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Biophysical and Genetic Aspects of Light-Potentiated Gravitropic Curvature in the Maize Primary Root

D.M. Fantin
(Ph.D. Thesis)

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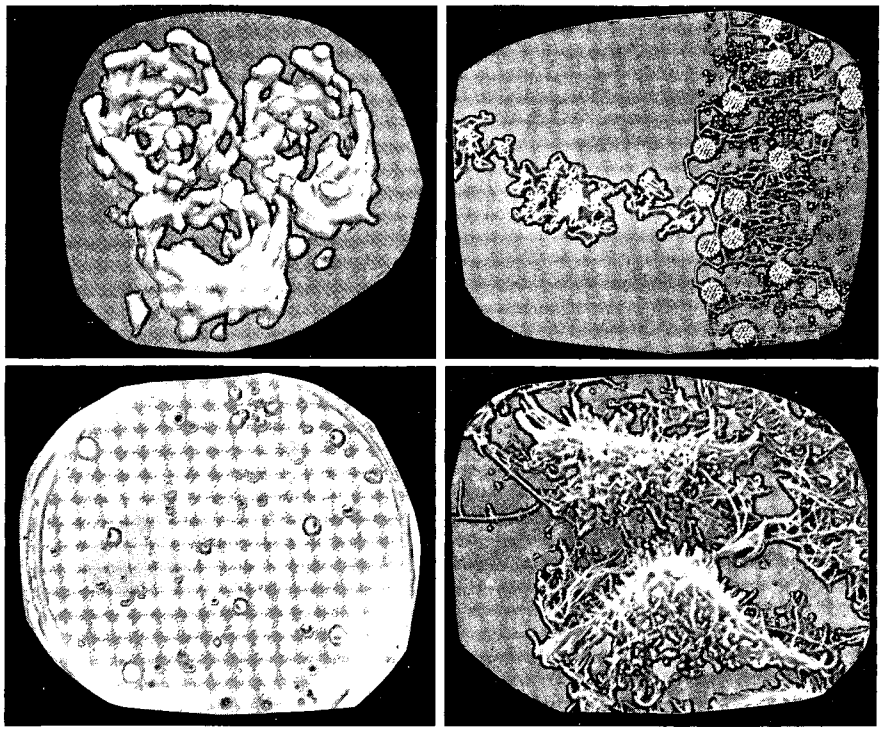

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BIOPHYSICAL AND GENETIC ASPECTS OF LIGHT-POTENTIATED GRAVITROPIC CURVATURE IN THE MAIZE PRIMARY ROOT

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Ph.D. Thesis

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To Emily and Willy:

Whose sense of wonder about the nature of their world

becomes the source of scientific curiosity in the adult.

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BIOPHYSICAL AND GENETIC ASPECTS OF LIGHT-POTENTIATED GRAVITROPIC CURVATURE IN THE MAIZE PRIMARY ROOT

by

Dennis M. Fantin

ABSTRACT

The thesis explores a number of issues related to the subject of light-induced gravitropic curvature in maize primary roots. Topics investigated include: 1. The role of the cap as the receiver of light in gravitropism; 2. The entrainment by light of nutational curvature in the gravistimulated root; and 3. A possible role for genetics in the bending response variation seen among individual gravistimulated roots.

The thesis is organized around a mathematical model which links the stages of light-stimulated gravitropic curvature in the primary root. The theory emphasizes the physical forces and molecular flows assumed to be involved in this process. The model traces the movement of a putative growth inhibitor. The inhibitor is: A. produced at the root cap under the influence of light and a sinking geosensor; B. transported by diffusion along the bottom margin of the root to the extension zone where it retards cell elongation; and C. transported by convection in a circumferential pattern around the extension zone where it retards growth at every point along its path at a rate equivalent to its concentration. Model-generated curves are presented with experimental data as one measure of the theory's efficacy.

The root cap was shown to be the site of the photo-active trigger which stimulates positive gravitropic curvature beyond the dark control level. This result was established by probing cv. Merit roots at various sites along their length with focused light from a helium neon laser.

In the presence of light, gravistimulated cv. Merit roots show a negative curvature phase after the initial descent. In relation to this finding we discovered that it is possible to stimulate secondary and tertiary cycles of root curvature by pulsing incandescent light every two to four hours. When the interval between light pulses is more frequent than two hours, however, secondary and tertiary cycles of curvature are suppressed.

We determined that there is no simple one- or two-gene expression system governing positive graviresponsiveness in the presence of light in cv. Merit or cv. Pioneer maize primary roots. This conclusion was reached after examining the results of selected genetic crosses.

