min, in order to raise the relative humidity in the chamber to 100%. Then the chamber was stoppered, and the competitive ethylene binding inhibitor, bicyclo [2.2.1] hepta-2,5-diene (NBD), was placed in a Petri dish in the bottom of the Plexiglas chamber and allowed to evaporate and come into equilibrium with the air in the chamber.

The initial quantity of NBD required was calculated from the temperature, volume of the chamber, molar gas constant, and desired final concentration. Generally, concentrations ranging from 1000-20,000 ppm were used.

To insure that the vaporized gas was readily diffusing evenly throughout the chamber, experiments were
repeated with a small, battery-operated fan placed in the back of the chamber.

Experiments were run for 2 h, with data being collected every 2 min. Growth and time course of curvature curves were generated as described in Chapter I.

**Treatment with AVG and Cobalt**

Roots were pretreated for 1 h in L-α-(2-aminoethoxyvinyl)-glycine (AVG) and cobalt nitrate, in 1 X 10⁻² M potassium phosphate buffer at pH 6.3. Roots were mounted vertically with Mortite caulking compound to the inside of a plastic beaker. The beaker was then filled with the pretreatment solution, to the level of attachment of the root to the grain. Oxygen was bubbled continuously into the solution during pretreatment. Both AVG and cobalt are inhibitors of ethylene biosynthesis, AVG blocking the conversion of SAM to ACC and cobalt blocking the conversion of ACC to ethylene. Concentrations ranged from 1-10 μM AVG and from 20-200 μM cobalt.

Roots were then marked and placed in the Plexiglas chamber in the vertical orientation (see Chapter I). Due to the evident difficulty in adaptation from submerged to atmospheric conditions, roots were allowed to acclimate in the Plexiglas chamber for 60 min. Humidity was
enhanced by placing additional wet paper toweling on the ceiling of the chamber. After 60 min, the roots were mounted in the desired (vertical or horizontal) orientation, and data collection was begun. Root extension was monitored at 2 min intervals for 2 h. Growth and curvature were plotted as described previously (Chapter I).

To verify the results obtained with the digitizer system, several straight growth experiments were performed with an auxanometer. An auxanometer is an adaptation of the position-sensor transducer method of straight growth measurement (Evans 1976). It is very sensitive and can be used on roots growing in solution. This allowed growth determination, while the roots were in the presence of the two ethylene inhibitors, rather than after pretreatment. Rapid changes in inhibitor concentrations being administered to the roots could be made, and the problem of acclimatization to atmospheric conditions after being submerged could be avoided. Roots were monitored over several hours, and inhibitor concentrations were elevated regularly, until the maximum concentration utilized with the digitizer experiments was reached.
Treatment with Silver Nitrate

Qualitative studies were made of growth and gravitropic curvature in the presence of the ethylene activity inhibitor, silver nitrate. Roots were placed vertically or horizontally in concentrations of silver ranging from 0.01 μM to 100 μM. Growth and curvature were monitored qualitatively for a period of 12 h.

Treatment with Ethylene

Roots were marked and placed vertically in the Plexiglas chamber. Ethylene was added in combination with air through the input port at the top of the chamber. Both gases passed through the plastic tubing and bubbled through the air stone into the standing water at the bottom of the chamber. The concentration of ethylene in the chamber was monitored by gas chromatography of samples taken periodically through a rubber stopper at the top of the chamber. Concentrations ranged from 1-6 ppm. Gas chromatography determinations were done using a Hewlett-Packard 517 gas chromatograph with an Al2O3 column (90 cm long x 2 mm inner diameter). The temperatures of the injection port, column and flame ionization detector were 110, 100, 110 degrees, respectively.
Results and Discussion

Hypobaric Studies

Roots of maize grown at 1/5 atmosphere with 100% oxygen (oxygen availability = 20%) exhibited an overall decrease in relative growth rate (Fig. 12). The maximal relative growth rate was reduced to 0.11 mm/mm/hr as compared to a maximal rate of 0.28 mm/mm/hr for control roots (see Fig. 5 of Chapter I). The slowed growth was not a result of decreased water availability, as even roots grown under the same conditions, but supplied with a steady stream of water along one side, exhibited the same reduced surface extension rates.

Interestingly, the maximal rate of extension of vertically-oriented roots was less than that of either surface of horizontally-oriented roots (Fig. 13). While the maximal rate of relative growth of vertically-oriented roots was 0.11 mm/mm/hr, the maximal rate along the upper surface of horizontally-oriented roots was 0.17 mm/mm/hr and the maximal rate along the lower surface was 0.12 mm/mm/hr. Curvature was similar to that for roots grown under normal atmospheric conditions (compare Fig. 14 with Fig. 7 of Chapter I). Maximal (75°) curvature occurred after about 3 h. These results show that gravitropic curvature occurs under conditions expected to eliminate or reduce endogenous gradients of ethylene.
Growth of Vertically-Oriented Roots under Hypobaric Conditions (Apple System)

The distribution of relative growth rates is indicated for roots grown at 1/5 atmosphere with 100% oxygen supplied (O₂ availability = 20%). Rates are expressed as (hundreths of mm)/mm/hr. The maximal growth rate is 0.11 mm/mm/hr.
Figure 12.
Figure 13.

Growth of Horizontally-Oriented Roots under Hypobaric Conditions (Apple System)

The distribution of relative growth rates is indicated for the upper surface (above) and lower surface (below) of roots grown at 1/5 atmosphere with 100% oxygen supplied ($O_2$ availability = 20%). Rates are expressed as (hundredths of mm)/mm/hr. The maximal rates are 0.17 mm/mm/hr for the upper surface and 0.12 mm/mm/hr for the lower surface.
Figure 13.
Curvature of Horizontally-Oriented Roots under Hypobaric Conditions (Apple System)

The time course of curvature development is shown for roots grown at 1/5 atmosphere with 100% oxygen supplied ($O_2$ availability = 20%). Maximal ($75^\circ$) curvature is reached after 3 h, as for normal graviresponding roots.
Figure 14.
However, under these conditions curvature results from an acceleration of growth on both the upper and lower surfaces of the root, with the acceleration being greater on the upper surface. Hence, the extent and rate of curvature is about the same for roots under hypobaric conditions as for roots under normal atmospheric conditions. However, the pattern of growth leading to curvature is different. Gravitropic curvature in roots growing under hypobaric conditions occurs as a result of a differential acceleration of growth, while in roots growing under normal atmospheric conditions curvature results from a differential deceleration of growth.

To establish whether the growth effects observed under hypobaric conditions were due primarily to reduced ethylene levels, physiological levels of ethylene (0.02-0.06 ppm) in oxygen were resupplied to roots growing at 1/5 atmosphere and 100% RH. Overall growth was slowed, under such conditions, to the same extent as for roots growing under hypobaric conditions without supplemental ethylene (Fig. 15). The maximal relative growth rate was 0.10 mm/mm/hr for these roots as compared to 0.11 mm/mm/hr for vertically-oriented roots growing at 1/5 atmosphere without the addition of ethylene (see Fig. 12). These results suggest that the reduction of growth under hypobaric conditions results from some effect of
Figure 15.

Growth of Vertically-Oriented Roots under Hypobaric Conditions with Supplemental Ethylene (Apple System)

The distribution of relative growth rates is indicated for roots grown at 1/5 atmosphere, with physiological levels (0.2-0.6 ppm) of ethylene, along with normal concentrations (20%) of oxygen available. Rates are expressed as (hundredths of mm)/mm/hr. The maximal growth rate is 0.10 mm/mm/hr.
Figure 15.
hypobaric conditions other than reduction of endogenous ethylene levels. In fact, since ethylene is believed to inhibit growth in roots, one would predict that reduction of endogenous ethylene under hypobaric conditions would result in an increase in growth, rather than a reduction in growth, as was observed. If, on the other hand, the level of ethylene present in roots is generally at an optimal concentration, one might expect a reduction in the level of ethylene by hypobaric conditions to result in an ethylene level inhibitory to growth (i.e. sub-optimal). However, if this were the case, one would expect readdition of supplemental ethylene under the same conditions to evoke an increase in growth. This did not occur.

The time course of curvature for roots growing at 1/5 atmosphere with supplemental ethylene was comparable to that of roots growing at reduced pressure without additional ethylene and also for normally (1 atm) graviresponding roots. However, the growth pattern leading to the curvature was not the same in roots at 1/5 atm pressure with and without supplemental ethylene (Fig. 16). The gravitropic growth pattern in roots growing under ethylene-supplemented hypobaric conditions mimicked that of normal, graviresponding roots, by exhibiting a differential inhibition of growth
The distribution of relative growth rates is indicated for roots grown at 1/5 atmosphere, with physiological levels (0.2-0.6 ppm) of ethylene, along with normal concentrations (20%) of oxygen available. Rates are presented as (hundredths of mm)/mm/hr. The maximal rates are 0.10 mm/mm/hr on the upper surface and 0.07 mm/mm/hr on the lower surface.
Figure 16.
(or at least an inhibition on the lower surface) as compared with vertical roots. While vertical roots, under these conditions, exhibited a maximal growth rate of 0.10 mm/mm/hr, the maximal rate on the upper surface of horizontally-oriented roots was 0.10 mm/mm/hr, and the maximal rate on the lower surface was 0.07 mm/mm/hr. Hence, it appears that under hypobaric conditions, if ethylene is resupplied to the roots, the growth pattern leading to gravitropic curvature returns to that of normal roots, i.e. a differential inhibition of growth on the upper and lower surfaces. Although these results indicate that the level of ethylene can strongly influence the growth pattern behind gravitropic curvature, they do not support the proposal that ethylene gradients per se are the primary cause of gravitropic curvature.

The first portion of these experiments was repeated with the IBM system, i.e. roots were grown at 1/5 atmosphere with 100% oxygen (Fig. 17). Again, normal vertical growth was significantly reduced at this low pressure, presumably by some physical effect on the growing root tissue, or by removal of some essential gas other than ethylene. Although maximal growth was reduced from about 42% h⁻¹ to about 8% h⁻¹ in vertical roots, gravitropism still occurred in horizontal roots under
Figure 17.

Growth of Vertically- and Horizontally-Oriented Roots under Hypobaric Conditions (IBM System)

The distribution of relative growth rates is indicated for vertical and graviresponding roots grown at 1/5 atmosphere with 100% oxygen supplied ($O_2$ availability = 20%). The maximal rate for vertical roots is 8% h$^{-1}$. For horizontal roots, the maximal rates are 11% h$^{-1}$ on the upper surface and 7% h$^{-1}$ on the lower surface.
Figure 17.
hypobaric conditions. The extent of curvature was the same as for normal graviresponding roots, but it occurred by a differential acceleration of growth (or at least by an acceleration of growth on the upper side). The maximal rate on the upper surface of horizontally-oriented roots was 11% h⁻¹ and on the lower surface was 7% h⁻¹. Again, these results suggest that ethylene does not directly control gravitropism, but may still affect the growth pattern leading to gravitropism. Clearly, hypobaric conditions have effects on root tissue other than removal of ethylene. Such effects complicate studies of the role of ethylene in gravitropism by this method.

Treatment with Norbornadiene (IBM System)

Growth of roots in the presence of low concentrations (1000-3000 ppm) of the competitive ethylene activity inhibitor, bicyclo [2.2.1] hepta-2,5-diene (norbornadiene), was slightly enhanced over that of control roots (Fig. 18). The increase in rate occurred all along the extending zone, beginning 1-2 mm from the root tip. The area under the curve (overall growth) and the maximal rate was shown to be significantly different (to the 95% confidence level) from that of the control at 1000 ppm NBD, using a Mann-Whitney Rank Sum Test. Higher
The distribution of relative growth rates is indicated for roots grown in the presence of concentrations of norbornadiene (NBD) ranging from 1000-20,000 ppm.
Figure 18.
concentrations (5000-20,000 ppm) significantly decreased growth below that of roots grown in the absence of NBD. This lowering of the relative growth rate also occurred all along the extending zone. When the same experiments were performed in the presence of a battery-operated fan, to ensure circulation of the NBD throughout the Plexiglas chamber, the same overall stimulation of growth at low concentrations and overall inhibition of growth at high concentrations occurred. These results suggest that the level of ethylene normally present in roots is supraoptimal, and somewhat inhibitory to growth, and therefore a slight reduction in ethylene activity by NBD results in a stimulation of growth. If one assumes that some minimal level of ethylene is somehow necessary for root growth, it seems possible that higher concentrations of NBD may competitively lower ethylene activity to levels insufficient to support growth. Alternatively, growth inhibition at high levels of NBD could result directly from NBD toxicity.

The time course of gravitropism was not affected by any concentration of NBD. However, growth during gravitropism was affected in a manner similar to that observed for vertical roots (Fig. 19). Low concentrations (1000-5000 ppm) resulted in a stimulation of growth all along the root surface as compared to control roots,
Figure 19.

**Growth of Horizontally-Oriented Roots Treated with Various Concentrations of Norbornadiene (IBM System)**

The distribution of relative growth rates is indicated for the upper surface (above) and the lower surface (below) of graviresponding roots, in the presence of various concentrations of norbornadiene (NBD).
Figure 19.
whereas higher concentrations (20,000 ppm) resulted in an overall inhibition of growth. This occurred on both the upper and the lower surfaces of graviresponding roots. The results refute any evidence for the direct involvement of ethylene in gravitropism, but do confirm ethylene's delicate control of growth. The finding that 5000 ppm NBD enhanced growth on both the upper and lower surfaces of graviresponding roots, a level which was inhibitory to vertical roots, suggests that the level of ethylene present in vertical and in graviresponding roots is different. Possibly, the already inhibitory level of ethylene increases to an even more inhibitory level when the roots are given a gravitropic stimulus. Therefore, a higher concentration of NBD is needed to inhibit ethylene's activity to the same extent. Higher concentrations of NBD thus result in a reduction of ethylene activity to a level stimulatory to growth, and still higher concentrations are required to reduce ethylene activity to a level low enough to be inhibitory to growth. NBD is clearly an effective agent for study of the involvement of ethylene in root growth and gravitropism.

Treatment with AVG and Cobalt Nitrate (IBM System)

When roots were pretreated in potassium phosphate buffer for 1 h, and then positioned in the chamber
in moist air, the growth rate compared with non-pretreated controls was reduced substantially, even after 60 min acclimation time (compare Fig. 20 with Fig. 10 of Chapter I). However, the addition of the two ethylene biosynthesis inhibitors, L-α-(2-aminoethoxyvinyl)-glycine hydrochloride (AVG) and cobalt nitrate, affected the growth of the roots further. Low concentrations (1-2 μM AVG + 20-40 μM cobalt) caused an overall enhancement of growth of vertical roots, while high concentrations (5-10 μM AVG + 100-200 μM cobalt) caused an overall inhibition of growth. The overall growth and maximal growth was shown to be significantly different from the control at 2 μM AVG + 40 μM cobalt according to the Mann-Whitney Rank Sum Test. These results again suggest that the ethylene level normally present in roots is supraoptimal and that reduction of that level brings it to a level which stimulates growth.

The effect of the various AVG and cobalt concentrations on the absolute growth of roots was observed with the auxanometer, to determine whether the same overall effects were found when roots were grown in the continuous presence of those concentrations, rather than after a brief pretreatment with those concentrations. The effects of the inhibitors on roots growing in
Growth of Vertically-Oriented Roots Treated with Various Concentrations of AVG and Cobalt Nitrate (IBM System)

The distribution of relative growth rates is indicated for roots grown after pretreatment for 1 h in various concentrations of AVG (1-10 µM) and cobalt nitrate (20-200 µM). Roots were allowed to acclimate for 60 min following pretreatment.
Figure 20.
the auxanometer system were the same as for pretreated roots growing in the humid chamber of the digitizer system. This suggests that the comparative effects of the two inhibitors observed with the digitizer system were real, despite the overall slow growth of the roots after shifting from submerged to atmospheric conditions. Also, it demonstrates that inhibition by AVG and cobalt is not readily reversible, and that pretreatment with the two inhibitors is an effective way of investigating the role of ethylene in growth.

Pretreatment in potassium phosphate buffer for 1 h had a similar inhibitory effect on the subsequent growth of roots mounted horizontally in moist air (compare Fig. 21 with Fig. 10 of Chapter I). Overall growth was much slower, even after 1 h acclimation time, than for roots which had never been submerged. Pretreatment with various concentrations of AVG and cobalt nitrate did not restrict gravitropism; it occurred as in normal, graviresponding roots. However, the various concentrations of the two inhibitors did affect the growth rate during gravitropism on both the upper and lower surfaces. Low concentrations (1 μM AVG + 20 μM cobalt) enhanced growth, while high concentrations (5μM AVG + 100 μM cobalt) inhibited growth. These results are in agreement with the norbornadiene results, suggesting that ethylene
Figure 21.

Growth of Horizontally-Oriented Roots Treated with Various Concentrations of AVG and Cobalt Nitrate (IBM System)

The distribution of relative growth rates is indicated for the upper surface (above) and lower surface (below) of roots, after pretreatment for 1 h in various concentrations of AVG (1-5 μM) and cobalt nitrate (20-100 μM). Roots were allowed to acclimate for 60 min following pretreatment.
Figure 21.
is not directly responsible for the gravitropic response. Both ethylene biosynthesis and ethylene activity inhibitors fail to prevent gravireaction. Yet, ethylene does have an obvious effect on growth of root tissue, and the level of ethylene in the tissue may indeed change during gravireaction, resulting in an effect on the growth pattern leading to gravitropism. In addition, since the level of ethylene normally present in root tissue appears to be supraoptimal, its level may be reduced to allow a stimulation of root growth. This supports a likely role for ethylene in mediation of root growth, and perhaps other physiological growth responses, aside from gravitropism.

Treatment with Silver Nitrate

Qualitative studies of roots grown in the presence of various concentrations of silver nitrate indicated that while higher concentrations (10-100 μM) were toxic to the roots, lower concentrations (0.1-1 μM) failed to restrict growth or gravitropism. This is convincing evidence against the involvement of ethylene metabolites in gravireaction. Not only do ethylene biosynthesis and competitive ethylene binding inhibitors fail to prevent gravireaction, but also an ethylene metabolism inhibitor is unsuccessful. The summation of all of the results on
ethylene provides convincing evidence against the direct involvement of ethylene in gravireponse, inspite of its likely involvement in growth regulation.