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CHAPTER 1

GENERAL LITERATURE REVIEW

Part One: Gravitropism

1.1: Introduction

Gravitropism (formerly, geotropism) is the means by which plants perceive and respond to gravity. This subject has drawn considerable attention from botanists and biophysicists in recent years and has been reviewed many times in the scientific literature since 1900. I have relied upon several of the more recent reviews for guidance in selecting relevant topics for examination. In particular, Juniper (1976), Audus (1979), Jackson and Barlow (1981), Feldman (1984), Wilkins (1984), Pickard (1985) and Moore and Evans (1986) all proved helpful to me in preparing the current chapter.

1.2: Graviperception Mechanisms

1.2.1: The Starch Statolyth Hypothesis

In 1900 Haberlandt and Nemec published papers independently demonstrating the existence of sedimentable starch granules in the cells of gravitropically sensitive plant organs (Haberlandt, 1900; Nemec, 1900).[1] These investigators theorized that, when the plant organ is inclined from its normal orientation with respect to the plumb line, the movement of starch granules toward the lower margin of cells acts as a trigger for gravitropically-induced curvature. Nemec showed that in roots the starch grains, which he believed served as statoliths, were localized in the central core of the root cap in a layer of cells known as the columella. Haberlandt

[1]According to Audus (1979) ideas about gravity perception in plants emerged directly from the suggestion of Noll (1892) that plant cells might contain microscopic gravity-sensing particles analogous to the statoliths in the autosomes of animals.

showed that in shoots the starch grains were located all along the shoot "most frequently in the cells that surround the vascular bundle, ... in specific cells located close to vascular bundles or in a specific layer of cells within the stems such as the inner cortical layer or endodermis". Hawker (1933), working with stems of *Lathyrus odoratus* (sweet pea) supported the starch statolith hypothesis by correlating the presentation time with temperature and rate of fall of starch grains within cells of the coleoptiles. Presentation time is defined as the minimum time of gravitropic stimulation required to produce a just detectible curvature. From 10°C-30°C the presentation time decreased as did the time required for starch grains to move across a cell in a gravitropically-stimulated coleoptile. From 30°C-40°C the presentation time increased along with the sedimentation rate of the putative statoliths. Hawker concluded that the mobility of starch grains depended on the viscosity of the cell cytoplasm which, she speculated, decreased in the range from 10°C-30°C and increased from 30°C-40°C. These findings lent correlational weight to the starch statolith hypothesis.

According to Wilkins (1984) it is now clear that each so-called statolith is not simply a naked starch grain but rather a group of starch grains enclosed within a membrane. The complex is known as an amyloplast. There may be from one to eight starch grains in each amyloplast and perhaps from four to twelve amyloplasts in each statocyte.

There is some evidence that amyloplasts are metabolically active. Northcote and Pickett-Heaps (1966) demonstrated that amyloplasts are characterized by very rapid turnover of monomers. Hinchman and Gordon (1964) reported that horizontal *Avena* coleoptiles, rotated along their own axis, produce starch grains larger than those contained in vertical controls. This result suggests that amyloplast turnover relates to the position of the amyloplasts within gravity perceptive cells.

The starch statolith hypothesis was called into question by Pickard and Thimann (1966)

who incubated young wheat coleoptiles in gibberellic acid (GA) and kinetin. The result was the complete hydrolysis of statolith starch. Even so, the gravitropic responsiveness of the coleoptiles was retained, albeit to a lesser degree than in controls.

Iversen (1969) showed that incubation of *Lepidium sativum* (cress) in a high concentration of 6-furfuryl-aminopurine (kinetin) and GA_3 completely removed starch grains from the tip regions of roots and coleoptiles. After this treatment the gravitropic sensitivity of roots was completely obliterated. Following this treatment, roots were shown to grow straight, irrespective of gravitropic stimulation when allowed to grow in light and water. Graviresponsiveness returned in twenty-four hours simultaneously with the reformation of starch grains. Iversen (1974) confirmed these results in the roots of red and white clover and in wheat (*Triticum durum*). He attempted to perform the same experiment with wheat coleoptiles as Pickard and Thimann (1966) and found traces of statolith starch. When Iversen changed the temperature of the experiment from 30° to 34°C however, he reported the abolition of statolith starch. At the same time he noted the elimination of geotropic activity. According to Audus (1979), confidence in the starch statolith theory was thus revived.

Another line of evidence pointing to the importance of amyloplasts in graviperception is the often-confirmed result that removal of the root cap or root tip eliminates the root's ability to respond to a gravitational stimulus (Darwin, 1880; Juniper et. al., 1966; Gibbons and Wilkins, 1970). The additional fact that gravitropic sensitivity returns in decapped roots some hours prior to the reformation of the root cap was explained in light of the starch statolith theory with reports from Grundwag and Barlow (1973), Barlow (1974) and Barlow and Grundwag (1974). These studies showed that, immediately after cap removal starch grains begin forming from proplastids

in cells of the quiescent center of the root apex.[2] Twenty-four hours later the starch grains in the quiescent center have grown in size and the roots have recovered gravitropic responsiveness but there is still no evidence of cap reformation (Figure 1.1 illustrates the relationship between various cell layers in the distil 0.16 cm of the root tip). Hillman and Wilkins (1982) extended this result by showing that, in *Zea mays*, decapped roots recover graviresponsiveness quite suddenly between twelve and twenty-four hours after decapping. Microscopic studies of those roots which regained gravitropic responsiveness showed that at least some cells in the root apex contain amyloplasts which have sedimented to the lower side of the horizontally-placed root. By contrast, even four hours of centrifugation at 25 g did not cause sedimentation of amyloplasts in apical root cells ten hours after decapping. Hillman and Wilkins conclude that there is a physical change in the state of the cytoplasm which occurs at the onset of gravitropic responsiveness. This allows sedimentation of a sufficient number of amyloplasts to begin the chain of events leading to reorientation of the root tip in the positive gravitropic direction. This result suggests that root graviresponsiveness is important enough to the plant to allow the development of a secondary system for graviperception in advance of the reformation of the root cap.[3] Whether the return

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[2]The quiescent center is composed of immature xylem and cortex cells of the apical meristem found immediately adjacent to the root cap.

[3]In reality, the entire body of evidence in support of the starch statolith theory is correlational. Amyloplasts may indeed be involved in graviperception but until a more defined and detailed mechanism for perception is elaborated, researchers (in my view) would be wise to resist defining the primary role of amyloplasts with such zeal, for example, as that demonstrated by Audus (1979). It is my impression that part of the reason for this much too-firm conviction is the very longevity of the theory. One wonders why such a vehement stance is taken in defense of a theory which is corelational and therefore unprovable.

Wilkins takes a more balanced view pointing out some of the weaknesses in the starch statolith theory but even he is tempted to reach somewhat far in support of the theory. For example, the finding of Hillman and Wilkins (1982) that a <u>few</u> apical cells contain <u>some</u> sedimentable amyloplasts at the time when decapped roots have regained gravitropic responsiveness and to suggest that this result is important evidence in support of the theory is, I think, stretching a point.

of graviperception really involves the sedimentation of amyloplasts in the cells of the apex rather than some other mechanism is not established unequivically.

Such results, for example, do not preclude the possibility that graviresponsiveness is stimulated by the movement of an organelle or substance other than the amyloplast. The evidence that sedimentable starch grains develop in the root apex after detachment of the cap at a time which is coincident with the onset of graviresponsiveness, and before the reformation of the cap, is a correlational rather than a causal result.

There is also some evidence drawn from the genetics literature in support of the starch statolith theory. Hertel, de la Fuente and Leopold (1969) working with coleoptiles of a maize mutant (amylomaize) whose amyloplasts were reduced in size from those of the wild type, found that the amyloplasts of the mutant sedimented to a lesser degree. The mutant also showed reduced lateral flow of indole-3-acetic acid (IAA) while also showing diminished graviresponsiveness.

Miles (1981) demonstrated with a non-photosynthetic mutant of *Zea mays* (hcf-3) that graviresponsiveness was lost after the endosperm starch reserves were exhausted. When the leaf-sheath cells were provided with sucrose the amyloplasts reappeared and the shoots regained gravitropic responsiveness.

1.2.2: Evidence in Opposition to the Starch Statolyth Hypothesis

There is a growing body of evidence which challenges the role of amyloplasts in graviperception. There are several examples of plants (*Arabidopsis*, *Zea mays*, *Isoetes* and *Selaginella*) with graviresponsive roots whose statocytes have greatly reduced amyloplasts (Peterson, Scott and Kott, 1979; Grenville and Peterson, 1981; Moore and McClelen, 1985; Caspar, Somerville and Pickard, 1985).