Application of Exogenous Ethylene (Apple System)

To further assess the effect of ethylene on surface extension in roots of maize, and its involvement in the gravitropic response, various levels of ethylene were supplied to roots growing under normal atmospheric conditions. Exogenous ethylene supplied at 1 ppm appeared to cause an overall stimulation of surface extension of vertically-oriented roots, whereas higher concentrations (3-6 ppm) caused a definite overall inhibition of growth (Fig. 22). These results are somewhat surprising in light of earlier results suggesting that a slight lowering of the ethylene level by ethylene inhibitors also causes a stimulation of growth. Perhaps there is more than one optimal ethylene level for root growth, due to changes in tissue sensitivity to ethylene. There is considerable evidence for large changes in tissue sensitivity to hormones. Alternatively it is possible that root tissue is very sensitive to rapid changes in ethylene level in either direction. One could imagine, for example, that a growth spurt occurs with a small change in ethylene level and a
The distribution of relative growth rates is indicated for roots grown in the presence of various concentrations (1-6 ppm) of exogenous ethylene. Rates are expressed as (tens of %)/hr.
Figure 22.
growth slump occurs with a large change in ethylene level. These growth rate changes may be transient, and roots may return to normal rates after changes in sensitivity to ethylene, or compensatory changes in ethylene production or metabolism occur.

Gravitropism was not delayed or eliminated by any level of ethylene, suggesting that gravitropism cannot be prevented even by swamping the system with ethylene. However, the growth rates during gravitropism were variable depending on the level of exogenous ethylene supplied (Fig. 23). Growth was enhanced by 1-3.5 ppm added ethylene on both the upper and lower surfaces. The stimulation was especially strong at 2 ppm. The fact that 1 ppm ethylene had little effect on the growth of horizontally-oriented roots, but strongly stimulated the growth of vertically-oriented roots (compare Figs. 22 and 23), suggests that gravistimulation changes either endogenous ethylene levels, tissue sensitivity to ethylene, or both.

All in all, the effects of ethylene on root growth, in the presence and absence of a gravitropic stimulus, are quite complex. It appears that tissue sensitivity to ethylene is not fixed, and that sudden changes in ethylene level can either stimulate or inhibit growth. Tissue sensitivity to ethylene appears to play a role in
Figure 23.

Growth of Horizontally-Oriented Roots with Various Concentrations of Exogenous Ethylene (Apple System)

The distribution of relative growth rates is indicated for the upper surface (above) and the lower surface (below) of roots grown in the presence of various concentrations (1-3.5 ppm) of exogenous ethylene. Rates are expressed as (tens of %)/hr.
Figure 23.
the growth effects caused by rapid changes in ethylene concentration. The fact that a higher concentration of exogenous ethylene was required for stimulation in horizontally-oriented roots as compared to vertically-oriented roots suggests that the internal ethylene content of roots can affect growth differently depending on the orientation of the roots, or, more likely, that endogenous ethylene levels change with tissue orientation. Nonetheless, it is clear that an ethylene gradient per se is not responsible for the growth gradient which occurs during graviresponse. Even swamping the system with the gas fails to eliminate gravitropism.
CHAPTER III
THE ROLE OF THE EPIDERmis IN GRAVIRESPONSE

Introduction

It was first made clear by Sachs (1882) that in growing plant tissues, the different layers of cells have tendencies to grow at different rates. He showed that when growing tissue, such as stems or petioles, are cut longitudinally or transversely, the epidermis and generally also the cortex and wood tend to contract, while the pith expands. In the intact tissue, therefore, the epidermis is under tension and the pith under compression. Sachs recognized that tissue tension is caused by differences in growth, especially differences in the lengthening of various layers of tissue.

Thimann and Schneider (1938) attempted to gain a better understanding of differential growth in Pisum stems and Avena coleoptiles. Recognizing that normal growth is controlled by the same factors (including auxin) as differential growth, they attempted to gain a better understanding of the latter in order to understand normal growth. They took direct measurements on the
elongation of isolated layers of tissue, and found that the inner and outer layers behaved differently in the presence of auxin. The inner layers showed most of their auxin response in very low concentrations, the outer layers in very high ones. These differences in auxin response of the different tissues were thought to be the principal cause of the tissue tensions reported earlier.

Based on studies of light grown pea seedlings, Masuda et al. (1972) found that auxin promoted extension primarily in the epidermis, with little effect on expansion of the inner tissue. This was confirmed by comparison of expansion between peeled and unpeeled segments, examination of growth patterns in stem segments split part way down the middle, and by measurement of stress-relaxation properties of the epidermal cell wall. They concluded that the induction of stem elongation by auxin, in at least its initial stage, is brought about by removal of restraint of the stem tissue by the epidermis, and that this is brought about by cell wall loosening in the epidermis. They regretted that no detailed studies had been reported as to the differences in the biochemical, chemical or physical properties between epidermal cells and parenchymatous cells. This would be necessary to connect structural differences and auxin responsiveness.
Firn and Digby (1977) found that the rate of elongation of sunflower (*Helianthus annuus* L.) hypocotyl sections was dependent on the rate of growth of the outermost cell layers (peripheral cell layers) of the tissue. Hypocotyl sections from which those layers had been peeled grew, but did not show typical gravicurvature. Hypocotyl sections were bisected longitudinally and the semicylindrical sections were oriented horizontally with the epidermis on the upper side or with the epidermis on the lower side. Those semicylinders with the epidermis on the lower side grew much faster than those with the epidermis uppermost. However, if the peripheral layers were removed from the semicylinders before they were placed horizontally, there was no effect of orientation on the rate of growth, though the sections did elongate considerably during the experiment. These observations further emphasize the central role of the peripheral layers in the response to gravity. Their model of gravitropic curvature proposed that the differential growth causing curvature is due to a differential rate of elongation between the upper and lower peripheral cell layers of a horizontal shoot. In the model, it is speculated that the peripheral cell layers are the site of both graviperception and graviresponse. Curvature is a result of a direct effect
of gravity on cell growth, and hence any chemical
gradients could be a consequence, rather than a cause, of
the gravity-induced growth pattern.

In studies of cucumber hypocotyls, Iwami and Masuda
(1974) discovered that the lower epidermis of
horizontally-oriented hypocotyls played a more important
role than the upper in the gravitropic response. They
found that the extensibility of the lower epidermal cells
was increased significantly by the geostimulus before the
onset of the curvature response.

These results are interesting, especially in light
of work by Sakai and Imaseki (1973), involving a
proteinaceous inhibitor of ethylene biosynthesis. They
found that the inhibitor could inhibit ethylene
biosynthesis in hypocotyls even when only applied to the
epidermal cells. This suggested that the site of auxin-
induced ethylene production is the epidermis. Recent
work by Todaka and Imaseki (1986) also suggested a
possible role for the epidermis in regulation of ethylene
production in mung bean (Vigna radiata) hypocotyl
sections. However, they found that IAA-induced ethylene
production in etiolated mung bean sections did not occur
in the epidermal cells. Still, a protein contained in
mungbean hypocotyls, which inhibits auxin-induced
ethylene biosynthesis, was effective in unpeeled sections
but not in peeled sections. Hence, it seems likely that
the epidermis plays some role in controlling ethylene production, which occurs in cells other than that of the epidermis.

In addition, Ohwaki and Tsurumi (1976, 1978) made autoradiographs of transverse sections of *Vicia faba* roots and showed that basipetal transport occurred mainly through the outer part of the root, whereas acropetal transport occurred mainly through the inner part, the central cylinder. They also inserted a wire longitudinally in the roots, and found that acropetal movement in the segment decreased distinctly after 2 h, whereas basipetal movement showed a tendency to increase. These results suggested to them that the basipetally transported IAA, passing through the outer part of the root, inhibits the elongation of the intact root. These results could also indicate a role for the epidermis in gravitropism, by providing a pathway for basipetal auxin transport, and hence carrying and eventually responding to a signal gradient across the root.

Unfortunately, detailed studies of the involvement of the epidermis in gravitropism in root tissue have not been made. This could be important to a more complete model of root graviresponse, especially if the epidermis is responsible for regulation of ethylene biosynthesis and auxin transport. These studies could prove critical
to an understanding of the mechanism of gravicurvature and the involvement of auxin and ethylene in this growth pattern. To date, the only work on root epidermal cells is that of Barlow (1983). He found that if the epidermis is stripped from roots of Zea mays, the underlying cortical cells in time acquire an appearance identical to the removed cell layer. By three days after epidermal peeling, a new epidermis has regenerated. Barlow did not carry his study further to include the effect of the removal of the epidermis on the gravitropic response, nor did he study the growth of semicylinders of root tissue. I have undertaken such studies with the digitizer system to attempt to reveal the necessity of the epidermis in root gravitropism.

Materials and Methods

Growth Measurement of Submerged Roots

For video digitization under submerged conditions, black block printer's ink was applied with a pin to the surface of the root tissue, forming distinct marks like the glass beads described in Chapter I. The grain of the seedling was attached with putty to the inside of an 8 X 8 X 11 cm Plexiglas chamber. A piece of Plexiglas was sealed to the open end of the chamber with vacuum grease
and heavy rubber bands. After the ink was allowed to dry for several minutes, water or the appropriate solution was added to the chamber to cover the entire root surface. The chamber could be mounted vertically or horizontally for straight growth or gravitropism studies. Only horizontally-oriented roots were used for the experiments in this study. Oxygen was supplied to the growing root tissue through an input port at the bottom rear of the chamber. This kept the oxygen availability high enough for effective growth of the tissue, but did not disrupt the video image by excessive turbulence. Growth rates and curvature patterns were monitored as described in Chapter I.

Studies of the Curvature of Submerged Roots

Roots were marked as described above and positioned horizontally under submerged conditions (either in double-distilled water or in 5 mM Mes (2[N-Morpholino]ethanesulfonic acid) buffer pH 6.2, with bubbling oxygen). They were monitored every 10 min for 12 h. The time courses of angle and radius of curvature development for these submerged roots were calculated and plotted as described in Chapter I. The final value and the time course of attainment of that value for each of the two parameters was compared with that for graviresponding
roots growing in atmospheric conditions with 100% relative humidity. This allowed illustration of the utilization of the two parameters to more completely characterize different types of gravitropic curvature.

**Half-Root Experiments**

To examine the involvement of the epidermis in graviresponse, half-roots were produced by cutting the roots longitudinally through the center with a razor blade. The growth and curvature of these roots was studied after they were placed either vertically or horizontally, and with their cut surface in various orientations. These studies mimicked those of Firn and Digby, which provided evidence for the involvement of the epidermis in the graviresponse of hypocotyls (see Introduction). To ensure that water loss, due to the removal of epidermal tissue, did not contribute to the observed results, roots were grown submerged, with bubbling oxygen. In some experiments, the entire root cap was left intact, while a semicylinder of tissue was excised from the elongation zone.

**Short-Term Studies of Roots after Complete or Partial Epidermal Abrasion**

The epidermis of roots was abraded by stroking with a paste made from 320 grit silicon carbide powder
(Buehler, Ltd., Evanston, IL) and water. For complete abrasion, roots were stroked evenly on all sides of the root. Some roots were abraded only on two opposing sides (partial epidermal abrasion). Abrasion was carried out, beginning at the point of connection of the root to the grain and continuing to the root cap. Although uniform abrasion of the root tip was often very difficult, especially without disturbing the root cap, abrasion of the region of surface extension was generally quite satisfactory and resulted in definitive removal of the single-celled epidermal layer. This was demonstrated by taking cross-sections of abraded and unabraded roots in the region of maximal extension (about 2-4 mm from the root tip). Hand sections were made of roots preserved in fixative (2% paraformaldehyde, 2% glutaraldehyde, 0.025 M NaH₂PO₄, 0.025 M Na₂HPO₄; at pH 6.7). The sections were mounted in distilled water and observed with a light microscope. Photographs (Kodak Panatomic-X film) were made of representative abraded and unabraded sections, using a Zeiss Photomicroscope I, with polarized light and low magnification (16.96 X), revealing the success of the epidermal abrasion technique (Plate V). Note that the distinctly oblong epidermal cells are present in the unabraded section but not in the abraded section.
Plate V.

Comparison of Unabraded and Abraded Root Cross-Sections

Unabraded and abraded roots were fixed and then sectioned by hand, in the region of maximal elongation (2-4 mm from the tip). Sections were mounted in water and then observed with a light microscope. Representative sections of unabraded (above) and abraded (below) roots were photographed (Kodak Panatomic-X film) with a Zeiss Photomicroscope I, under polarized light and 16.96 X magnification.
Plate V.
Studies of overall angle of root curvature were made for roots oriented horizontally for 2 h under submerged conditions. Some experiments were performed on roots with an intact epidermis; others were performed on roots with the entire epidermis abraded. In addition, experiments were undertaken using roots with two sides of the epidermis abraded and two sides left intact. These roots were oriented with either the intact epidermis facing up and down (i.e. with intact top and bottom epidermis) or facing to the two sides (i.e. with intact side epidermis). After abrasion, roots were marked and positioned horizontally as described above. They were monitored every 2 min for 2 h. The relative growth rate distribution and the time course of curvature were plotted as described in Chapter I.

Long-Term Studies of Roots after Complete Epidermal Abrasion

Roots with their epidermis completely abraded were marked as described above and positioned horizontally under submerged conditions. They were monitored for 12 h at 10 min intervals and the time course of overall angle of curvature development was compared with that for control (unabraded) roots grown under the same conditions.
To determine the contribution of physical bending ("sagging") to the limited curvature observed with abraded roots (see below), some abraded roots were submerged in 10 mM potassium cyanide to prevent growth. They were monitored like the others every 10 min for 12 h, and the time course of curvature development was compared to that for abraded roots grown submerged without potassium cyanide.

Curvature of horizontally-oriented abraded roots was then followed for an additional 12 h (at 10 min intervals), to achieve a time course of curvature for abraded roots over a total of 24 h. The return of epidermal cells to the region of surface extension, due to migration from the meristematic zone, was monitored during the same overall time period. Roots were abraded and positioned horizontally under submerged conditions. The method of abrasion was varied with each root, to ascertain that random patterns of abrasion occurred. Also, the roots were positioned in different orientations with respect to the grain, to ascertain that the pattern of return of the epidermis was not dependent on root orientation. Every 2 h for 24 h, several roots were fixed as described above. Later, they were sectioned by hand and the sections were mounted in distilled water and observed under a light microscope. Sections were taken
primarily from the beginning of the extension zone (about 3 mm from the tip), but sections were also made from regions further from the tip, including the maturation zone. Representative specimens were photographed (Kodak Panatomic-X film) with the Zeiss Photomicroscope I, under polarized light and low magnification (16.96 X).

For comparative purposes, the return of the epidermis into the region of surface extension was also monitored for vertically-oriented, abraded, submerged roots. Roots were fixed every 2 h for 24 h, and then sectioned and observed with the light microscope as described above.

Short-Term Studies of Roots Grown in Humid Air after Partial Epidermal Abrasion

Because of the slower gravireponse of submerged roots as compared with roots growing in humid air, the difference in curvature between roots with and without epidermis, or between partially abraded roots oriented different ways, was not always large. It was therefore important to try to repeat the studies in humid air, where the gravireponse of intact roots is strong. Unfortunately, roots with their epidermis fully abraded did not grow well under these conditions, presumably due
to water loss. However, partially abraded roots were capable of growing in humid air, although at a rate still somewhat less than that of intact roots.

Roots were partially abraded, and marked according to Chapter I. They were positioned vertically until growth was noticeable (about 30 min), and then switched to the horizontal position, with either the intact epidermal tissue on the top and bottom, or on the two sides. Roots were monitored every 2 min for 2h, and the time course of curvature plotted and compared for the two orientations.

Results and Discussion

Curvature of Submerged Roots

Roots positioned horizontally under submerged conditions (either in double-distilled water or in Mes buffer) exhibited much slower graviresponse than roots grown in moist atmospheric conditions (Fig. 24). Maximal (50°) curvature did not occur until almost 12 h, as compared to the same curvature in 2 h for roots growing in humid air (see Fig. 11 of Chapter I). The radius of curvature did not drop below about 13 mm, and
Figure 24.

Curvature of Horizontally-Oriented Roots under Submerged Conditions

Above: The time course of degree curvature development is indicated for graviresponding roots grown submerged. Curvature began after 30 min, and maximal (50°) curvature was attained in 12 h.

Below: The time course of radius of curvature establishment is indicated for graviresponding roots grown submerged. The radius of curvature dropped rapidly and stabilized at about 13 mm after 3 h.
Figure 24.
this minimal radius was reached in 3-4 h. This suggests that the appropriate growth differential was established early and maintained until gravicurvature was complete. Although the ultimate angle of curvature attained by submerged roots is similar to that attained by roots growing in humid air, the radius of curvature assumed is much larger (compare Fig. 24 with Fig. 11 of Chapter I). The radius of curvature assumed by roots growing in humid air was about 5 mm. Together the results indicate that the extent of the curving zone is much greater for submerged roots. Even though the same angle of curvature is attained by submerged roots as for roots in moist air, the radius of curvature is much larger in submerged roots, indicating that the arc of root tissue contributing to gravicurvature is much greater. The comparison demonstrates nicely the utilization of overall radius and overall angle of curvature to distinguish between curvature over a small region of root tissue and curvature over a large region of root tissue. It is not clear why roots exhibit such strikingly different curvature patterns in different growth media. It seems unlikely that the difference results simply from the buoyant force of water since roots establish strong gravicurvature in soils. Perhaps the difference results from a partial dilution of a gravistimulation-induced
chemical gradient across submerged roots. In any event, it is interesting that the growth medium can alter the expression of the graviresponse in roots. The slowed graviresponse of roots growing under submerged conditions need be kept in mind during discussion of the following experiments.

Curvature of Half-Roots

Roots which were bisected longitudinally always grew away from the remaining epidermis, both in roots suspended in moist air and in submerged roots. This occurred when the half-roots were oriented vertically, or when they were oriented horizontally with either the cut-face up or cut-face down. It also occurred, at all orientations, in half-roots in which the entire root cap was left intact.