The mere sedimentation of amyloplasts in columella cells does not necessarily insure that

a root is graviresponsive (Ransom and Moore, 1983; Olsen and Iversen, 1980; McNitt and Shen-Miller, 1978; Moore, 1985). For examle, McNitt and Shen-Miller (1978) showed that amyloplasts sediment to the bottom of columella cells of gravistimulated roots of *Zea mays L.* (Wisconsin hybrid 64Ax22R). The sedimentation occured in both light-exposed (661 nm, 60-seconds exposure) and dark-treated roots irrespective of whether the roots curved under the influence of gravity.

Wilkins (1984) suggests that the discovery of at least one growth regulating substance produced in root caps leads to the possibility that one of these substances and not the starch grains actually triggers gravitropic stimulation. He suggests that an experiment should be conducted in which roots are first destarched and then half decapped. If curvature develops toward the remaining half cap then the role of starch grains in the stimulation of gravicurvature would be undercut.

1.2.3: Amyloplasts as Geosensors in Combination with Other Organelles

There is also evidence supporting the theory that amyloplasts act as geosensors through contact with other organelles rather than by simple sedimentation onto the plasmalemma. Perbal (1978) presents electron micrographic studies showing that the amyloplasts in *Lens culinaris* roots do not actually sediment to make contact with the lateral plasmalemma. He showed that after forty-two hours geostimulation, amyloplasts had sedimented to a point between 0.135 and 0.26 μm from the lower plasmalemma while some had already sedimented to this point after only twenty minutes. As a result, Perbal concludes that perhaps the geostimulation consists in the amyloplasts making contact with peripheral ER or microtubules. Juniper (1976) supports Perbal's observations: "...there is no evidence that the amyloplasts of any statocyte cells ever come in contact with the plasmalemma at any time. The observations of all those workers who have

studied statocyte cells under the EM are in agreement that the amyloplasts, regardless of their position in the cell, the degree of stimulus applied to that statocyte, or the species in which they are studied, lie at distances from 0.5 μm to several microns from the plasmalemma..." (Juniper, 1976, p. 394).

Juniper (1976) has advanced a theory of graviperception involving interaction between amyloplasts and plasmodesmata. The theory links the concurrent minimal displacements of the statoliths and the very short presentation times observed in several species, e.g., ten seconds in *Lipidium* (Sievers and Volkmann, 1979). Juniper's model takes into account the attachment between plasmodesmotubules and the membranes of the rough ER. The ER appears capable of movement under gravitational stimulation in spite of being attached to the plasmodesmotubules on either side of the cell wall.

In the normal vertical position the amyloplasts rest on a "heap" of ER on the distal transverse walls of the statocytes. In this position, according to Juniper's theory, pressure of the amyloplasts on the ER causes closure of the plasmodesmata on the transverse walls while the plasmodesmata remain open on the longitudinal walls. When the root is oriented horizontally, the "lose arms" of the ER move under the pressure of the amyloplasts to become resituated onto the plasmodesmotubules on the lower longitudinal cell walls. Pressure of the amyloplast-ER complex now cause a successive closing of the longitudinal plasmodesmata and the opening of the lateral plasmodesmata (Figures 1.2-1.3).

Larsen (1969) illustrates one difficulty with Juniper's model: namely, that the maximal gravitropic curvature in roots is stimulated not by horizontal exposure but rather by inclination of the root 45° above the horizontal. This fact is hard to reconcile with Juniper's model. It is even more perplexing to attempt to accommodate Juniper's theory with the strongly supported supposition that root geocurvature is stimulated by hormone flow from the cap to the extension

zone through longitudinal channels in the under side of the root. This does not seem to be accounted for in Juniper's model.

The theory of graviperception advanced by Sievers and Volkmann (1972, 1977) and Sievers and Schmitz (1975) also relates graviperception in roots to the sedimentation of starch statoliths. Working with *Lepidium sativum L.*, Sievers, et. al., note the following molecular and anatomical facts. Three distinct parabolic cell layers are visible in the root tip. These consist of an outer secretory layer of cells whose purpose is the production of polysaccharide slime which aids the movement of the root through the soil. An apical band of cells lies inside the secretory layer. Finally, in the central core of the tip are statolith cells whose primary function is graviperception.

There is a polar arrangement of organelles within the statocytes with the nucleus at the proximal end and the rough ER at the distal end. The outer statocyte cells are symmetrically oriented at an angle of 55° from the root axis such that the distal ends of these cells are bowed outward from the main root axis. In the normal tip-down root orientation, the starch statoliths rest on the ER complex causing equal pressure from one side of the central root axis to the other. The result is straight orthogravitropic movement. When the root is inclined 90° from the vertical the ER complex remains at the distal margin of the statocytes but the starch statoliths now become redistributed in the direction of gravity such that an unequal stress is put on the ER in the cells above and below the root axis. This differential pressure, relatively greater in the cells below the root axis, leads, according to Sievers, to asymmetrical hormone distribution and eventually to reorientation of the root in the positive gravitropic direction (Figure 1.4). The short, ten second presentation time discovered in *Lepidium* roots is consonant with this theory of geoperception because the amyloplasts need to move very little to cause a difference in pressure on the two sides of the root axis. Schneider and Sievers (1981) have proposed that communication between the

sedimented amyloplasts and the ER might involve glycoprotein surface receptors on the ER complex.

Two factors make it difficult to generalize Sievers' model to other plant species. First, as noted above, sedimented amyloplasts do not typically contact the ER (Perbal, 1978; Moore and McClelen, 1983; Barlow, et. al., 1984). Secondly, columella cells of most roots examined do not contain a distal ER complex (Juniper, 1976; Moore and Pasieniuk, 1984).

In discussing the required size of subcellular particles necessary to sediment sufficiently to be likely candidates as plant geosensors, Audus (1962) noted that according to Stokes' law organelles the size of mitochondria could sediment to distances close to their own diameter during reported short presentation times. Gordon (1963) agreed it was unlikely that particles smaller than mitochondria could serve as geosensors.

The density of subcellular particles is an important quantity because sedimentation can only occur when the density of the particle under consideration is greater than that of the surrounding cytoplasm. Bouck (1963a, 1963b) ranked organelles of pea root tip in descending order of density after centrifugation of the intact roots as follows: amyloplast, nucleus, rough ER, mitochondria, dictyosomes, vacuole and lipid droplets. After centrifugation the dictyosomes remain midway in the cell and thus appeared to have a density approximating that of the surrounding cytoplasm.[4]

Another class of theories for geoperception relates to direct metabolic or other changes in the amyloplast. Barlow (1974) suggests that amyloplasts produce a hormone inhibitor after sedimenting onto the ER. The inhibitor is thought to be responsible for differential growth above

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[4] The trigger for this process might, for example, involve the buoyant movement of a particle such as the vacuole towards the upper margin of graviperceptive cells. This possibility seems to have received little attention and yet it remains a viable, alternative first step in the process of graviperception.

and below the central root axis. The plasmodesmotubules adjacent to the ER then provide channels for hormone flow.

Milborrow (1974) shows evidence that chloroplasts, which are related to amyloplasts, can synthesize abscisic acid (ABA). This hormone (see below) may be involved in the gravitropic reaction mechanism by effecting growth in the extension zone of the root.

Aasheim and Iversen (1972) and Iversen (1974) claim to have located indole-3-acetic acid oxidase in the amyloplast membranes of root tips of cress, cabbage and maize. Audus (1979) thinks this is unlikely because, as he notes, gradients within individual cells do not integrate to give a gradient of growth over a series of cells and this would be required in the case of root curvature. This would become a more plausible explanation if, for example, such a theory were proposed in conjunction with coordinated hormone movement between cells. To wit, Hager (1967) proposed that amyloplasts might carry with them RNA templates to the sides of cells for the purpose of synthesizing permeases which would in turn promote the downward movement of hormone.

Wilkins (1978) proposed that if amyloplasts were electrically charged their sedimentation could induce an electronic polarity which might effect permeability and transport properties of the near-by plasmalemma. Sack, Priestley and Leopold (1983) discovered that intact amyloplasts have a negative potential of approximately 19.4 millivolts. This result should prompt further exploration of Wilkins' hypothesis.

1.2.4: A Biophysical Approach to Geosensitivity

Tobias, Risius and Yang (1973) have approached the matter of sedimentation or floatation of possible graviceptors from a fundamental, physical point of view. The gravitational force exerted on a spherical particle of given density and radius which is suspended in an isotropic,

liquid medium of known density is:

$$F_g=\frac{4\pi}{3}r^3(\rho-\rho_s)g,$$

where r is the radius of the particle, ρ the density of the particle, ρ_s the density of the supporting medium and g the gravitational acceleration. When the particle is moving with average velocity v the Stokes' shearing force F_{sh}, that serves to retard the free fall of the particle, is:

$$F_{sh}=6\pi\eta rv,$$

where η is the viscosity of the suspending medium. The two forces can be equated to give the steady-state sedimentation velocity:

$$v=\frac{2}{9}\frac{r^2(\rho-\rho_s)}{\eta}g.$$

Though the mean thermal velocity, v_{th}, is much greater than the steady state sedimentation velocity, the sedimentation effect is dominant over time due to its directional property:

$$v_{th}=(\frac{9}{4\pi}\frac{kT}{\rho r^3})^{1/2}.$$

In a steady state situation when sedimentation and thermal effects balance, the concentration of uncharged graviceptors as a function of height in the cell is given by the barometric altitude formula:

$$c(h)=c(0)\exp(\frac{-h}{H}),$$

where:

$$H=\frac{3kT}{4\pi r^3(\rho-\rho_s)g}.$$

Tobias hypothesizes a linear relationship between graviceptor concentration and growth rate. He assumes that a 1% concentration gradient of uncharged graviceptors can be produced across a 20 μm cell with $\rho=2\rho_s$. He derives a minimum graviceptor radius of 0.4 μm at a field strength of $0.01g$, the minimum field strength needed to produce geotropic effects. Tobias also investigates the sedimentation of charged graviceptors in an acqueous medium. For example, he shows that particles of 1 μm diameter may develop a 10 millivolt potential at 1g across a distance of 100 μm (the diameter of the growth zone of the sporangiophore of Phycomyces blakesleeanus). This result is derived from the equation relating the electric and gravitational fields:

$$E=\frac{\zeta\varepsilon(\rho-\rho_s)g}{3\pi\eta\kappa},$$

where ζ is the potential of the Gouy-Chapman boundary layer. ζ depends on the net electric charge of the sedimenting particle and on the concentrations of the various ionic species contained in the cytoplasm.

1.3: Gravity Response Mechanisms

1.3.1: Gravireaction Mechanisms in the Shoots of Monocotyledonous Plants

Cholodny and Went proposed independently in 1926 that a growth promoting substance was produced in the tips of vertically growing shoots which moved symmetrically down the shoot to the sub-apical elongation zone. When the shoot was turned horizontally the growth promoting substance moved both laterally and basipetally to accumulate preferentially in the cells of the underside of the growth zone. In theory, the total extent of growth was fixed by the concentration of growth promoting substance and would not change in the event of horizontal exposure. The

growth promoting substance would instead become partitioned based on concentration levels between the upper and lower half of the inclined shoot.

Boysen-Jensen (1936) and Went and Thimann (1937) supported the Cholodny-Went hypothesis when they discovered that the tip of the coleoptile was the source of a growth promoting substance, auxin. Later, Greenwood, Shaw, Hillman, Richie and Wilkins (1972) used mass spectroscopy to establish unequivocally the presence of auxin (IAA) in coleoptiles of *Zea mays*.

Goldsmith and Wilkins (1964) showed that IAA moves preferentially in a downward lateral direction in horizontal maize coleoptiles. They used agar blocks supplied with radioactive IAA applied to only one side of the tips of maize coleoptiles. The coleoptiles were oriented either vertically or horizontally with a receiver agar block placed laterally across half of the cut basel end. The striking result was that twice as much IAA accumulated in the receiver blocks with the coleoptile horizontal, the donor block on the upper side of the coleoptile tip and the receiver block located on the lower lateral side of the basel cut end as compared to the IAA level found with the coleoptile oriented vertically downward, the donor on one side of the coleoptile tip and the receiver on the opposite side of the basel end.

Shaw, Gardner and Wilkins (1973) used a micro-application technique to introduce tritiated IAA into the apices of intact maize coleoptiles. No damage appeared to result from the small point of entrance. Tracking the radioactive hormone as a result of time and gravitropic orientation showed that IAA became redistributed in a lateral downward direction in the horizontally-placed shoot. Opposition to the Cholodny-Went hypothesis has been provided by Digby and Firn (1979) and Hall, Digby and Firn (1980). They observed that the upper halves of etiolated, horizontal maize coleoptiles cease growing completely almost immediately while the lower sides almost double their growth rate. These results differ from the smaller growth rate

changes noted by Weber (1931) and Navez and Robinson (1933) and according to Wilkins "is not what would be expected on the basis of the Cholodny-Went hypothesis unless there was a really massive movement of auxin from the upper to the lower half of the organ". (Wilkins, 1984, p. 173) Hall, et. al., (1980) suggests that the magnitude of the growth differential is too great to be explained by the changes in auxin concentration alone in light of the established fact that the growth rate of maize stems is dependent on the log of the external auxin concentration.

Wilkins (1984) points out that without knowing the absolute auxin concentration in the cells of the growing zone (as opposed to the external auxin concentration) it is impossible to truly assess the contribution of auxin in determining growth rate of the upper and lower halves of horizontal stems. Because there is no current method for determining internal auxin concentration, the criticism of Hall, et. al., (1980) is impossible to assess. "...[T]he concentration of the compound in the cell or organ cannot be measured. Only the amount is determined and this cannot be converted to a concentration unless its distribution throughout the volume of the cell or organ is known precisely." (Wilkins, 1984, p. 173).