These experiments were performed with the intent of imitating the work of Firn and Digby (1977) on sunflower hypocotyls (see above). It was desirable to determine whether semicylinders of root tissue placed horizontally behaved as did shoot tissue, by growing at different rates depending on whether the cut surface was oriented up or down. The attainment of such results would suggest that transverse movement of a growth regulator is not necessary for graviresponse. Then, study of the growth
of semicylinders of root tissue with their epidermis removed could test the involvement of the epidermis in graviresponse.

Unfortunately, the results were not as straightforward as those for sunflower hypocotyls. All half-roots appeared to curve, even without gravistimulation. This curvature was always toward the cut surface, and indicated that a growth differential occurred across the semicylinder of root tissue. Slower growth was evident along the cut surface, as compared to the surface with an intact epidermis. Since, the same curvature occurred when the entire root cap was present, a differential release of some growth regulator due to bisection of the root cap cannot explain the growth differential leading to curvature. Also, the inhibition of growth on the cut surface could not have occurred simply from a decrease in water availability, since submerged half-roots exhibited the same curvature. Two remaining possibilities are that growth was inhibited along the cut surface by injury during bisection, or that it was slowed due to the lack of an intact epidermis.

To test the involvement of the epidermis in growth and graviresponse, the epidermis could not be peeled as in hypocotyls. However, it could be abraded. The results from studies on the effect of epidermal removal on gravicurvature are discussed below.
Short-Term Effects of Complete and Partial Epidermal Abrasion

Roots in which the epidermis was completely abraded, and which were then submerged and positioned horizontally, curved about $10^\circ$ in 2 h (Fig. 25). This was much less than roots with an intact epidermis, which curved about $25^\circ$ in 2 h. These results suggest that the epidermis is important, and perhaps essential, for graviresponse. However, one problem with the results is the fact that the abraded roots did respond to gravity to some extent. This could suggest that not all of the epidermis was successfully abraded near the tip (i.e. the region which would next move into the extension zone), or perhaps some sagging (nongrowth-induced bending) of the root occurred, since the epidermis was not present to add support to the root structure. An alternative explanation for bending of the abraded roots is simply that the epidermis is not solely responsible for graviresponse, and hence some, gravicurvature can occur in the absence of the epidermis. One additional problem with the results is that although the roots which had been abraded did grow, they did not grow as rapidly as intact roots. Thus it seems possible
Figure 25.

Short-Term Curvature of Roots With and Without Complete Epidermal Abrasion

The time course of curvature is indicated for roots with their epidermis completely abraded and for roots with their epidermis intact, both grown in submerged conditions. In 2 h, 25° curvature was attained by roots with an intact epidermis, whereas 10° curvature was attained by roots whose epidermis had been removed by abrasion.
Figure 25.
that the reduction of gravicurvature by epidermis removal may have resulted from a general suppression of growth rather than from removal of tissue playing a special role in graviresponse. In order to test this idea, gravitropism experiments were done with roots from which the epidermis had been removed from two sides but left intact on the remaining two sides.

Partial abrasion of roots did not slow growth significantly, and all roots abraded on two sides grew at about the same rate. However, roots oriented horizontally with the abraded surfaces on the sides curved much more rapidly than those with the abraded surfaces on the top and bottom (Fig. 26). Those roots with the two sides abraded achieved curvature like intact roots (25° in 2 h), whereas those with the top and bottom abraded achieved curvature like those with the entire epidermis abraded (15° in 2 h). It is possible that the partial curvature of those roots with their top and bottom epidermal surfaces abraded could be due to physical sagging of the roots (since the support of the top and bottom epidermal surfaces is absent). Alternatively, the partial curvature may have been due to incomplete epidermal abrasion near the tip. It is also possible that the side epidermal surfaces contribute somewhat to graviresponse via a gradient in growth
Figure 26.

**Short-Term Curvature of Roots with Partial Epidermal Abrasion (in Two Orientations)**

The time course of curvature is indicated for roots whose epidermis has been partially abraded (on two opposing sides), and then oriented with the remaining epidermis on the top and bottom (upper curve), or on the two sides (lower curve). After 2h, roots with the two sides abraded (top and bottom present) exhibited 25° curvature, whereas roots with the top and bottom abraded (two sides present) exhibited 10° curvature.
Figure 26.
occurring from the top to the bottom of those surfaces. Still another possibility, of course, is that gravicurvature may occur, at least to some extent, by a means not under epidermal control. Strangely, those roots with the side epidermal surfaces abraded did not exhibit the expected 30 min lag before the onset of curvature. Perhaps the side epidermal surfaces actually impede graviresponse, allowing faster response upon their removal. It is also possible that the lower precision of the submerged monitoring technique prevented detailed assessment of the time course of curvature.

Long-Term Effects of Complete Epidermal Abrasion

Long-term studies of roots from which the epidermis was completely removed by abrasion revealed that a maximum of 20° curvature was attained in horizontally-oriented abraded roots after 4 h and this curvature was maintained for 12 h, as compared to the 50° reached after 12 h in intact control roots (Fig. 27). To determine to what extent the curvature observed in abraded roots might be due to physical sagging, some abraded roots were placed in solutions of cyanide at a concentration that completely inhibited growth. Their curvature was compared to that of abraded roots growing in the absence of cyanide. As can be seen in Figure 28, the curvature of
Figure 27.

Long-Term Curvature of Roots With and Without Complete Epidermal Abrasion

The time course of curvature is indicated for roots with their epidermis completely abraded and for roots with their epidermis intact, both grown in submerged conditions. After 12 h, roots with their epidermis intact exhibited 50° curvature, whereas roots whose epidermis had been removed by abrasion exhibited 20° curvature.
Figure 27.
Figure 28.

Long-Term Curvature of Abraded Roots in the Presence and Absence of Potassium Cyanide

The time course of curvature is indicated for completely abraded roots, grown under submerged conditions either in the presence or in the absence of 10mM potassium cyanide.
Figure 28.
abraded roots in cyanide was almost like that of abraded roots without cyanide (around 20°), suggesting that most, if not all, of the initial curvature of abraded roots occurs by nongrowth-induced bending of the root tissue due to the absence of the supportive epidermal cells.

Observation of abraded roots for an additional 12 h, revealed that just after 12 h another phase of curvature began, continuing until 24 h, when 50° was attained (Fig. 29). This time course of curvature was correlated with the return of epidermal cells into the region of surface extension by taking cross-sections of the root tissue in the beginning of the extending zone during the same time period (Plates VI-VII). It was evident from the cross-sections that some return of the epidermal layer into the region of surface extension did occur within 2-4 h, but substantial development of the epidermal layer did not occur until 12-16 h, and complete return of the epidermis occurred after about 24 h. Regeneration of new epidermal cells did not take place in the maturation zone, even after 24 h.

Interestingly, the new epidermis consistently returned initially on two opposing sides, and gradually expanded to meet and form a complete epidermal layer. This characteristic pattern of epidermal renewal was observed for vertically- as well as horizontally-
Figure 29.

**Extended-Term Curvature of Roots with Complete Epidermal Abrasion**

The time course of curvature is indicated for roots with their epidermis completely abraded, and grown in submerged conditions.
Figure 29.
Plate VI.

Cross-Sections of Abraded Roots after 2-12 Hours

Abraded roots were fixed after 2, 4, 8 and 12 hours oriented horizontally in submerged conditions. Hand sections were made at the beginning of the extending zone (2-3 mm from the tip), and these sections were mounted in water and observed with a light microscope. Representative sections were photographed (Kodak Panatomic-X film) with a Zeiss Photomicroscope I, under polarized light and 16.96 X magnification.
Plate VI.
Cross-Sections of Abraded Roots after 16-24 Hours

Abraded roots were fixed after 16, 20 and 24 hours oriented horizontally in submerged conditions. Hand sections were made at the beginning of the extending zone (2-3 mm from the tip) (for 16, 20 and 24 h roots) and in the maturation zone (for 24 h roots). These sections were mounted in water and observed with a light microscope. Representative sections were photographed (Kodak Panatomic-X film) with a Zeiss Photomicroscope I, under polarized light and 16.96 X magnification. For the 24 h sections: E = extending zone; m = maturation zone.
Plate VII.
oriented roots, indicating that it did not occur as a differential response to gravity, whereby roots preferentially developed new epidermal layers on the top and bottom. Still, roots which were oriented horizontally for over 12 h almost always showed the new epidermis on the top and bottom sides. During earlier stages, those with the new epidermis on the top and bottom responded to gravity, and those with the new epidermis on the two sides did not. It appeared that those roots which had grown for 12 h, were long enough that twisting of the root could occur, and the new epidermis, even if it first appeared on the two sides, could rotate to the upper and lower surfaces. No matter how much the abrading technique and the original positioning of the roots was varied, it still appeared that most roots after 12 h carried new epidermis on the top and bottom sides. Why the epidermis seemed to return to the extending zone in two distinct regions, is difficult to explain. Morphologically, it would appear that there may be two "growing points," or meristematic initiation regions. However, considering the evident difficulty in abrading (particularly near the root tip), it seems more likely that the root was consistently abraded or injured differentially at the tip, and hence the new epidermis returned differentially.
Nonetheless, it is important to note that the return of the new epidermal tissue coincided perfectly with the onset of the second phase of the curvature response of abraded roots. After 12 h, a sizeable epidermis was present on the upper and lower sides of the abraded roots (looking much like partially abraded roots), and at the same time the graviresponse was reinstated. This indicates a role for the epidermis in gravireaction. Completely abraded roots show little or no graviresponse until the epidermis returns to the extending region at least on the top and bottom surfaces.

Effects of Partial Epidermal Abrasion of Roots Growing in Humid Air

To compare with the results from submerged roots, partial abrasion was performed on roots which were then observed positioned horizontally in humid air. Figure 30 indicates that even under these conditions, the same discrepancy is observed for roots with the two sides abraded (and hence the top and bottom epidermal surfaces intact) versus roots with the top and bottom abraded (and hence the two side epidermal surfaces intact). Those roots with only the sides abraded responded even more than normally to gravity (65° in 2 h), whereas those roots with the top and bottom abraded exhibited almost no
Curvature of Roots with Partial Epidermal Abrasion (in Two Orientations) in Humid Air

The time course of curvature is indicated for roots whose epidermis has been partially abraded (on opposing sides), and then oriented, in humid air, with the remaining epidermis on the top and bottom, or on the two sides. After 2 h, roots with the two sides abraded (top and bottom present) exhibited 70° curvature, whereas roots with the top and bottom abraded (two sides present) exhibited 10° curvature.
PARTIAL EPIDERMAL ABRASION (HUMID AIR)

CURVATURE (DEGREES)

TIME (MIN)

SIDES ABRADED

TOP AND BOTTOM ABRADED

Figure 30.
graviresponse (10° in 2 h). The exaggerated curvature of roots containing only the top and bottom epidermal surfaces may result from a greater apparent growth differential across the roots since the side epidermal surfaces are not present to dilute or restrain that differential. The difference in curvature observed for roots in the two opposing horizontal orientations is even more significant than that observed for partially abraded roots under submerged conditions. This provides additional evidence for a special role of the epidermis in gravireaction. Just what that role may be, is still open to speculation. Perhaps the epidermis is simply the mechanical control tissue for growth. However, epidermal cells are certainly not the only cells in the root capable of growth. It thus seems equally probable that the epidermis may play some other role, either as a transport channel for some growth regulator, or as the control site for the synthesis of, metabolism of, or determination of tissue sensitivity to some growth regulator. Any of these factors could ultimately lead to an apparent gradient in that regulator and a consequential differential in growth. Moore and Evans (1986b) have suggested a role for the root mucilage in transport of calcium. During graviresponse, calcium accumulates in the mucilage along the lower side of the root (Moore et
al. 1986a). Perhaps the epidermal layer is also involved in that transport, and provides a pathway for development of a calcium gradient. This accumulation of calcium on the lower side could either act as a sink for auxin, or sensitize the tissue to auxin, leading to an apparent auxin gradient across the root tissue and a resulting growth differential. Whatever its means may be the epidermis clearly plays an important role in root gravitropism, just as it does in shoot gravitropism.
CHAPTER IV

TEMPORAL AND SPATIAL GROWTH AND CURVATURE PATTERNS DURING GRAVITROPISM

Introduction

The beauty of plant form has delighted and inspired poets, artists and scientists throughout recorded history. It is perhaps less immediately obvious that the forms, or at least the dimensions, of many parts of a plant are continually changing throughout the plant's life (Silk and Erickson 1979). A root of maize oriented vertically presumably grows linearly, that is, using terms of fluid dynamics, its growth is steady. This means that the local growth rates are invariant with time and the Eulerian description of growth is constant. However, each material element expands more rapidly and then less rapidly as it is displaced from the root tip, i.e. material growth rates are continually changing. Even so, if these material growth rates are plotted against distance from the tip, they too are invariant with time, and the Eulerian (spatial) and Lagrangian
(material) descriptions of growth coincide. This assumption of time invariance has been made during many studies of growth, especially that of root and shoot apices. Erickson, in defining the relative elemental growth rate in maize roots, used a second derivative of X, the distance of a point from the tip (Erickson and Sax 1956). While X varies with time alone, its derivative, dX/dt, may vary with time and with distance from the tip. Therefore, differentiation should theoretically yield the expression:

\[ dX' = \frac{X'}{X} + \frac{X'}{t} \frac{dt}{X} \]

However, Erickson assumed that the mode of elongation did not change with time, and he simplified the expression by eliminating the time element. The derivative was rewritten as d/dX (dX/dt), and used in analysis of steady growth in roots of maize.

While it is not difficult to find examples of steady growth in which the local temporal derivative is zero, or negligible, many developmental systems require specification of rates of change in both spatial and material terms for their complete description. Very little detailed growth data of this sort is available, for the majority of morphogenetic systems. It is
important for a proper understanding of plant morphogenesis to give close attention to growth patterns which result in the characteristic forms of plants and their organs. However, relatively little attention has been given to quantitative analysis of spatial and temporal relationships in developmental morphology (Silk and Erickson 1978). Knowledge of the spatial and temporal variation in growth rates within tissues is essential for an understanding of the morphological aspects of plant development. For example, during hypocotyl opening, the pattern of growth is nonsteady (varying in time), and the local rates of change must be evaluated in growth analysis. The application of this growth analysis can also be a powerful tool in physiological studies (Silk and Erickson 1979).

Some caution must be made in interpreting the spatial and material specifications of the growth of a plant in continuum mechanical terms, i.e. as fluid flow. Mathematically, this interpretation is a rich one, but the analogy to fluids cannot be taken too far beyond the kinematics. While ordinary fluids are both passive and spatially homogenous, tissues composed of living cells are neither (Goodall et.al. 1986). Nonetheless, it is important to recognize that growth field variables are functions of both position in the plant and of time, and
can be specified in either Eulerian (spatial) or Lagrangian (material) terms (Erickson 1980). While these two specifications are the same when growth is steady, during nonsteady growth, they can be quite different. During steady growth, the convective rate of change is often greater than the local rate of change, but during nonsteady growth, the local rate of change can be significant. Most of the experimental data in the literature is specified in Eulerian terms. Not much data on the spatial distributions of growth velocities or of growth trajectories of material elements can be found. Much remains to be discovered about temporal and spatial variation in developmental variables (Silk 1984). We do not know the extent to which biochemical and organelle distributions are steady in growing tissues or even the extent to which geometrical organs themselves are steady structures. We are ignorant of the way in which patterns are changed by perturbations in temperature or water stress within the physiological range (Erickson 1980), or by other environmental stimuli such as light or gravity. As Silk diagnoses, analysis of the finer-scale fluctuations and periodicities evident in the growth records of single roots and leaves will require autoregressive or time series analysis (Silk 1984).
Graviresponding roots are clearly not steady growth structures, yet nothing has been done to attempt to describe the growth during their curvature in both Eulerian and Lagrangian terms. Although Eulerian specifications of growth are becoming more common, it must be recognized that during gravicurvature the Eulerian and Lagrangian specifications of growth are different, and moreover the Eulerian specification is constantly changing. Therefore, a description of the changing growth rates of each material element as it passes through the length of tissue is as important as a description of the spatial distribution of growth rates, since these local rates are continually changing and hence the two specifications do not coincide. However, probably as instructive and much easier to visualize is simply a time series of Eulerian specifications of growth, i.e. a 3-dimensional image of growth, showing its dependence on both time and on distance from the tip (the two parameters which Erickson so insightfully recognized as the two determinants of relative elemental growth rates, one of which he assumed to be invariant in his studies of nongraviresponding roots). The digitizer system, along with some specially designed software should allow production of such revealing 3-dimensional descriptions of growth during gravicurvature.
In observing a change in form like gravitropism, not only are the temporal and spatial growth patterns on the two sides of interest, but also the changing form of the curve itself. Botanists have frequently defined curvature simply as an angle, but it is possible to find a value of curvature at any point on the edge of a plane projection of a plant organ. Curvature, K, is defined in calculus as the rate of change of the tangent angle with respect to the arc length of a curve. The radius of curvature, R, is defined as the radius of the circle which just fits the curve at a given point. The radius of curvature is the reciprocal of K:

\[ K = \frac{d\alpha}{ds}, \quad R = \frac{1}{K} \]

where \( \alpha \) is the angle (in radians) of inclination of the tangent to the curve, and s is the arc length. Radially symmetrical plant structures which bend in a plane, such as stems and branches, are therefore well represented by a graph of curvature vs. distance along the generative axis (Silk and Erickson 1978). Silk and Erickson applied this knowledge of calculus to mapping the point curvature of lettuce hypocotyls during hook maintenance and opening. Differentiation formulas based on fitting of second degree polynomials were used to obtain values for:
\[ x_\bullet = dx/ds; \quad x^{\bullet\bullet} = d^2x/ds^2; \quad y_\bullet = dy/ds; \quad y^{\bullet\bullet} = d^2y/ds^2, \] where \( x \) and \( y \) are the coordinates of points along the curving axis. Curvature was then determined from the formula:

\[ K = \frac{(x^{\bullet\bullet}y_\bullet) - (y^{\bullet\bullet}x_\bullet)}{(x^{\bullet\bullet} + y^{\bullet\bullet})^3/2} \]

During hook maintenance, the Eulerian distribution of curvature remained fairly constant (and the Eulerian and Lagrangian distributions of curvature coincided), but during hook opening, the spatial distribution of curvature changed. Hence, local as well as convective changes occurred during hook opening, while only convective changes occurred during hook maintenance. Point curvature is hence subject to the same Eulerian and Lagrangian specifications as growth. Silk (1984) recommends that the material derivative of curvature and the possible importance of convective curvature changes should be investigated in studies of phototropism and bending. In non-growing systems, such as the touch sensitive mimosa leaflet, she predicted that the convective change would be negligible and that curvatures are described simply by local changes. In tropic responses, on the other hand, in which curvature is brought about by growth, she expected that convective changes would be important and should be considered, along with local changes.
Clearly it is important to develop a more detailed description of curvature in graviresponding roots. Rather than simply a single overall angle of curvature measurement, point determinations of curvature can be made. But since curvature is the reciprocal of the length of the radius of the circle which just fits in the plane of the curve, the radius of curvature is an equally valid parameter. As was pointed out by Silk, \( K = \frac{d\alpha}{ds} \), \( \alpha \) being the tangent angle (in radians) and \( s \) being the arc length. By analogy, \( R = \frac{ds}{d\alpha} \). However, it is equally legitimate to determine \( R \) as the distance between the center of the circle which just fits the curve and some point on the curve. This center can be determined as the intersection of two perpendiculars to the two tangent lines on the curve. In addition, since the Eulerian description of curvature is changing through time during graviresponse, it is also essential to describe the material (Lagrangian) aspects of curvature. As with growth, it is equally informative and more sensible visually simply to illustrate the changing Eulerian specifications of curvature through time, rather than map the convective changes in curvature as an element of root tissue travels down the axis. This can be done by plotting a series of Eulerian curvature descriptions
at various times during graviresponse. The resulting image is thus 3-dimensional, with curvature being plotted against both time and distance from the tip (the origin of growth and hence also the origin of curvature). This complete imaging of curvature is possible with some specialized software along with the digitization system. I have made these studies in order to unravel the temporal changes in surface extension rates along the root surface during graviresponse, as well as to more definitively describe the nature of the curve observed during gravitropism.

**Materials and Methods**

**Measurement of Temporal and Spatial Distribution of Growth**

Using the Apple System, four sequential 1 h experiments were run on the same vertically-oriented roots. Roots were marked and their growth monitored as described in Chapter I. Marker positions were stored every 15 min for 1 h. Then, a new experiment was begun and run for another hour, etc. This was continued until 4 sequential 1 h experiments had been run on the same roots. The growth rates were compiled separately for each hour of growth, and plotted as described in Chapter
I. The growth distribution could then be compared through time for vertical roots, to ascertain whether root growth is indeed temporally constant for normal, undisturbed, nongravistimulated roots.

With the IBM System, 4 h growth experiments were run on vertically-oriented roots and 8 h experiments were run on horizontally-oriented roots. Roots were marked and monitored as described in Chapter I. Growth was measured every 2 min for a total of 4 h (vertical and horizontal roots). For horizontal roots, the markers were readjusted, new markers were added and the chamber was rehumidified at this time. Then, growth was monitored every 2 min for an additional 4 h, these representing hours 5–8 following gravistimulation. Growth rates were calculated and separated according to the hour interval in which they occurred. Growth distribution curves were plotted individually for each hour of growth, as described in Chapter I. These temporal plots were made for vertically-oriented roots as well as for the upper and lower surfaces of horizontally-oriented roots. This allowed determination of temporal changes in maximal growth rates, growth distributions, and growth boundaries in normal and graviresponding roots. Of course, it did not allow identification of short-term fluctuations in growth which might have occurred within each hour interval.
Measurement of Temporal and Spatial Distribution of Curvature

Using the IBM System, the radii of curvature were determined at points along the surface of graviresponding roots. The radii were calculated much like the overall radius of curvature, by finding the intersection of the perpendicul ars to the two tangent lines bordering a given point (which was, in essence, the center of the best fit circle), and then averaging the distances between this point and the midpoints of the two tangent segments.

Roots were marked, oriented horizontally, and monitored as described in Chapter I. Marker positions were determined every 2 min for a total of 4 h. Radii of curvature were calculated at the positions of all of the markers on the upper surface, except the two on the ends (since two tangent lines could not be determined for these points). Each radius of curvature was stored with its respective value for the distance of the associated marker from the root tip. These pairs of values (distance from the tip, radius of curvature) were stored in separate files for each time period of 30 min. These values were averaged, smoothed and then fitted with a second degree polynomial as described in Chapter I. A separate plot of radius of curvature versus distance
from the tip was made for 8 consecutive 30 min time intervals, and a description of both the spatial and the temporal distribution of curvature was hence obtained. This allowed identification of changes in the shape of the curve (i.e. the distribution of differential growth) in roots, while they are responding to gravity. Clearly, some difference may be evident between the shape of the curve along the upper side and that along the lower side. However, assuming the width of the root is fairly uniform, the two curving surfaces should be parallel, and either should yield a representative description of the curvature pattern in the root.

Results and Discussion

Spatial and Temporal Distribution of Growth in Vertical Roots (Apple and IBM System)

According to the data from the Apple System, the spatial distribution of relative growth rates in vertically-oriented roots did not vary substantially over time (Fig. 31). The maximal rate of surface extension during each hour did not deviate sharply from 0.28 mm/mm/hr, and occurred at about 3-4 mm from the root tip. The results indicate that under normal conditions nongraviresponding roots do not exhibit slowed growth in
The distribution of relative growth rates in vertically-oriented roots of maize is indicated for the 1st hour (upper left), the 2nd hour (bottom left), the 3rd hour (upper right), and the 4th hour (bottom right) of growth. Rates appear as (hundredths of mm)/mm/hr or as (tenths of mm)/mm/hr, as indicated on each graph. The maximal rate during all hours is about 0.28 mm/mm/hr and occurs at 3-4 mm from the root tip.
Figure 31.
the first hour due to the trauma of mechanical stimulation, nor in the last hour due to maturation of the tissue. The results do not, however, eliminate the possibility that shorter, more transient changes in growth rate or distribution may occur. It is certainly possible that growth may not be the smooth, continuous process suggested by the similarity of the growth curves for each hour time period. However, streak photographs by Erickson and Sax (1956) indicate that the material growth of root cells is indeed a steady, even process. The combination of his results, and the longer-term findings of this report, tend to suggest that growth of vertical roots is smooth and continuous for relatively long periods of time.

Results from the IBM System, likewise suggest that little variability in maximal growth rate and in growth distribution occur in vertically-oriented roots (Fig. 32). However, at this higher resolution, a slight drop in maximal rate did seem apparent during the 1st and 4th hours, indicating that in fact some drop in growth rate may occur during the first hour due to the mechanical stimulation of marker application and root positioning. Also, some drop in rate may occur during the final hour, either as a result of maturation of the tissue or more probably due to a slight lowering of the humidity within the Plexiglas chamber at that time.
Figure 32.

**The Spatial and Temporal Distribution of Growth in Vertically-Oriented Roots (IBM System)**

The distribution of relative growth rates in vertically-oriented roots of maize is indicated for the 1st-4th hours. The maximal rate and distribution of rates is similar for all hours, except a slight lowering of rates is apparent during the 1st and 4th hours.
Figure 32.
Spatial and Temporal Distribution of Growth in Graviresponding Roots (IBM System)

A basipetal shift is apparent in the location of both the maximal rate and the basal border of the extension zone, on both the upper and lower surfaces of graviresponding roots during the first hour (Fig. 33). This basipetal shift in the maximal rate is reduced (i.e. it shifts back apically) on both surfaces, during each consecutive hour until the 5th hour (Fig. 34). However, the basal border of the extending zone shifts even more basipetally during hours 3 and 4 on the upper surface. The maximal rate of extension is somewhat reduced during the 1st hour on both surfaces, as was true of vertically-oriented roots, due perhaps to the mechanical stimulation during preparation. The maximal rate also drops during the 1st and 4th hours of the second observation period (i.e. the 5th and 8th hour of root monitoring), similar to vertical roots. Whether a reduction is observed during the 4th hour of the first observation period is unclear, due to the net enhancement of maximal rate, resulting from the completion of curvature and the resumption of normal (resembling vertical) growth patterns on both surfaces. Interestingly, during the 3rd hour, when curvature is just being completed and some
The relative growth rate distribution is indicated for the upper surface (above) and the lower surface (below) of graviresponding roots, during the 1st-4th hours. A basipetal shift in the location of the maximal rate and the basal boundary of the extension zone is apparent on both surfaces during the first hour. The locations of the maximal rates gradually shift apically during the following hours. A sharp drop in the maximal rate, along with a further basipetal shift in the basal end of the extension zone, on the upper surface is apparent during the third hour. This occurs concomitant with a sharp increase in maximal rate on the lower surface.
Figure 33.
Figure 34.

The Spatial and Temporal Distribution of Growth in Horizontally-Oriented Roots During Hours 5-8 (IBM System)

The relative growth rate distribution is indicated for the upper surface (above) and the lower surface (below) of graviresponding roots, during the 5th–8th hours. The maximal rates and the boundaries of the extension zone are like those in vertical roots by the 5th hour. The roots continue to grow as vertical roots during the remaining hours.
Figure 34.
signs of autotropism are apparent (see Fig. 11 of Chapter I), the rates on the upper surface are strongly lowered and more spread out (i.e. with a greater overall extension zone), while the maximal rate on the lower side is even higher than that on the upper side. This pattern continues during the 4th hour, although the rates on the upper side begin to increase. By the 5th hour, the growth rates and locations of those rates approach those of vertical roots, and from hours 6-8 those growth patterns are maintained.

The results indicate again that gravitropic curvature occurs by a differential inhibition of growth, and by a basipetal shift in the region of surface extension, particularly on the lower side. It therefore seems essential to look for a growth inhibitor as the mediator in root gravitropism. In addition, it is important to explain a change in the growth zone as well as in growth rates, in accounting for gravicurvature. In other words, a mechanism utilizing control of the on/off growth switch, as well as control of the degree of growth, must be identified to explain graviresponse.

In addition, the results suggest that as curvature is completed, an autotropic response occurs by further inhibition of growth on the upper side in combination with a basal extension of the growing zone on that
surface. At the same time, the lower side exhibits a noticeable stimulation (lesser inhibition as compared to vertical roots) of growth. Hence, the complete gravitropic response must also incorporate some explanation for the increase in growth rates on the lower side after a certain overall degree of curvature is achieved, as well as an explanation for the sharp drop in the maximal rate of growth concommitant with the increase in the extent of the growing zone along the upper surface. The delicate control of both growth rates and boundaries of growth during gravitropism illustrate the intricate nature of this morphological process. Obviously, differences in the growth differential between the upper and lower surfaces occur at different positions along the curving surface at different times during graviresponse. The eventual return of both extension rates and extension zone boundaries to those of vertical roots after attainment of maximal curvature emphasizes the ability of roots to perceive and respond not only to the horizontal orientation, but to the near-vertical orientation as well. Hence, roots apparently can perceive the exact direction of the gravistimulus, and respond accordingly. Clearly, the spatial and temporal nature of growth during gravitropism is complex. The subtleties revealed in this study need to be considered
in future biochemical investigations, as well as in additional kinematic characterizations of root gravitropism.

**Spatial and Temporal Distribution of Curvature in Graviresponding Roots (IBM System)**

Curvature began to appear within 30 min in graviresponding roots (Fig. 35). This curvature was evident near the tip. As time progressed the center of the curvature response (i.e. the point of minimal radius) was transported basipetally, and the distribution of curvature became symmetrical. This demonstrates the nature of the curvature response responsible for gravitropism. It illustrates where the differential growth response begins and how it is propagated through the root tissue. As growth is generally inhibited during graviresponse, the growth differential which leads to curvature is a differential inhibition of growth. The point of maximum curvature, or minimal radius, is hence the point of greatest differential in growth inhibition. The transport of this differential basipetally could provide supporting evidence for the basipetal transport of some growth inhibitor, most likely from the root cap. However, the graph suggests that curvature moves basipetally at about 2 mm/hr, during the initial stages
Figure 35.

The Spatial and Temporal Distribution of Curvature in Horizontally-Oriented Roots (IBM System)

The distribution of radii of curvature is indicated for graviresponding roots after 30-240 min. Early in the curvature response (30-60 min), most of the curvature (differential growth) occurs near the apical end of the extending region. As this curvature is transported basipetally by growth (90-120 min), the growth differential reverses in the apical portion of the extending region although it continues more basipetally. From 150-240 min, the region and distribution of curvature is well established, and is simply pushed away from the tip by growth occurring more apically.
Figure 35.
of graviresponse. This is somewhat slower than the rates in the literature for basipetal auxin transport in roots (Davies and Mitchell 1972, Davies et al. 1976, Konings 1969). Still, the possibility exists for changes in auxin sensitivity or lateral auxin transport, in combination with basipetal movement of auxin, causing the described changes in growth differential between the upper and lower sides over time.

Clearly, a 0 mm radius never occurs, even though such is indicated by the 30 and 60 min plots. This is due to the sharp drop in radius as the root tip is approached. Hence the best-fit curve appears as almost a straight line and runs off the bottom of the graph, rather than bending sharply to the left at some small radius of curvature, as is probably the real distribution of curvature. The minimal radius of curvature appears to increase through time. This, too, is most likely an artifact, resulting from the method of measuring radii of curvature. Since one is limited to the marks present on the root surface, and as time progresses they move apart, the radius of curvature at any point in the extending region may appear to increase even though the two tangent angles do not change (and hence the best-fit circle remains the same). Once a given point moves out of the extending region this apparent increase in radius ceases,
as can be seen by the slowing in that increase in the minimal radius at 240 min, when it approaches a location of 5 mm from the tip.

Inspite of the slight inaccuracies of the radius of curvature calculation and the selected curve fitting technique, the data do demonstrate the spatial distribution of curvature along the root surface, and the temporal shifts in that distribution during graviresponse. They indicate that curvature begins most prominently near the beginning of the extending zone, and then this curvature shifts basipetally. The data on differential growth, discussed above, support this. As was noted, a greater basipetal shift in the region of growth occurred on the lower side than on the upper side, especially of the apical border. This growth response would hence result in the greatest differential growth occurring in the front of the extending region (although a smaller differential would also occur further back on the root), as is apparent on the spatial map of curvature. Most of the curvature would be complete by 120 min. At that time there is a sudden drop in the growth on the upper side, concomitant with an increase in growth on the lower side. This serves to reverse the growth differential (to one of higher growth on the lower side) in the apical end of the extending zone, although
the same differential (higher growth on the upper side) is maintained in the basal region of the extending zone, by the basipetal shift in the basal boundary on the upper side. By 4 h, growth is similar to that of vertical roots (i.e. no growth differential is present). Hence, curvature is no longer being transported basipetally in growing cells. Instead, the pattern of curvature is established in a particular piece of mature tissue, and growth is occurring in front of that curve to produce new root tissue.

In general, the distribution of curvature data correlate closely with the growth data, through time. The radius of curvature map gives an easier approach to visualizing the distribution of differential growth, and hence a way to locate where on the responding tissue growth regulator gradients may occur. Future work on graviresponding roots ought to take into account the insights divulged by this study, namely that early in the curving process the sharpest growth differential occurs in the apical end of the extending zone and later on, during the beginning of autotropism, a reverse (negative) growth differential occurs in that zone, while the same positive growth differential occurs more basipetally in the extending region. These complexities in growth gradients must be accounted for in any model of root gravitropism.
A computer-based video digitizer which allows calculation of relative growth rates, by Blackman's formula, at points along a plant surface is described for the Apple II+ computer and for the IBM Personal Computer. The digitizer also allows determination of the angle and radius of curvature for graviresponding organs. The Apple System requires manual identification of marker locations at given time intervals. The IBM System searches automatically for the markers and stores them at a predetermined time interval. Both systems were used in studies of growth and gravitropism in root tissue.

Relative growth rates were calculated along the surface of roots of Zea mays. The region of extension was found to extend from about 1 to 6 mm from the root tip, with maximal growth occurring at about 3 mm from the tip. The extending zone on both the upper and lower surfaces shifted basipetally in graviresponding roots. The maximal growth rate was reduced from that of vertical roots during graviresponse, with the maximal rate on the lower surface being less than that on the upper surface. These results indicate that gravitropism in roots of
maize (B73 x Missouri 17 hybrid) occurs by a differential inhibition of growth, concomitant with a shift in the zone of surface extension on both the upper and the lower surfaces.

Reduction of endogenous ethylene by hypobaric conditions did not eliminate gravitropism in maize roots. Inhibition of ethylene binding by 2,5-norbornadiene (NBD), of ethylene biosynthesis by L-α-(2-aminoethoxyvinyl)-glycine (AVG) and cobalt nitrate, and of ethylene activity by silver nitrate also failed to block gravitropic response in roots. Swamping of any existing ethylene gradients by addition of ethylene under atmospheric conditions did not alter gravitropism. The results indicate that ethylene is not necessary for normal gravitropic curvature. However, hypobaric conditions did change the growth pattern leading to gravitropism to one involving a differential acceleration of growth on the upper and lower surfaces. This growth pattern returned to a differential inhibition of growth by addition of supplemental ethylene under hypobaric conditions. Low concentrations of NBD, or of AVG and cobalt nitrate, stimulated growth of maize roots, while higher concentrations inhibited growth. Addition of small amounts of ethylene under atmospheric conditions also stimulated growth, while higher concentrations
inhibited growth. These results indicate that ethylene may be involved in growth, and that the level of ethylene normally present in root tissue is supraoptimal for growth. Rapid changes in that level may bring about changes in tissue sensitivity to ethylene, and consequential changes in growth rate.