1.3.2: Gravireaction Mechanisms in Dicotlyledonous Shoots

Gillespie and Thimann (1963), used externally applied C14 IAA, to demonstrate the redistribution of auxin in a downward lateral direction in horizontal hypocotyl segments of *Helianthus annuus*.

Phillips (1972) reported in horizontal *Helianthus* hypocotyl apical segments that a substantial difference in gibberellin concentrations (as great as ten to one in favor of the lower half of the segment) could be observed using receiver blocks at the cut basal ends of the segments. Concentrations were measured after twenty hours in the horizontal position, whereas the geotropic response can be detected after one hour. Still, this points to the possible contribution of other

hormones in the regulation of the growth asymmetry in shoots as a result of gravitropic stimulation.

Bode (1959, 1960) examined the ash of horizontal *Helianthus* hypocotyls and discovered a redistribution of potassium in favor of the lower halves of hypocotyls while calcium was found in greater proportion in the cells of the upper side. The calcium asymmetry in favor of the upper side was confirmed by Goswami and Audus (1976) who demonstrated a maximum of twenty percent increase in favor of the upper sides of *Helianthus* hypocotyls after one hour, at which time curvature was just beginning.

More doubt as to the efficacy of the Cholodny-Went hypothesis was cast after findings of Mertens and Weiler (1983) who found no asymmetry in upper and lower halves of *Helianthus* hypocotyls of IAA, ABA or GA after two hours gravistimulation by which time substantial upward curvature had already developed.

1.3.3: Gravireaction Mechanisms in Roots

In roots, the growth promoting substances ABA, IAA, gibberellins and cytokinins have all been detected unequivocally. The movement of IAA occurs in the stellar core of the root and is highly polarized towards the tip (Scott and Wilkins, 1968). There also exists at least one growth inhibitor elaborated in the root cap which is thought to be involved in gravicurvature.

In *Zea mays*, removal of the root cap eliminates gravitropic curvature (Juniper, 1966). Gibbons and Wilkins (1970) found that removal of half of the primary root cap caused curvature towards the remaining half cap no matter which way the root was oriented. This finding indicates that a growth inhibitor, produced by the root cap, is translocated to the extension zone. Insertion of mechanical barriers, perpendicular to the longitudinal root axis, confirm such findings and shows that inhibitor movement is from the cap to the extension zone rather than acropetally to the

growth zone from a point on the basal side of this growth zone. Shaw and Wilkins (1973) showed in *Zea mays* and *Pisum sativum* that whereas, removal of half of the cap caused curvature towards the remaining half-cap, removal of half of the root apex did not produce curvature in roots whose caps had been entirely removed. Thus, the cap as distinct from the apex appears to be the source of an inhibitor. The possibility that curvature arises as a response to surgical trauma in decapping experiments is eliminated by the finding that replacement of the half-cap immediately after its removal also eliminates curvature in the vertically-oriented root (Pilet, 1973).

The chemical nature of the inhibitor from the cap has not been unequivocally determined but there is strong evidence opposing the role of IAA as the principal inhibitor. Bridges, Hillman and Wilkins (1973) have documented the presence of IAA in the stele with very little found in the cortex or the apex. Scott and Wilkins (1968) showed that IAA movement in the roots of *Zea mays* is polarized from the base to the tip rather than in the opposite direction. If correct this result would preclude the possibility that IAA is the inhibitory compound involved in *Zea mays* root gravicurvature. Kundu and Audus (1974) showed the presence of a cap inhibitor at an r_f close to that of ABA and definitely not IAA in *Zea mays* roots. Wilkins and Wain (1974) showed the presence of ABA and two other inhibitors, but not IAA, in the caps of *Zea mays* roots exposed to light. None of these inhibitors were found with dark treatment. Rivier and Pilet (1974) by contrast did find IAA in *Zea mays* root caps using mass spectroscopy. According to Wilkin (1984), this is the only published study pointing to the possibility that IAA is indeed the chemical inhibitor produced by the cap.

Evidence that ABA is the inhibitor from the cap is as follows: ABA at 10^{-8}M to 10^{-4}M causes inhibition of growth in the roots of *Zea mays* while asymmetric application of 10^{-8}M ABA causes significant curvature away from the point of application. Similar asymmetric application

of IAA does not produce curvature. (Pilet, 1975)

Wilkins and Wain (1974) showed that, in a cultivar of *Zea mays* requiring light to express root gravicurvature (cv. LG 11), ABA was present in the caps of light-exposed roots but not in etiolated roots. Wilkins and Wain (1975) working with the same light-requiring cultivar showed that application in solution of 10^{-9}M ABA caused horizontal dark grown intact roots to bend downward while untreated roots remained horizontal.