Half-roots (obtained by longitudinal bisection of roots) always curved toward the cut surface, whether submerged or suspended in humid air, positioned horizontally or positioned vertically, possessing a complete root cap or only half of a root cap. Abrasion of the epidermis slowed graviresponse in submerged maize roots, but also slowed growth. Roots abraded on two sides and then positioned horizontally with the remaining epidermis either on the top and bottom or on the two sides showed different graviresponsiveness. Those with top and bottom epidermis intact responded like control (unabraded) roots, while those with side epidermis intact responded like fully abraded roots. However, even for unabraded roots, graviresponse under submerged conditions was not large. After about 12 h, fully abraded roots began to reattain graviresponsiveness. This correlated well with the return of epidermal cells into the extending region. Epidermal tissue always returned on two opposing surfaces. Most frequently, after 12 h, the
surfaces possessing intact epidermis were the upper and lower surfaces of the root. Those roots with intact epidermis on the sides failed to respond significantly to gravity. Fully abraded roots did not grow well in humid air. Roots abraded on two sides did grow under such conditions, and those oriented horizontally with epidermis on the top and bottom surfaces demonstrated greater gravireponse than those with epidermis on the two sides. The data indicate that the epidermal layer is necessary for normal gravicurvature. It may play a mechanical role in controlling growth, it may act as a transport channel for some growth regulator, or it may aid in effecting an apparent growth regulator gradient.

Temporal, as well as spatial, variability in growth rates during graviresponse was discerned by mapping growth curves at 1 h intervals for 8 h. It was discovered that the basipetal shift in extending zones was transient, and was stronger on the lower side, so that the greatest growth differential occurred initially near the root tip. As time progressed, the region of extension shifted apically on the lower side and the maximal growth rate increased significantly. Hence, the greatest differential occurred more basipetally. Gradually, the growth curves on both surfaces evolved to those like vertical roots. Curvature was complete and
growth continued in front of the curving region. The spatial distribution of curvature was described by determining the radius of curvature at points along the root surface. These radii were mapped every 30 min to create a temporal image of the changes in curvature distribution. These results correlated well with the temporal growth data, indicating initial strong curvature near the tip and gradual propagation of that curvature basipetally. The direction, although not the rate, of curvature propagation supports the theory of auxin transported back from the root cap being involved in gravitropism.

In conclusion, the digitizer system provides a highly accurate means of assessing the growth patterns leading to various plant forms. However, such a kinematic description of growth, inspite of its direct application to biochemical studies, is highly incomplete. As Silk (1984) so aptly stated: "A complete understanding of growth involves dynamics, i.e. knowledge of the forces and energies which produce the observed growth pattern. In moving from the kinematic to the dynamic analysis of growth we encounter some new problems, for kinematics is a problem of accurate measurement and not a problem of fundamental conceptual difficulty. Growth dynamics, in contrast, must involve a hypothesis of causality and
proof of the hypothesis." Presumably the spatial and temporal gradients in growth discussed in these studies depend on one or more biophysical parameters governing growth. Unfortunately, there is little direct evidence on this matter (Cosgrove 1986). Until a more thorough understanding of biophysical control of growth is attained, and the changes in biophysical parameters which bring about spatial and temporal gradients in growth rates are determined, a good biochemical understanding of development of plant form will be exceedingly difficult.
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APPENDIX A: Listing of the Computer Program, HOLD6, Used to Track Marker Locations with the Apple System

The program was written in Applesoft Basic on an Apple II+ microcomputer. It allows semiautomatic tracking of marker locations on a plant surface using the Apple-Based Video Digitizer System.

```
10 CLEAR: RTIMES$="": SA=1: CALL-936: SCALE=2: ROT=0
20 ONERR GOTO 780
30 ADRS=0
40 PRINT CHR$(4); "BLOAD RBOOT"
50 CALL 520: ADRS=USR(0),"HRCG": CALL ADRS: POKE 216,0
60 PRINT ""
70 PRINT "ROOT-IMENTARY GROWTH PROGRAM": PRINT:
     PRINT "----------------------------------------
     *****************: PRINT: PRINT: INPUT "COORDINATE/TIME FILE NAME?"; FILNAMES
80 PRINT: PRINT: PRINT: INPUT "USE LIGHT PEN? (Y/N)"; YP$
84 PRINT: PRINT: PRINT: INPUT "HOW MANY SEGMENTS/ROOT?"; YV
86 PRINT: PRINT: PRINT: INPUT "HOW MANY ROOTS?"; YH
90 PRINT CHR$(4); "OPEN"; FILNAMES$";D1"
94 PRINT CHR$(4); "WRITE"; FILNAMES
96 PRINT YV: PRINT YH
100 PRINT "CLOSE"; FILNAMES
110 PRINT "";
120 HGR2
130 GOSUB 570
140 PRINT "BLOAD TIMER"
150 PRINT "BLOAD DSCAN 4.2.OBJ"
160 POKE M,1
170 XDRAW 2 AT X2,Y2
180 GOSUB 390
190 POKE ADRS + 10,00: POKE ADRS + 11,03: PRINT"
200 VTABLE(1): HTABLE(17): PRINT CHR$(PEEK(521));
      CHR$(PEEK(522)); ":"; CHR$(PEEK(525)); ":";
      CHR$(PEEK(527)); CHR$(PEEK(528))
210 GOSUB 960
220 IF X<11 THEN X=11
230 IF X>272 THEN X=272
240 IF Y<7 THEN Y=7
```

238
239

250 IF Y>152 THEN Y=152'
260 XDRAW OBJ AT X,Y: IF CROSS=1 THEN X1=X: Y1=Y
270 IF CROSS=2 THEN X2=X: Y2=Y
280 DIS=SQR(((X1-11)-(X2-11))^2)+((Y1-7)-(Y2-7))^2): IF T=195 THEN 360
290 IF T=210 THEN GOSUB 360
310 VTAB(23): PRINT " ": SA-1; TAB( 12); X-11; TAB( 20); Y-7; TAB( 27); INT(DIS); SPC( 4);
320 IF T=210 THEN 330
330 XDRAW OBJ AT X,Y: POKE L, PDL(0): POKE C, (255-PEEK(L)) * PDL(1)/255
340 IF T>176 AND T<185 THEN POKE M,T-176
350 T=0: GOTO 180
360 GOSUB 390
370 IF T=196 THEN 160
380 POKE S, PDL(0): FOR I=1 TO 120: NEXT I: POKE E, PEEK(S) + (255-PEEK(S)) * PDL(1)/255: POKE ADRS + 10,00: POKE ADRS + 11,31: PRINT "": CALL DE: GOTO 360
390 GOSUB 760: IF T<128 THEN RETURN
400 IF T=194 THEN INC=10
410 IF T=214 THEN INC=1
420 IF T=209 THEN 630
430 POKE -16368,0: IF T=155 THEN SA=SA-1
440 IF T=211 THEN HOME: INPUT "SAVE AS PIC.PIC (Y/N?";
450 IF GYS="Y" THEN AVS="PIC.PIC": GOSUB 540
460 IF T=216 THEN GOSUB 840
470 IF T=205 THEN Y=Y+INC
480 IF T=202 THEN X=X-INC
490 IF T=201 THEN Y=Y-INC
500 IF T=203 THEN X=X+INC
510 IF T=200 THEN 660
520 IF T=215 THEN HOME: INPUT "NAME?": AVS: GOSUB 540
530 RETURN
540 HOME: PRINT" ": HOME
550 HTAB(1): HTAB(9): PRINT CHR$(PEEK(521)); CHR$(PEEK(522)); CHR$(PEEK(523)); CHR$(PEEK(524)); CHR$(PEEK(525)); CHR$(PEEK(526)); SPC( 9): AVS
560 PRINT CHR$(4); "BSAVE": AVS; ",A$4000,L$2000": HOME: PRINT" ": RETURN
570 DS=CHR$(4): DS=7168: DE=7936: M=0: L=2: C=1: S=0: E=1
580 X=11: Y=7: ROT=0: CROSS=1: X2=272: Y2=152: T=2:
PRINT CHR$( 4); "BLOAD CROSS 1+2,A$1700"
240

590 POKE 232,00: POKE 233,23: INC=10: OBJ=1: HCOLOR=7:
SCALE=2
600 PRINT ""
610 PRINT ""
620 RETURN
630 VTAB(23): HTAB(1)
640 PRINT: PRINT: INPUT "DO YOU REALLY WANT TO QUIT?
(Y/N)"; ZE$: IF ZE$="Y" THEN 990
650 GOTO 160
660 VTAB(23): HTAB(1)
670 HGR: HGR2: HOME: PRINT: PRINT" ROOT-IMENTARY
GROWTH MENU": PRINT: PRINT "C CONTOUR OR
EDGE DISPLAY"
680 PRINT "D DITHER (GREY LEVEL) DISPLAY": PRINT "H
HELP DISPLAYS THIS PAGE": PRINT "Z SWITCH CROSS-
HAIRS": PRINT "V MOVES BY UNITS OF 1": PRINT" B
MOVES BY UNITS OF 10"
690 PRINT "I MOVES CROSS-HAIR UP": PRINT "J MOVES
CROSS-HAIR LEFT": PRINT "K MOVES CROSS-HAIR RIGHT":
PRINT "M MOVES CROSS-HAIR DOWN": PRINT " X DITHERS
CURRENT VIDEO IMAGE"
700 PRINT "W NAMED FILE SAVE OF HI-RES SCREEN": PRINT
"R SAVES COORDINATES AND TIME": PRINT "Q QUIT
PROGRAM": PRINT "S SAVE OF HI-RES SCREEN AS
<PIC.PIC>"
710 PRINT "<ESC> DELETES LAST TIME/CORD VALUE": PRINT
"1-8 SET DITHER MATRIX SIZE": PRINT "------------
------------------"
720 PRINT "HAND CONTROL FOR BLACK LEVEL <0>": PRINT "
AND FOR CONTRAST <1>"
730 GOSUB 760: IF T<128 THEN 730
740 FOR A=1 TO 24: PRINT: NEXT A
750 GOTO 180
760 T=PEEK(-16384): IF T>127 THEN POKE -16368,0
770 RETURN
780 PRINT "ERROR ON CHAR LOAD": STOP
790 IF CROSS=2 THEN CROSS=1: GOTO 820
800 X1=X: Y1=Y: XDRAW 1 AT X1,Y1: OBJ=2: X=X2: Y=Y2:
XDRAW 2 AT X2,Y2: CROSS=2
810 RETURN
820 X2=X: Y2=Y
830 XDRAW 2 AT X2,Y2: OBJ=1: X=X1: Y=Y1: XDRAW 1 AT
X1,Y1: RETURN
840 POKE ADRS + 10,00: POKE ADRS + 11,28
850 PRINT ""
860 XDRAW OBJ AT X,Y+8: XDRAW 2 AT X2,Y2+8: XDRAW 1 AT
X1,Y1+8: RETURN
870 TEMP=FRE(X): VTAB(23): HTAB(34): PRINT TAB( 34);
CHR$(PEEK(521)); CHR$(PEEK(522)); CHR$(PEEK(524));
CHR$(PEEK(525)); CHR$(PEEK(527)); CHR$(PEEK(529));
880  RTIME$ = CHR$(PEEK(521)) + CHR$(PEEK(522)) +
     CHR$(PEEK(524)) + CHR$(PEEK(525)) + CHR$(PEEK(527))
     + CHR$(PEEK(528))
890  R1(SA)=X-11: R2(SA)=Y-7: R3(SA)=INT(DIS*100+.05)/
     100: TM$=RTIME$: R4$(SA)=TM$: SA=SA+1
900  IF SA=YV+1 THEN GOTO 920
910  RETURN
920  PRINT: PRINT CHR$(4); "APPEND"; FILNAME$
930  PRINT CHR$(4); "WRITE"; FILNAME$
940 FOR TEMP=1 TO SA-1: PRINT R1(TEMP): PRINT R2(TEMP):
     PRINT R3(TEMP): NEXT TEMP
950 PRINT D$: "CLOSE"; FILNAME$: SA=1: RETURN
960 POKE - 16176,0: IF PEEK(- 16175)<128 THEN 970
970 IF PEEK(- 16170)<128 THEN RETURN
980  ZY=PEEK(- 16173): Y=ZY: ZX=PEEK(- 16174) + 256 *
     (PEEK(- 16172)>127): X=ZX: RETURN
990 GOSUB 920
     PRINT: PRINT "DO YOU WISH TO PRINT DATA? (Y/N)";
     WZ$: IF WZ$="Y" THEN PRINT CHR$(4); "RUN
     PRINTER1": END
APPENDIX B: Listing of the Computer Program, RATECALC, Used to Calculate Relative Growth Rates with the Apple System

The program was written in Applesoft Basic on an Apple II+ microcomputer. It allows determination of relative growth rates along a plant surface based on the change in segment lengths (distance between markers) over time.

```
10 CLEAR
20 DIM A(300), B(300), C(300), E$(300), F(L(300),
   IL(300), DL(300), R(300), T$(300), D(300), ZZ(300),
   Z(300), T(300), X(300), QR(300), TF(300), TI(300)
30 D$=CHR$(4)
40 HOME: DF$=""
50 HTAB(6): PRINT "ROOT-IMENTARY GROWTH RATE PROGRAM"
60 VTAB(2): HTAB(8): PRINT " GROWTH RATE CALCULATION"
70 VTAB(4): PRINT "--------------------------------------"
80 VTAB(10): INPUT "NAME OF DATA PILE?"; DF$
90 PRINT D$: "OPEN"; DF$: ",D1"
100 PRINT D$: "READ"; DF$
110 ONERR GOTO 160
120 INPUT S,N
130 FOR H=1 TO 999999
140 INPUT A(H), B(H), C(H), E$(H)
150 NEXT H
160 POKE 216,0: PRINT D$: "CLOSE"
170 Z=N*S
180 FOR K=1 TO W-l
190 GOSUB 210
200 NEXT K: GOTO 470
210 FOR J=1 TO N
220 HF=0: MF=0: SF=0: HI=0: MI=0: SI=0: QP=0: TF$=""
230 GOSUB 240
240 NEXT J: GOTO 460
250 FOR I=(K*Z)+((J-1)*S)+1 TO (K*Z)+((J-1)*S)+S
260 FL(I)=0.0860*C(I): REM FINAL SEGMENT LENGTH
270 IL(I)=0.0860*C(I-Z): REM INITIAL SEGMENT LENGTH
280 DL(I)=FL(I)-IL(I): REM CHANGE IN SEGMENT LENGTH
290 TF$=E$: T1$=E$(I-Z)
300 HF=(VAL(LEFT$(TF$,2))*3600): HI=(VAL(LEFT$(T1$,2))
```

242
300  \text{MF} = (\text{VAL(MID$(TF$, 3, 2))} \times 60): \text{MI} = (\text{VAL(MID$(TI$, 3, 2))} - \ast 60)
310  \text{SF} = (\text{VAL(RIGHT$(TF$, 2))}): \text{SI} = (\text{VAL(RIGHT$(TI$, 2))})
320  \text{TF}(I) = \text{HF} + \text{MF} + \text{SF}; \text{TI}(I) = \text{HI} + \text{MI} + \text{SI}
330  \text{T}(I) = \text{TF}(I) - \text{TI}(I)
340  \text{Z}(I) = \text{T}(I) / 2
350  \text{X}(I) = (\text{TF}(I) - \text{TI}(I - ((K - 1) \ast Z))) - \text{Z}(I)
360  \text{R}(I) = ((\text{LOG}(\text{FL}(I)) - \text{LOG}(\text{IL}(I))) \ast (1 / \text{T}(I))) \ast 3600
370  \text{QR}(I) = \text{IL}(I) / 2
380  \text{QP} = \text{IL}(I) / 2
390  \text{D}(I) = \text{QP} - \text{QR}(I)
400  \text{QP} = \text{D}(I) + \text{QR}(I)
410  \text{VTAB}(22): \text{HTAB}(14): \text{PRINT} "\text{*WORKING*}"
420  \text{FOR XZ} = 1 \text{ TO 80: NEXT XZ}
430  \text{VTAB}(22): \text{HTAB}(14): \text{PRINT} " "
440  \text{NEXT I}
450  \text{RETURN}
460  \text{RETURN}
470  \text{HOME: PRINT} "\text{TURN ON PRINTER}": \text{PR#1: PRINT: HOME}
480  \text{PRINT} "\text{ROOT-IMENTARY GROWTH DATA FILE}": \text{PRINT: PRINT}
490  \text{PRINT} "\text{FILE:} \text{DF$}: \text{PRINT: PRINT: PRINT}
500  \text{PRINT} "\text{SAMPLE#", "DISTANCE", "RATE", "TIME}"
510  \text{PRINT} "\text{-----------------------------}
520  \text{FOR Q=Z+1 \text{ TO H-1}}
530  \text{PRINT Q-Z, D(Q), R(Q), X(Q)/3600}
540  \text{PRINT}
550  \text{NEXT Q}
560  \text{PR#0: HOME}
570  \text{PRINT: INPUT} "\text{DO YOU WISH TO SAVE FOR CURFIT? (Y/N)}": \text{BS}
580  \text{IF BS="Y" THEN 600}
590  \text{IF BS="N" THEN 698}
600  \text{PRINT CHR$(4): "OPEN": DF$; "CURFIT"
610  \text{PRINT CHR$(4): "WRITE": DF$: "CURFIT"
620  \text{PRINT (H-Z-1)*2: FOR WW=Z+1 \text{ TO H-1: PRINT D(WW):}
630  \text{PRINT R(WW): NEXT WW)
640  \text{PRINT CHR$(4): "CLOSE": DF$: "CURFIT"
650  \text{HOME: INPUT} "\text{DO ANOTHER CALCULATION? (Y/N)}": \text{A$}
660  \text{IF A$="Y" THEN 10}
660  \text{IF A$="N" \text{ THEN 695}
695  \text{PRINT: INPUT} "\text{DO YOU WISH TO RUN CURFIT? (Y/N)}":}
UU$: IF UU$="Y" THEN CLEAR: PRINT CHR$(4); "RUN CURFIT"

698 PRINT: INPUT "DO YOU WISH TO QUIT? (Y/N)"; KU$: IF KU$="N" THEN GOTO 10

700 HOME: PRINT "PLEASURE DOING BUSINESS WITH YOU": END
APPENDIX C: Listing of the Computer Program, DEGCURV, used to Calculate Overall Angle of Curvature with the Apple System

The program was written in Applesoft Basic on an Apple II+ microcomputer. It allows determination of the overall angle of curvature (in degrees) of a plant surface at all measurement times during the course of gravitropic response.

10 CLEAR
20 DIM A(500), B(500), C(500), E$(500), AN(500), G(500), TF(500), TI(500)
30 DS=CHR$(4)
40 HOME: DF$=""
50 HTAB(6): PRINT "ROOT-IMENTARY GROWTH PROGRAM"
60 VTAB(2): HTAB(6): PRINT "DEGREE CURVATURE CALCULATION"
70 VTAB(4): PRINT "---------------------------------------------"
80 VTAB(10): INPUT "NAME OF DATA FILE?"; DF$
90 PRINT DS; "READ"; DF$
100 ONERR GOTO 160
120 INPUT S,N
130 FOR H=1 TO 999999
140 INPUT A(H),B(H),C(H),E$(H)
150 NEXT H
160 POKE 216,0: PRINT DS; "CLOSE"
180 QQ=0
190 Z=N*S
200 FOR K=1 TO W
210 GOSUB 230
220 NEXT K: GOTO 390
230 FOR J=1 TO N
240 JJ=J-1
250 SA=((K-1)*N)+(J-1)*S+1
260 SA=(A(JJ+1)-A(JJ))/B(JJ+1)-B(JJ)
270 SB=(A(JJ+S-2)-A(JJ+S-1))/B(JJ+S-2)-B(JJ+S-2)
280 QQ=QQ+1
290 IF SA*SB=-1 THEN AN(QQ)=90
245
300 AN(QQ)=(ATN((SB-SA)/(1+(SB*SA))))*420/(2*3.1416)
310 TF$=E$(JJ);  TI$=E$(1)
320 HF=(VAL(LEFT$$(TF$,2))*3600);  HI=(VAL(LEFT$$(TI$,2) *3600)
330 MF=(VAL(MID$$(TF$,3,2))*60);  MI=(VAL(MID$$(TI$,3,2)) *60)
340 SF=(VAL(RIGHT$$(TF$,2)));  SI=(VAL(RIGHT$$(TI$,2))
350 TF(JJ)=HF+MF+SF;  TI(JJ)=HI+MI+SI
360 G(QQ)=((TF(JJ)-TI(JJ))/3600
370 NEXT J
380 HOME:  PRINT"TURN ON PRINTER":  PR#1:  PRINT:  HOME
390 PRINT  "ROOT-IMENTARY GROWTH DATA FILE":  PRINT:  PRINT
"FILE:";  DF$:  PRINT:  PRINT
400 PRINT  "---------------------------------------------"  "="
410 PRINT  "SAMPLE#",  "DEGREE CURVATURE",  "TIME"
420 PRINT  "---------------------------------------------  "  
430 PRINT  "---------------------------------------------  "
440 FOR Q=1 TO 2*W
450 PRINT QQ, AN(QQ),  "  ",  G(QQ)
460 PRINT
470 NEXT QQ
480 PR#0:  HOME
490 PRINT:  INPUT  "DO YOU WISH TO SAVE FOR CURFIT?  
(Y/N)";  BS
500 IF BS="Y" THEN 520
510 IF BS="N" THEN 560
520 PRINT  DFS;  "OPEN";  DF$;  "CURFIT"
530 PRINT  DFS;  "WRITE";  DF$;  "CURFIT"
540 PRINT  ((H-l/S)*2:  FOR QQ=1 TO 2*W:  PRINT  G(QQ): PRINT  AN(QQ):  NEXT QQ
550 PRINT  DFS;  "CLOSE";  DF$;  "CURFIT"
560 PRINT:  INPUT  "DO ANOTHER CALCULATION? (Y/N)";  AS
570 IF AS="Y" THEN 10
580 IF AS="N" THEN 590
590 HOME:  HTAB(16):  PRINT  "THE END"
600 END
APPENDIX D: Listing of the Computer Program, LOCATE, Used to Identify Marker Locations with the IBM System

The program was written in IBM Basic on an IBM Personal Computer. It allows identification of the locations of all of the markers along a plant surface before running the SCAN program.

10 REM *** LOCATE:841006 ***
20 CLS : PRINT : PRINT: WIDTH 40
30 PRINT TAB(10);"GROWTH PROGRAM" :PRINT
40 PRINT : PRINT
50 INPUT "ENTER NUMBER OF TARGETS ",N
60 DIM T(400,2)
70 CLS
80 PRINT : PRINT
90 PRINT "USE FI KEY TO LIST ", N
100 PRINT " PIXEL LIGHT LEVEL."
110 PRINT
120 PRINT "USE F2 KEY TO STOP FI FUNCTION"
130 PRINT " AND TURN ON THE CURSOR"
140 PRINT " KEYS FOR MOVEMENT."
150 PRINT
160 PRINT "USE F3 KEY TO RECORD THE PIXEL"
162 PRINT " LOCATION FOR THE SPECIFIC"
164 PRINT " TARGET. LOAD TARGETS FROM"
166 PRINT " THE TIP BACK. IF TARGETS"
168 PRINT " ARE ON BOTH SIDES OF THE"
170 PRINT " ORGAN, LOAD THE UPPER SIDE"
172 PRINT " FIRST, THEN THE LOWER SIDE."
174 PRINT: PRINT
180 PRINT : PRINT
190 X=&H80
200 Y=&H80
210 B=1
220 ON KEY(1) GOSUB 440: ON KEY(2) GOSUB 590: ON KEY(3)
230 ON KEY(4) GOSUB 930
240 ON KEY(11) GOSUB 660
250 ON KEY(12) GOSUB 700
260 ON KEY(13) GOSUB 740
270 ON KEY(14) GOSUB 780
280 KEY(3) ON
290 KEY(4) ON
300 IF EFLAG=1 THEN 980
310 A=&HF14
320 REM X=&H80
330 REM Y=&H80
340 KEY(14) ON
350 KEY(13) ON
360 KEY(12) ON
370 KEY(11) ON
380 KEY(1) ON
390 OUT A,X
400 OUT A+1,Y
410 D%=INP(A+3)
420 GOSUB 820
430 GOTO 430
440 KEY(1) OFF
450 KEY(11) OFF
460 KEY(12) OFF
470 KEY(13) OFF
480 KEY(14) OFF
490 KEY(2) ON
500 D%=INP(A+3)
510 GOSUB 820
520 RETURN 530
530 OUT A+1,Y
540 T=INP(A+1) AND 1
550 IF T=0 GOTO 540 ELSE 560
560 D%=INP(A+3)
570 PRINT D%,X,Y : FOR G=1 TO 500: NEXT
580 GOTO 530
590 KEY(1) ON
600 KEY(11) ON
610 KEY(12) ON
620 KEY(13) ON
630 KEY(14) ON
640 KEY(2) OFF
650 RETURN 430
660 IF Y=&H1 GOTO 680
670 Y=Y-1
680 OUT A+1,Y
690 RETURN 430
700 IF X=&H1 GOTO 720
710 X=X-1
720 OUT A,X
730 RETURN 430
740 IF X=&HFE GOTO 760
750 X=X+1
760 OUT A,X
770 RETURN 430
780 IF Y=&HFE GOTO 800
790 Y=Y+1
800 OUT A+1,Y
810 RETURN 430
820 T=INP(A+1) AND 1
830 IF T=0 GOTO 820 ELSE 840
840 D%=INP(A+3)
850 RETURN
860 REM *** STORE TARGET DATA ***
870 LET T(B,1)=X : LET T(B,2)=Y
880 PRINT "TARGET ";B
890 LET B=B + 1
900 IF B>N THEN 920
910 RETURN
920 REM *** DATA TO DISK ***
930 OPEN "B:GDATA" FOR OUTPUT AS 1
940 WRITE #1, N,N,N
950 FOR TN=1 TO N
960 WRITE #1,T(TN,1),T(TN,2),0
970 NEXT TN
980 REM *** CALL SCAN ***
990 CLOSE 1
1000 LOAD "A:SCAN",R
1010 END
APPENDIX E: Listing of the Computer Program, SCAN, Used to Track Marker Locations with the IBM System

The program was written in IBM Basic with an IBM Personal Computer. It allows automatic tracking of marker locations with the IBM Video Digitizer System.

10 REM *** SCAN:850130 ***
20 CLEAR
30 NEWTM$="00:00:00"
40 A=$HF14
50 REM *** GET TIME DATA ***
60 LET TL=1 : CLS
70 INPUT "NAME OF DATA FILE:",DF$
80 PRINT
90 INPUT "DISTANCE FROM TARGET #1 TO TIP (MM):",DT
100 PRINT
110 INPUT "CONVERSION FACTOR (UM/CU):",CF: CF=CF/1000
120 PRINT
130 INPUT "TOTAL RUN TIME (MIN):",TEMPUS
140 PRINT
150 FUG$=NEWTM$
160 INPUT "TIME LAPSE BETWEEN RECORDINGS (MIN):",FT
170 IF FT>99 OR FT<.1 THEN 190
180 XT=INT(FT):IF XT<>FT THEN XT=FT-INT(FT):GOTO 220 ELSE R=4:GOTO 200
190 PRINT"Enter any interval between 0.1 and 99 min":GOTO 160
200 IF FT<10 THEN FUGIT$="0"+RIGHT$(STR$(FT),1) ELSE FUGIT$=RIGHT$(STR$(FT),2)
210 GOTO 270
220 IF FT<10 THEN FUGIT$="0"+RIGHT$(STR$(INT(FT)),1):GOTO 240
230 FUGIT$=RIGHT$(STR$(INT(FT)),2)
240 MIDS(FUG$,4)=FUGIT$
250 FUGIT$=STR$(CINT(60*XT))
260 FUGIT$=RIGHT$(FUGIT$,2):R=7
270 MIDS(FUG$,R)=FUGIT$ 'PLACE INTERVAL IN STRING
280 FUG=VAL(FUGIT$)
290 IT=TEMPUS/FT 'INTERVAL CALCULATION
300 LOCATE 25,10:PRINT "DATA OKAY ?(y/n)";
310 A$=INKEY$:IF A$="" THEN 310
320 IF A$="y" OR A$="Y" THEN 350

250
330 IF A$="n" OR A$="N" THEN 340 ELSE 300
340 CLS: GOTO 30
350 PRINT "PROGRAM START : TIME INITIALIZATION 00:00:00"
360 TIMES=NEWTM$
370 CLS
380 REM *** LOAD WORKING TARGET LOCATIONS ***
390 OPEN "B:GDATA" FOR INPUT AS 1
400 INPUT #1, N,N,N: DIM WTL(N,2),E(N)
410 IF EOF(1) THEN 450
420 X=X + 1
430 INPUT #1,WTL(X,1),WTL(X, 2),E(X)
440 GOTO 410
450 CLOSE #1
460 OPEN "B:" + DFS$ FOR OUTPUT AS 1
470 WRITE#1,N,IT,DT
480 WRITE #1,CF,CF,CF
490 FOR X91  TO N
500 WRITE #1,WTL(X,1),WTL(X,2),E(X)
510 NEXT X
520 PRINT ",# OF TARGETS=";N
530 REM *** SCAN LOGIC ***
540 FOR LOOP = 1 TO N
550 E(LOOP)=TL*FUG
560 OUT A , WTL(LOOP,1) :  OUT A+l, WTL(LOOP,2) '  SETS X,Y OF WTL
570 T=INP(A+1) AND 1
580 IF T=0 THEN 570
590 D%=INP(A+3)
600 IF D%=0 THEN 750
610 LET WTL(LOOP,1) = WTL(LOOP,1) - 2
620 LET WTL(LOOP,2) = WTL(LOOP,2) - 2
630 FOR Y=WTL(LOOP,2) TO (WTL(LOOP,2) + 4)
640 FOR X=WTL(LOOP,1) TO (WTL(LOOP,1) + 4)
650 OUT A,X :  OUT A+1,Y
660 T=INP(A+1) AND 1
670 IF T=0 THEN 660
680 D% = INP(A+3)
690 IF D% = 0 THEN 700 ELSE 710
700 LET WTL(LOOP,1) = X : LET WTL(LOOP,2) = Y : GOTO 750
710 NEXT X
720 NEXT Y
730 LET WTL(LOOP,1)=WTL(LOOP,1) + 2
740 LET WTL(LOOP,2)=WTL(LOOP,2) + 2
750 NEXT LOOP
760 REM *** TIME CHECK ***
770 LOCATE 8,10:PRINT "TOTAL RUN TIME (MIN):";TEMPUS
780 LOCATE 10,10 : PRINT "INTERVAL TIME: "; TIMES
790 PRINT " ELAPSED TIME: "; (TL-1)*FUG
800 REM *** INTERVAL CHECK ***
810 IF TIMES$>=FUG$ THEN 830
820 GOTO 540 'NEXT LOOP
830 FOR LOOP$=1 TO N
840 WRITE #1,WTL(LOOP$),E(LOOP$) 'DATA
      FORMAT IS X,Y
850 NEXT LOOP
860 IF TL$>IT THEN 900
870 TL$=TL$+1
880 TIMES$=NEWTM$  
890 GOTO 540
900 CLS
910 PRINT "DONE"
920 CLOSE
930 PRINT:PRINT
940 PRINT "RUN ANOTHER? (y/n)"
950 A$=INKEY$:IF A$="" THEN 950
960 IF A$="Y" OR A$="y" THEN 980
970 IF A$="N" OR A$="n" THEN 990 ELSE 940
980 LOAD "A:LOCATE",R
990 PRINT "CALCULATE RATES? (y/n)"
1000 A$=INKEY$:IF A$="" THEN 1000
1010 IF A$="Y" OR A$="y" THEN 1030
1020 IF A$="N" OR A$="n" THEN 1040 ELSE 990
1030 LOAD "A:RATES",R
1040 END
APPENDIX F: Listing of the Computer Program, RATES, Used to Calculate Relative Growth Rates with the IBM System

The program was written in IBM Basic with an IBM Personal Computer. It allows determination of relative growth rates along a plant surface based on the change in segment lengths (distance between markers) over time.

```
10 REM *** RATES:860606 ***
20 DIM LX(2900),LY(2900),E(2900),RGR(2700),DD(2700)
30 CLS: H=0: J=0:DD=0
40 PRINT TAB(8); "GROWTH RATE CALCULATION": PRINT
50 PRINT: PRINT
60 INPUT "NAME OF DATA FILE:", DF$
70 INPUT "FOR (3)- OR (2)-DIMENSIONAL PLOT?", HF
80 IF HF=2 THEN 110
90 IF HF=3 THEN 100
100 INPUT "NUMBER OF LINES IN PLOT:", NL
110 INPUT "(V)ERTICAL OR (H)ORIZONTAL GROWTH?", GF$
120 INPUT "CALCULATION INTERVAL:",X
130 IF GF$="V" THEN 180
140 IF GF$="H" THEN 525
150 REM *******************************
160 REM CALCULATION FOR VERTICAL GROWTH
170 REM *******************************
180 OPEN "B:" + DF$ FOR INPUT AS 1
190 INPUT #1,NT,IT,DT ' # OF TARGETS, # OF INTERVALS,
200 INPUT #1,CFV,CFV,CFV 1 CONVERSION FACTOR (MM/CU)
210 CFH=1.22*CFV
220 IF EOF(1) THEN 270
230 H=H+1
240 INPUT #1,LX(H),LY(H),E(H) ' INPUT X,Y,TIME
250 GOTO 220
260 PRINT H "DATA POINTS": CLOSE #1
280 FOR J=X TO IT STEP X
290 DD=DT
300 FOR H=(J*NT)+2 TO (J+1)*NT
310 ET=(E(H)-E(H-(NT*X)))/60 ' TIME TRANSPIRED (HRS)
315 FLS=SQR(((LY(H-1)-LY(H))*CFV)^2 + (((LX(H-1)-
320 LX(H))^2)) ' SEGMENT LENGTH (MM)
325 ILS=SQR(((LY(H-(NT*X)-1)-LY(H-(NT*X)))*CFV)^2 +
326 (((LX(H-(NT*X)-1)-LX(H-(NT*X)))*CFH)^2)) ' SEGMENT
```
LENGTH (MM)
330 IF ILS<=0 OR FLS<=0 THEN 340 ELSE 350
340 RGR(H)=0: GOTO 370
350 RGR(H)=100*(LOG(FLS)-LOG(ILS))/ET ' RELATIVE GROWTH RATE (%/HR)
360 RGR(H)=INT((RGR(H)*100)+.5)/100 ' ROUNDED VALUE
370 DD(H)=(FLS/2)+DD 'DISTANCE FROM TIP
380 DD(H)=INT((DD(H)*100)+.5)/100
390 DD=FLS+DD
400 NEXT H
410 NEXT J
420 IF HF=3 THEN 1070
430 OPEN "B:" + DFS + ".GR" FOR OUTPUT AS 2
440 FOR J=X TO IT STEP X
450 FOR H=(J*NT)+2 TO (J+1)*NT
460 WRITE #2,DD(H),RGR(H)
470 NEXT H
480 NEXT J
490 GOTO 910
500 REM *********************************
510 REM CALCULATION FOR HORIZONTAL GROWTH
520 REM *********************************
525 IF HF=3 THEN 1170
530 OPEN "B:" + DFS:" FOR INPUT AS 1
540 INPUT #1,NT,IT,DT ' # OF TARGETS, # OF INTERVALS,
550 INPUT #1,CFV,CFV,CFV ' CONVERSION FACTOR (MM/CU)
560 CFH=1.22*CFV
570 IF EOF(1) THEN 620
580 H=H+1
590 INPUT #1,LX(H),LY(H),E(H) ' INPUT X,Y,TIME
600 GOTO 570
610 NT=NT/2 ' SET # OF WTLS TO # OF WTLS PER SIDE
620 PRINT H "DATA POINTS":CLOSE #1
630 NT=NT/2 ' SET # OF WTLS TO # OF WTLS PER SIDE
640 FOR J=(2*X) TO (2*IT)
650 DD=DT
660 FOR H=(J*NT)+2 TO ((J+2)*NT)
670 ET=(E(H)-E(H-/(2*NT*X)))/60 ' TIME TRANSPRIRED (HRS)
675 FLS=SQR(((LY(H-1)-LY(H))*CFV)^2 + (((LX(H-1)-
680 LX(H))*CFH)^2)) ' SEGMENT LENGTH (MM)
685 ILS=SQR(((LY(H-/(2*NT*X)-1)-LY(H-/(2*NT*X)))*CFV)^2 + 
690 (((LX(H-/(2*NT*X)-1)-LY(H-/(2*NT*X)))*CFH)^2)) ' 
700 SEGMENT LENGTH (MM)
690 IF ILS<=0 OR FLS<=0 THEN 700 ELSE 710
700 RGR(H)=0: GOTO 730
RGR(H) = \frac{100 \times (\log(FLS) - \log(ILS))}{ET} \quad \text{RELATIVE GROWTH RATE} \ (%/HR) \\
RGR(H) = \text{INT}\left(\frac{(RGR(H) \times 100) + .5}{100}\right) \quad \text{ROUNDED VALUE} \\
DD(H) = (FLS/2) + DD \quad \text{DISTANCE FROM TIP (MM)} \\
DD(H) = \text{INT}\left(\frac{(DD(H) \times 100) + .5}{100}\right) \\
DD = FLS + DD \\
NEXT H \\
NEXT J \\
OPEN "B:" + DF$ + ".GRL" FOR OUTPUT AS 1 \\
FOR J=1 TO IT STEP X \\
FOR H=(2*J*NT)+NT+2 TO ((2*J*NT)+(2*NT)) \\
WRITE #1, DD(H), RGR(H) \\
NEXT H \\
NEXT J \\
OPEN "B:" + DF$ + ".GRU" FOR OUTPUT AS 2 \\
FOR J=1 TO IT STEP X \\
FOR H=(2*J*NT)+2 TO ((2*J*NT)+NT) \\
WRITE #2, DD(H), RGR(H) \\
NEXT H \\
NEXT J \\
CLOSE \\
CLS: PRINT DF$ " DONE": PRINT: PRINT \\
PRINT "CALCULATE MORE RATES?" \\
A$=INKEY$: IF A$="" THEN 940 \\
IF A$="Y" OR A$="y" THEN 30 \\
IF A$="N" OR A$="n" THEN 970 \\
PRINT: PRINT \\
PRINT "MODIFY DATA?" \\
A$=INKEY$: IF A$="" THEN 990 \\
IF A$="Y" OR A$="y" THEN 1020 \\
IF A$="N" OR A$="n" THEN 1500 \\
LOAD "A: MODDATA", R \\
REM ************************* \\
REM CALCULATION FOR 3-D PLOT \\
REM ************************* \\
FOR N=1 TO NL \\
PRINT "FILE" N: INPUT "NAME OF FILE: ", AF$ \\
OPEN "B:" + AF$ FOR OUTPUT AS 1 \\
FOR J=(N-1)*(IT/NL)+X TO N*(IT/NL) STEP X \\
FOR H=(J*NT)+2 TO (J+1)*NT \\
WRITE #1, DD(H), RGR(H) \\
NEXT H \\
NEXT J \\
CLOSE #1 \\
NEXT N \\
GOTO 920 \\
REM ************************* \\
REM SEPARATION OF HORIZONTAL DATA \\
REM ************************* \\
OPEN "B:" + DF$ FOR INPUT AS 1
1180 INPUT #1, NT, IT, DT
1190 INPUT #1, CFV, CFV, CFV
1200 IF EOF(1) THEN 1240
1210 H=H+1
1220 INPUT #1, LX(H), LY(H), E(H)
1230 GOTO 1200
1240 N=H: PRINT H "DATA POINTS": CLOSE #1
1250 NT=NT/2
1260 OPEN "B:" + DFS + "U" FOR OUTPUT AS 1
1270 WRITE #1, NT, IT, DT
1280 WRITE #1, CFV, CFV, CFV
1290 FOR J=1 TO (2*IT)+1 STEP 2
1300 FOR H=((J-1)*NT)+1 TO (J*NT)
1310 WRITE #1, LX(H), LY(H), E(H)
1320 NEXT H
1330 NEXT J
1340 OPEN "B:" + DFS + "L" FOR OUTPUT AS 2
1350 WRITE #2, NT, IT, DT
1360 WRITE #2, CFV, CFV, CFV
1370 FOR J=2 TO (2*IT)+2 STEP 2
1380 FOR H=((J-1)*NT)+1 TO (J*NT)
1390 WRITE #2, LX(H), LY(H), E(H)
1400 NEXT H
1410 NEXT J
1420 CLOSE
1430 PRINT: PRINT
1440 PRINT "HORIZONTAL DATA IS DIVIDED INTO TWO FILES"
1450 PRINT "THE FILE NAME + "U" FOR THE UPPER SIDE"
1460 PRINT "THE FILE NAME + "L" FOR THE LOWER SIDE"
1470 PRINT "CALCULATE RATES FOR EACH SEPARATELY"
1480 PRINT "USE VERTICAL 3-D ROUTINE"
1490 INPUT "PUSH "R" WHEN READY ", ANS$"
APPENDIX G: Listing of the Computer Program, ERICK, Used to Calculate Relative Growth Rates with the IBM System

The program was written in IBM Basic with an IBM Personal Computer. It allows determination of relative growth rates along a plant surface based on the method of Erickson and Sax (1956), involving smoothing and differentiation of the position data.

10 REM *** ERICK:860527 ***
20 DIM LX(1530), LY(1530), E(1530), DD(1530), LS(1530),
  AGR(1530), XX(1530), YY(1530), RGR(1530)
30 CLS
40 PRINT "GROWTH RATE CALCULATION"
50 PRINT: PRINT
60 INPUT "NAME OF DATA FILE:1 1 , DF$"
70 INPUT "VERTICAL(V) OR HORIZONTAL(H) GROWTH?", GF$
75 INPUT "DESIRED X-INTERVAL (IN 1/100'S OF A MM):", S:
     S=S/100
80 IF GF$="V" THEN 120: IF GF$="H" THEN 1080
90 REM ****************************
100 REM POSITION VS TIME CALCULATION
110 REM ****************************
120 OPEN "B:" + DF$ FOR INPUT AS 1
130 INPUT #1,NT,IT,DT '# TARGETS, # INTERVALS, DISTANCE
  BETWEEN FIRST TARGET AND TIP (MM)
140 INPUT #1,CFV,CFV,CFV 'CONVERSION FACTOR (MM/CU)
150 CFH=1.22*CFV
160 IF EOF(1) THEN 204
170 H=H+1
180 INPUT #1, LX(H),LY(H),E(H) 'INPUT X,Y,TIME
190 LS(H)=SQR(((LY(H-1)-LY(H))*CFV)^2 + ((( LX(H-1)-
  LX(H)))*CFH)^2)) 'SEGMENT LENGTH (MM)
200 GOTO 160
204 ET=E(H-1)/(IT*60) 'TIME BETWEEN MEASUREMENTS (HR)
205 CLOSE #1
206 CLOSE #1
210 FOR J=1 TO IT+1
220 DD=DT
230 FOR H=((J-1)*NT)+2 TO J*NT
240 DD(H)=LS(H)+DD
250 DD=DD(H)
260 NEXT H

257
258
270 NEXT J
290 REM ****************************************************
300 REM ABSOLUTE GROWTH RATES BY 7 PT S&D FORMULA
310 REM *****************************************************
315 PRINT "CALCULATING ABSOLUTE RATES"
320 FOR K=2 TO NT
330 FOR J=3 TO IT-3
340 PA=DD(((J-3)*NT)+K): PB=DD(((J-2)*NT)+K): PC=DD(((J-
1)*NT)+K)
PG=DD(((J+3)*NT)+K)
360 AGR((J*NT)+K)=(1/(ET*252)) *((22*PA)-(67*PB)-(58*PC)+(58*PE)+(67*PF)-(22*PG))
364 NEXT J
366 NEXT K
370 OPEN "B:" + DF$ + ".A" FOR OUTPUT AS 1
380 FOR K=2 TO NT
390 FOR J=3 TO IT-3
400 WRITE #1, DD((J*NT)+K), AGR((J*NT)+K)
404 NEXT J
406 NEXT K
410 CLOSE #1
420 REM ***************
430 REM SORT ABSOLUTE RATES
440 REM ****************
445 N=0
446 PRINT "SORTING ABSOLUTE RATES"
450 OPEN "B:" + DF$ + ".A" FOR INPUT AS 1
460 IF EOF(1) THEN 500
470 N=N+1
480 INPUT #1,DD(N), AGR(N)
490 GOTO 460
500 CLOSE #1
510 KM=N: KT=N
520 KM=INT(KM/2)
530 IF KM=0 THEN 662
540 KK=KT-KM: KJ=1
550 KI=0
560 KL=KI+KM
570 IF DD(KI)<DD(KL) THEN 640
620 KI=KI-KM
630 IF KI>=1 THEN 560
640 KJ=KJ+1
650 IF KJ>KK THEN 520
660 GOTO 550
662 OPEN "B:" + DF$ + ".A" FOR OUTPUT AS 1
664 FOR K=1 TO N
666 WRITE #1, DD(K), AGR(K)
259

668 NEXT K
670 CLOSE #1
671 GOSUB 1420
672 REM *****************************************************
674 REM LEAST SQUARES PARABOLA FOR EVEN X-INTERVALS
676 REM *****************************************************
678 PRINT "INTERPOLATING EVEN X-INTERVALS"
680 OPEN "B:" + DF$ + ",A" FOR INPUT AS 1
681 H=0
682 IF EOF(1) THEN 689
683 H=H+1
684 INPUT #1,DD(H),AGR(H)
685 GOTO 682
689 N=H: CLOSE #1
690 H=3: T=1: XX(T)=(INT((DD(H)*100)+.5))/100
XE=DD(H+2)
700 YA=AGR(H-2): YB=AGR(H-1): YC=AGR(H): YD=AGR(H+1):
YE=AGR(H+2)
710 A=YA+YB+YC+YD+YE
720 B=(XA*YA)+(XB*YB)+(XC*YC)+(XD*YD)+(XE*YE)
730 C=(XA*XA*YA)+(XB*XB*YB)+(XC*XC*YC)+(XD*XD*YD)+(XE*XE-
YE)
740 D=XA+XB+XC+XD+XE
750 E=(XA`2)+(XB`2)+(XC`2)+(XD`2)+(XE`2)
760 F=(XA`3)+(XB`3)+(XC`3)+(XD`3)+(XE`3)
770 G=(XA`4)+(XB`4)+(XC`4)+(XD`4)+(XE`4)
780 CC=((C*D)-(A*F)-(B*D)+(A*E))/((E*E)-(F*D)-
(E*F)+(G*D))
790 AA=(((C*D)-(A*F))+(CC*(((E*F)-(G*D))))/((D*D)-(N*E))
800 BB=(A-(AA*N)-(CC*E))'/D
810 YY(T)=AA+(BB*XX(T))+(CC*(XX(T)`2))
820 IF(XX(T)+S) > DD(H+1) THEN 830 ELSE 850
830 H=H+1: IF H=N-1 THEN 870 ELSE 840
840 IF (XX(T)+S) < DD(H+2) THEN 850 ELSE 695
850 T=T+1
860 XX(T)=XX(T-1)+S: XX(T)=(INT((XX(T)*100)+.5))/100:
GOTO 695
870 P=T
880 REM *****************************************************
890 REM RELATIVE GROWTH RATES BY 7 PT S&D FORMULA
900 REM *****************************************************
905 PRINT "CALCULATING RELATIVE RATES"
910 FOR T=4 TO P-3
PFF=YY(T+2): PG=YY(T+3)
930 RGR(T)=(1/(S*252))*(((22*PA)-(67*PB)-
(58*PC)+(58*PE)+(67*PF)-(22*PG))*100
940 NEXT T
950 OPEN "B:" + DF$ + ",R" FOR OUTPUT AS 1
260 FOR T=4 TO P-3
265 WRITE #1, XX(T), RGR(T)
266 NEXT T
267 CLOSE #1
268 PRINT T "RATES CALCULATED"
269 PRINT: PRINT

1000 INPUT "CALCULATE MORE RATES?", ANS$
1010 IF ANS$="Y" THEN 30: IF ANS$="N" THEN 1020
1020 INPUT "PREPARE FOR ENERGRAPHICS?", ANS$
1030 IF ANS$="N" THEN 1050: IF ANS$="Y" THEN 1040
1040 LOAD "A:MODDATA",R
1050 PRINT "TSCHUSS!": GOTO 1410
1060 OPEN "B:" + DF$ FOR INPUT AS 1
1070 INPUT #1,NT,IT,DT
1080 INPUT #1,CFV,CFV,CFV
1090 IF EOF(1) THEN 1150
1100 H=H+1
1110 INPUT #1,LX(H),LY(H),E(H)
1120 GOTO 1110
1130 N=H: CLOSE #1
1140 NT=NT/2
1150 OPEN "B:" + DF$ + "U" FOR OUTPUT AS 1
1160 WRITE #1,NT,IT,DT
1170 WRITE #1,CFV,CFV,CFV
1180 FOR J=1 TO IT+1 STEP 2
1190 FOR H=(((J-1)*NT)+1) TO (J*NT)
1200 WRITE #1,LX(H),LY(H),E(H)
1210 NEXT H
1220 NEXT J
1230 GOTO 1110
1240 NEXT H
1250 OPEN "B:" + DF$ + "L" FOR OUTPUT AS 2
1260 WRITE #2,NT,IT,DT
1270 WRITE #2,CFV,CFV,CFV
1280 FOR J=2 TO IT +2 STEP 2
1290 FOR H=(((J-1)*NT)+1 TO (J*NT)
1300 WRITE #2,LX(H),LY(H),E(H)
1310 NEXT H
1320 NEXT J
1330 CLOSE
1340 PRINT: PRINT
1350 PRINT "HORIZONTAL DATA IS DIVIDED INTO TWO FILES"
1360 PRINT "THE FILE NAME + "U" FOR THE UPPER SIDE"
1370 PRINT "THE FILE NAME + "L" FOR THE LOWER SIDE"
1380 PRINT "CALCULATE RATES FOR EACH SEPARATELY"
1390 INPUT "PUSH "R" WHEN READY", ANS$
1400 IF ANS$="R" THEN 30
1410 GOTO 1890
1420 REM **********************
1430 REM SMOOTH ABSOLUTE RATES
1440 REM **********************
1445 PRINT "AVERAGING ABSOLUTE RATES"
1450 A=7 'NUMBER OF VALUES TO BE AVERAGED
1460 N=0: H=0: J=0
1470 OPEN "B:" + DFS + ".A" FOR INPUT AS 1
1480 IF EOF(1) THEN 1520
1490 N=N+1
1500 INPUT #1,DD(N),AGR(N)
1510 GOTO 1480
1520 CLOSE #1
1530 Z=INT(N/A)
1540 FOR H=1 TO Z
1550 M=0: Q=0
1560 FOR J=1 TO A
1570 K=DD((((H-1)*A)+J): P=AGR(((H-1)*A)+J)
1580 M=M+K: Q=P+Q
1590 NEXT J
1600 DD(H)=M/A: AGR(H)=Q/A
1610 NEXT H
1615 KILL "B:" + DFS + ".A"
1620 OPEN "B:" + DFS + ".A" FOR OUTPUT AS 1
1630 FOR H=1 TO Z
1640 WRITE #1,DD(H),AGR(H)
1650 NEXT H
1660 CLOSE #1
1670 B=7
1680 N=0
1690 OPEN "B:" + DFS + ".A" FOR INPUT AS 1
1700 IF EOF(1) THEN 1740
1710 N=N+1
1720 INPUT #1,DD(N),AGR(N)
1730 GOTO 1700
1740 CLOSE #1
1750 FOR H=1 TO (N+1-B)
1760 M=0: Q=0
1770 FOR J=1 TO B
1780 K=DD(H+J-1): P=AGR(H+J-1)
1790 M=M+K: Q=P+Q
1800 NEXT J
1810 DD(H)=M/B: AGR(H)=Q/B
1820 NEXT H
1830 OPEN "B:" + DFS + ".A" FOR OUTPUT AS 1
1840 FOR H=1 TO (N+1-B)
1850 WRITE #1,DD(H),AGR(H)
1860 NEXT H
1870 CLOSE #1
1880 RETURN
1890 END
APPENDIX H: Listing of the Computer Program, CURVES, Used to Calculate Overall Angle and Radius of Curvature with the IBM System

The program was written in IBM Basic with an IBM Personal Computer. It allows determination of the overall angle (in degrees) and radius (in mm) of curvature of a plant surface at all measurement times during the course of gravitropic curvature.

```
10 REM *** CURVES:850401 ***
20 DIM LX(3000),LY(3000),E(3000),AN(500),R(500)
30 CLS
40 PI=3.1416: H=0
50 PRINT TAB(10); "CURVATURE ANALYSIS"
60 PRINT: PRINT
70 INPUT "NAME OF DATA FILE:",DF$
80 REM ****************************
90 REM DEGREE CURVATURE CALCULATION
100 REM ****************************
110 OPEN "B:" + DF$ FOR INPUT AS 1
120 INPUT #1,NT,IT,DT ' # OF TARGETS, # OF INTERVALS, DISTANCE FROM FIRST TARGET TO THE TIP
130 INPUT #1,CFV,CFV,CFV ' CONVERSION FACTOR (MM/CU)
135 CFH=1.22*CFV
140 IF EOF(1) THEN 180
150 H=H+1
160 INPUT #1,LX(H),LY(H),E(H) ' INPUT X,Y,TIME
170 GOTO 140
180 CLOSE #1
190 FOR J=1 TO (IT+1)
200 D=((J-1)*NT)+2: C=((J-1)*NT)+3
210 B=((J-1)*NT)+(NT-1): A=((J-1)*NT)+NT
220 E(J)=E(A)
230 IF LX(A)=LX(B) THEN LX(B)=LX(B)+.001: GOTO 270
240 IF LX(C)=LX(D) THEN LX(D)=LX(D)+.001: GOTO 270
250 SLA=(LY(B)-LY(A))/(LX(B)-LX(A))
260 SLB=(LY(D)-LY(C))/(LX(D)-LX(C))
270 IF SLA>=0 THEN 280: IF SLA<0 THEN 310
280 IF SLB>=0 THEN AN(J)=(ATN(SLB)-ATN(SLA))*(180/PI): GOTO 300
290 IF SLB<0 THEN AN(J)=-(ATN(ABS(SLB))+ATN(SLA))*(180/PI)
```

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300 GOTO 330
310 IF SLB>=0 THEN AN(J)=(ATN(ABS(SLA))+ATN(SLB))*(180/-PI): GOTO 330
320 IF SLB<0 THEN AN(J)=-(ATN(ABS(SLB))-
ATN(ABS(SLA)))*(180/PI)
330 IF AN(J)<0 THEN AN(J)=0
340 GOSUB 640
344 IF J>50 THEN 346 ELSE 350
346 IF AN(J)=0 THEN AN(J)=AN(J-1): IF R(J)=100 THEN
R(J)=R(J-1)
350 NEXT J
360 OPEN "B:" + DF$ + ".DC" FOR OUTPUT AS 1
370 FOR J=1 TO (IT+1)
380 WRITE #1,E(J),AN(J)
390 NEXT J
400 CLOSE #1
410 OPEN "B:" + DF$ + ".RC" FOR OUTPUT AS 1
420 FOR J=1 TO (IT+1)
430 WRITE #1,E(J),R(J)
440 NEXT J
450 CLOSE #1
460 INPUT "PRINT DATA?",WNS$
470 IF WNS$="Y" THEN 490
480 IF WNS$="N" THEN 540
490 LPRINT "TIME(MIN)","DEGREE CURVATURE","RADIUS CURVATURE(MM)"
500 LPRINT: LPRINT
510 FOR J=1 TO (IT+1)
520 LPRINT E(J),AN(J)," ",R(J)
530 NEXT J
540 PRINT: PRINT
550 INPUT "RUN ANOTHER?",ANS$
560 IF ANS$="Y" THEN 30
570 IF ANS$="N" THEN 580
580 PRINT: PRINT: INPUT "MODIFY DATA?", BNS$
590 IF BNS$="Y" THEN 610
600 IF BNS$="N" THEN 630
610 LOAD ".MODDATA", R
620 GOTO 740
625 REM ****************************
630 REM RADIUS CURVATURE CALCULATION
635 REM ****************************
640 IF SLA<1E-30 THEN 644 ELSE 646
644 SLA=+1E+30: GOTO 650
646 SLA=+1/SLA
650 IF SLB<1E-30 THEN 654 ELSE 656
654 SLB=+1E+30: R(J)=+1E+30: GOTO 724
656 SLB=+1/SLB
670 XA=(LX(A)+LX(B))/2: XB=(LX(C)+LX(D))/2
680 YA=(LY(A)+LY(B))/2: YB=(LY(C)+LY(D))/2
690 BA=YA-(SLA*XA):BB=YZ-(SLB*XB)
700 X=(BB-BA)/(SLA-SLB)
710 Y=(SLB*X)+BB
714 RA=SQR(((YA-Y)*CFV)^2)+(((XA-X)*CFH)^2))
716 RB=SQR(((YB-Y)*CFV)^2)+(((XB-X)*CFH)^2))
720 R(J)=(RA+RB)/2
724 IF R(J)>100 THEN 726 ELSE 730
726 R(J)=100
730 RETURN
740 END
APPENDIX I: Listing of the Computer Program, MODDATA, Used to Modify Data with the IBM System

The program was written in IBM Basic with an IBM Personal Computer. It allows data files, containing growth or curvature data, to be compiled, sorted, averaged, smoothed, and then formatted to be read by the ENERGRAPHICS or ENERCHARTS software.

10 REM *** MODDATA:850310 ***
20 DIM XX(5000), YY(5000), X(8, 200), Y(8, 200), DOT%(8), NIT%(8), SSR%(8), SSC%(8), SUB%(8), MN(8), MX(8), ST(8), O%(8), -
     DF%(8)
30 CLS
40 PRINT TAB(10); "DATA FILE MODIFICATION"
50 LOCATE 10, 10: PRINT "1. COMBINE FILES"
60 PRINT
70 PRINT TAB(10); "2. SORT DATA"
80 PRINT
90 PRINT TAB(10); "3. AVERAGE AND SMOOTH DATA"
100 PRINT
110 PRINT TAB(10); "4. FORMAT DATA"
120 PRINT: PRINT: PRINT: PRINT: PRINT
130 PRINT TAB(10)
140 INPUT "SELECTION: ", S
150 CLS
160 IF S=1 THEN 210
170 IF S=2 THEN 480
180 IF S=3 THEN 810
190 IF S=4 THEN 1380
200 REM
210 CLS: PRINT TAB(10); "COMBINING FILES"
220 REM
230 PRINT: PRINT
240 INPUT "FIRST FILE: ", DF$
250 PRINT
260 INPUT "NEXT FILE: ", EF$
265 H=0: N=0
270 OPEN " B: " + EF$ FOR INPUT AS 1
280 IF EOF(1) THEN 320
290 H=H+1
300 INPUT #1, XX(H), YY(H)
310 GOTO 280

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320 CLOSE #1
330 OPEN "B:" + DF$ FOR APPEND AS 1
340 FOR N=1 TO H
350 WRITE #1,XX(N),YY(N)
360 NEXT N
370 CLOSE #1: KILL "B:" + EF$
380 PRINT
390 INPUT "COMBINE ANOTHER?",A$
400 IF A$="Y" THEN 250
410 IF A$="N" THEN 420
420 INPUT "NAME OF THE COMBINED FILE ":,D$
430 NAME "B:" + DF$ AS "B:" + D$
440 INPUT "COMBINE OTHER FILES?",B$
450 IF B$="Y" THEN 210
460 IF B$="N" THEN 30
470 REM
480 CLS: PRINT TAB(10); "SORTING DATA"
490 REM
500 PRINT: PRINT
510 INPUT "NAME OF FILE?",DF$
514 PRINT: PRINT: PRINT: PRINT
520 N=0
530 OPEN "B:" + DF$ FOR INPUT AS 1
540 IF EOF(1) THEN 580
550 N=N+1
560 INPUT #1,XX(N),YY(N)
570 GOTO 540
580 CLOSE #1
590 KM=N: KT=N
600 KM=INT(KM/2)
610 IF KM=0 THEN 720
620 KK=KT-KM: KJ=1
630 KI=KJ
640 KL=KI+KM
650 IF XX(KI)<XX(KL) THEN 690
660 T=XX(KI): XX(KI)=XX(KL): XX(KL)=T: T=YY(KI): YY(KI)=YY-
   (KL): YY(KL)=T
670 KI=KI-KM
680 IF KI=1 THEN 640
690 KJ=KJ+1
700 IF KJ>KK THEN 600
710 GOTO 630
720 OPEN "B:" + DF$ FOR OUTPUT AS 1
730 FOR K=1 TO N
740 WRITE #1,XX(K),YY(K)
750 NEXT K
760 CLOSE #1
764 PRINT: PRINT
766 PRINT "NUMBER OF X,Y PAIRS="N
PRINT: PRINT
INPUT "SORT ANOTHER FILE?", CS
IF CS="Y" THEN 480
IF CS="N" THEN 30
REM
CLS: PRINT TAB(10); "AVERAGING AND SMOOTHING DATA"
REM
PRINT: PRINT
INPUT "NAME OF FILE:", DF$
PRINT: PRINT
INPUT "# OF VALUES TO BE AVERAGED:", A
INPUT "# OF VALUES TO BE SMOOTHED:", S
IF A=0 THEN 1120
N=N+1
OPEN "B:" + DF$ FOR INPUT AS 1
IF EOF(1) THEN 940
N=N+1
INPUT #1, XX(N), YY(N)
GOTO 900
CLOSE #1
Z=INT(N/A)
FOR H=1 TO Z
M=0: Q=0
FOR J=1 TO A
K=XX(((H-1)*A)+J): P=YY(((H-1)*A)+J)
M=K+M: Q=P+Q
NEXT J
XX(H)=M/A: YY(H)=Q/A
NEXT H
KILL "B:" + DF$
OPEN "B:" + DF$ FOR OUTPUT AS 1
FOR H=1 TO Z
WRITE #1, XX(H), YY(H)
NEXT H
CLOSE #1
PRINT: PRINT
IF S=0 THEN 1340
N=N+1
OPEN "B:" + DF$ FOR INPUT AS 1
IF EOF(1) THEN 1190
N=N+1
INPUT #1, XX(N), YY(N)
GOTO 1150
CLOSE #1
FOR H=1 TO (N+1-S)
M=0: Q=0
FOR J=1 TO S
K=XX(H+J-1): P=YY(H+J-1)
M=K+M: Q=P+Q
NEXT J

1260 XX(H)=M/S: YY(H)=Q/S
1270 NEXT H
1280 KILL "B:" + DF$
1290 OPEN "B:" + DF$ FOR OUTPUT AS 1
1300 FOR H=1 TO (N+1-S)
1310 WRITE #1,XX(H),YY(H)
1320 NEXT H
1330 CLOSE #1: PRINT: PRINT
1340 INPUT "AVERAGE OR SMOOTH ANOTHER FILE?",E$
1350 IF E$="Y" THEN 810
1360 IF E$="N" THEN 30
1370 REM
1380 CLEAR: PRINT TAB(10); "DATA FORMATTING"
1390 REM
1400 PRINT: PRINT
1401 INPUT "(G)ROWTH RATE, (C)URVATURE OR (R)ADII DATA?", RES$
1402 IF RES$="C" THEN 1403 ELSE 1406
1403 INPUT "(D)EGREE OR (R)ADIUS OF CURVATURE DATA?", AES$
1404 RES$=AES$ + "C"
1406 PRINT: PRINT
1410 INPUT "TITLE OF CHART: ", TITLE$
1420 PRINT: PRINT
1430 INPUT "NUMBER OF GRAPHS? (<=6)",NG%
1440 FOR J%=1 TO NG%
1450 PRINT: PRINT
1460 PRINT "LINE #" J%
1470 INPUT "NAME OF DATA FILE: ", DF$
1480 INPUT "SUBTITLE OF LINE: ", SUB$(J%) 
1490 INPUT "ROW LOCATION (3-23) ", SSR%(J%) 
1500 INPUT "COLUMN LOCATION (12-75) ", SSC%(J%) 
1510 I%=0
1520 OPEN "B:" + DF$ FOR INPUT AS 1
1530 IF EOF(1) THEN 1570
1540 I%=I%+1
1550 INPUT #1,X(J%,I%),Y(J%,I%)
1560 GOTO 1530
1570 CLOSE #1
1580 NIX%(J%)=I% ' NUMBER OF ITEMS IN GRAPH (<=200)
1590 OK(J%)=0 ' PIE SLICE OFFSET FLAG
1600 MN(J%)=0 ' Y AXIS MINIMUM VALUE
1610 MX(J%)=50 ' Y AXIS MAXIMUM VALUE
1620 ST(J%)=5 ' Y AXIS STEP VALUE
1624 IF RES$="DC" THEN 1627 ELSE 1625
1625 IF RES$="RC" THEN 1628 ELSE 1626
1626 IF RES$="R" THEN 1629 ELSE 1630
1627 GOSUB 2000: GOTO 1630
1628 GOSUB 3000: GOTO 1630
1629 GOSUB 4000

1630 NEXT J%
1640 VAXL$=" RELATIVE GROWTH RATE (%/HR)"
1650 HAXL$="DISTANCE FROM TIP (MM)"
1660 TYP$="M" ' DETERMINES TYPE OF LINE CHART
1670 MS%=0 ' MULTIPLE SCALES FLAG
1680 XMN=0 ' X AXIS MINIMUM VALUE
1690 XMX=10 ' X AXIS MAXIMUM VALUE
1700 XST=1 ' X AXIS STEP VALUE
1704 IF RES$="DC" THEN 1707 ELSE 1705
1705 IF RES$="RC" THEN 1708 ELSE 1706
1706 IF RES$="R" THEN 1709 ELSE 1710
1707 GOSUB 2020: GOTO 1710
1708 GOSUB 3020: GOTO 1710
1709 GOSUB 4020
1710 PAT%=0: COL%=1
1714 PRINT: PRINT
1716 INPUT "NAME OF FORMATTED FILE: ", FF$
1720 OPEN "B:" + FF$ + ".ENG" FOR OUTPUT AS 2
1730 PRINT #2,TITLE$
1740 PRINT #2,"LINE"
1750 PRINT #2,VAXL$
1760 PRINT #2,HAXL$
1770 WRITE #2,2,NG%
1780 PRINT #2,TYP$
1790 WRITE #2,MS%,1
1800 WRITE #2,XMN,XMX,XST
1810 MNIT%=9999
1820 FOR J%=1 TO NG%
1830 DOTS(J%)=16*PAT% + COL%
1840 IF MNIT% > NIT%(J%) THEN MNIT%=NIT%(J%)
1850 WRITE #2,NIT%(J%),DOT%(J%),0%(J%),SSR%(J%),SSC%(J%)
1860 PRINT #2,SUB$(J%)
1870 WRITE #2,MN(J%),MX(J%),ST(J%)
1880 FOR I%=1 TO NIT%(J%): WRITE #2,X(J%,I%),Y(J%,I%): NEXT
1890 NEXT
1900 FOR I%=1 TO MNIT%: PRINT #2,"": NEXT
1910 FOR I%=1 TO 10: WRITE #2,0: NEXT
1920 CLOSE
1930 INPUT "FORMAT ANOTHER FILE?",PF$
1940 IF PF$="Y" THEN 1380
1950 IF PF$="N" THEN 30
1960 GOTO 4050
2000 MX(J%)=100: ST(J%)=10
2010 RETURN
2020 VAXL$=" CURVATURE (DEGREES)"
2030 HAXL$=" TIME (MIN)"
2040 XMX=120: XST=30
2050 RETURN
3000 MX(J%)=16: ST(J%)=2
3010 RETURN
3020 VAXLS=" RADIUS OF CURVATURE (MM)"
3024 HAXLS=" TIME (MIN)"
3026 XMX=120: XST=30
3030 RETURN
4000 MX(J%)=40: ST(J%)=5
4010 RETURN
4020 VAXLS=" RADIUS OF CURVATURE (MM)"
4030 XMX=8: XST=1
4040 RETURN
4050 END
APPENDIX J: Listing of the Computer Program, RADII, Used to Calculate Point Radii of Curvature with the IBM System

The program was written in IBM Basic on an IBM Personal Computer. It allows the determination of radii of curvature at points along the plant surface, at all measurement times during the course of gravitropic response.

```
10 REM *** RADII:860214 ***
20 DIM LX(2900),LY(2900),E(2900),R(2700),DD(2700)
25 CLS:H=0; J=0; LS=0; DD=0
30 PRINT TAB(8); "CALCULATION OF RADII OF CURVATURE"
40 PRINT: PRINT
50 INPUT "NAME OF DATA FILE:", DF$
54 INPUT "NUMBER OF LINES IN PLOT: ", NL
56 INPUT "CALCULATION INTERVAL:", XX
60 OPEN "B:" + DF$ FOR INPUT AS 1
70 INPUT #1, NT, IT, DT 'TARGETS, INTERVALS, DISTANCE FROM TIP (MM)
80 INPUT #1, CFV, CFV, CFV 'CONVERSION FACTOR (MM/CU)
90 CFH=1.22*CFV
100 IF EOF(1) THEN 140
110 H=H+1
120 INPUT #1, LX(H), LY(H), E(H) 'INPUT X,Y,TIME
130 GOTO 100
140 PRINT H "DATA POINTS": CLOSE #1
150 FOR J=XX TO IT STEP XX
160 DD=DT
170 FOR H=((J-l)*NT)+2 TO (J*NT)-1
180 IF LX(H+1)=LX(H) THEN LX(H+1)=LX(H+1)+.001
190 IF LX(H)=LX(H-1) THEN LX(H-1)=LX(H-1)-.001
200 SLA=(LY(H)-LY(H+1))/(LX(H)-LX(H+1))
210 SLB=(LY(H-1)-LY(H))/(LX(H-1)-LX(H))
220 IF SLA<1E-30 THEN 224 ELSE 226
224 SLA=+1E+30: GOTO 230
226 SLA=-1/SLA
230 IF SLB<1E-30 THEN 234 ELSE 236
234 SLB=-1E+30: R(H)=+1E+30: GOTO 304
236 SLB=-1/SLB
250 XA=(LX(H+1)+LX(H))/2: XB=(LX(H)+LX(H-1))/2
260 YA=(LY(H+1)+LY(H))/2: YB=(LY(H)+LY(H-1))/2
270 BA=YA-(SLA*XA): BB=YB-(SLB*XB)
```
275 IF SLA=SLB THEN SLA=SLA+.001
280 X=(BB-BA)/(SLA-SLB)
290 Y=(SLB*X)+BB
296 RA=SQR(((YA-Y)*CFV)^2)+(((XA-X)*CFH)^2))
298 RB=SQR(((YB-Y)*CFV)^2)+(((XB-X)*CFH)^2))
300 R(H)=(RA+RB)/2 'RADIUS OF CURVATURE
304 IF R(H)>100 THEN 306 ELSE 310
306 R(H)=100
310 LS=SQR(((LY(H-1)-LY(H))*CFV)^2+(((LX(H-1)-LX(H))*CFH)^2))
320 DD(H)=LS+DD 'DISTANCE FROM TIP
330 DD=LS+DD
340 NEXT H
350 NEXT J
360 FOR N=1 TO NL
365 PRINT "FILE" N: INPUT "NAME OF FILE:" , AF$ FOR OUTPUT AS 1
370 OPEN "B:" + AF$ FOR OUTPUT AS 1
375 FOR J=(N-1)*(IT/NL)+XX TO N*(IT/NL) STEP XX
380 FOR H=((J-l)*NT)+2 TO (J*NT)-1
390 WRITE #1,DD(H),R(H)
400 NEXT H
410 NEXT J
420 CLOSE #1
425 NEXT N
430 CLS: PRINT DF$ " DONE": PRINT PRINT
440 INPUT "CALCULATE MORE RADII OF CURVATURE?", CNS$
450 IF CNS$="Y" THEN 25
460 IF CNS$="N" THEN 500
500 END