In order to accept ABA as the inhibitor from the cap it must be shown that downward lateral transport of ABA in horizontal roots does occur. It must also be shown that an asymmetry of naturally occurring ABA is established in intact horizontal roots. Hartung (1976, 1981) was unable to demonstrate either an asymmetric distribution or lateral transport of radioactively-labeled ABA supplied by micro-application to root caps of *Phaseolus coccineus*, *Vicia faba* or *Zea mays*. Suzuki, Kondo and Fugii (1979) showed no such ABA asymmetry in maize roots in either the light or in the dark using a bioassay procedure. In fact, these authors demonstrated that a 1.6/1 ratio of ABA exists from the upper to the lower side of light-exposed horizontal roots. (They did, however, discover another unidentified growth inhibitor in the cap in greater concentration in the lower region of the cap when roots were light-treated but not in the dark. This inhibitor was located in the acid fraction of their extract.) Mertens and Weiler (1983) showed no asymmetry in either ABA or IAA in horizontal roots.

Contradictory evidence on this point is supplied by Rivier and Pilet (1981) who showed a just significantly detectible greater concentration of ABA in the bottoms of horizontal roots which had developed a downward curvature whereas those which had not responded to gravity did not show such an asymmetry.

Feldman (1981) showed the presence of ABA in *Zea mays* root caps exposed to incadescent light ($1.3W/m^2$). ABA was however, not present in the dark. In addition, Feldman

reported the presence of another inhibitory compound in the neutral fraction in the caps of etoliated roots while a second neutral fraction inhibitor appeared upon exposure to light. These findings were made in intact cv. Merit roots, a maize cultivar requiring light to express root gravicurvature. He also found that when the root caps are detached in darkness and cultured in the light ABA is not found but the second-mentioned neutral inhibitor is present. Feldman's results taken together seem to rule out ABA as the inhibitor in gravicurvature but leaves open the possibility that the inhibitory compound in the neutral fraction may play a critical role in the process leading to asymmetrical growth in the extension zone, and thus to downward gravitropic bending.

Moore and Smith (1984) observed that fluridone-treated *Zea mays* primary roots exhibited normal gravitropic curvature in spite of the fact that fluridone treatment eliminates the presence of ABA. Fluridone is a pyridinone herbicide which inhibits the de-saturation reactions converting phytoene to phytofluene thus blocking keratogenesis (Bartels and Watson, 1978; Fong and Schiff, 1979; Fong, et. al., 1982). The production of ABA is also prevented by fluridone treatment since ABA is synthesized via the carotinoid pathway. ABA levels were measured with a gas chromatograph-mass spectroscope (GC-MS). ABA concentration was detectable above six nanograms per gram fresh weight.

Moore and Smith (1985) also found normal gravicurvature among the carotinoid deficient mutants w-3, vp-5 and vp-7. Using GC-MS no ABA was detectable in any of the mutants tested. Gravicurvature was measured in flourescent light ($3.1\times10^{-1}W/cm^2$). There is a body of evidence in support of the role of calcium as an effector of root gravicurvature (Moore and Evans, 1986; Pickard, 1985; Miyazaki and Fugii, 1987). To begin, asymmetric application of calcium to the tips of vertically-oriented primary roots causes curvature toward the calcium source (Lee, Mulkey and Evans, 1983). Secondly, EDTA which serves as a calcium chelator, can be used to abolish

gravicurvature when applied to the tips of horizontally-oriented roots. When calcium is added the roots regain gravisensitivity (Lee, et. al., 1983). Finally, there are reports that a calcium gradient develops between the upper and lower sides of horizontally-oriented roots. Lee, Mulkey and Evans (1983) have shown that calcium preferentially accumulates in the under halves of root caps upon horizontal treatment. Miyazaki and Fugii (1987) have demonstrated that from 15 to 30 minutes after the start of gravistimulation (and thirty seconds exposure of white light) *Zea mays* roots exhibit increased levels of calcium in the lower sides of the caps while a similarly directed gradient occurs in the growth zone from 1 to 2.5 hours after the beginning of light exposure and gravistimulation. No such calcium gradient was found in either root caps or growth zone when gravistimulation was imposed in the absence of light.

Pickard (1985) has proposed that amyloplast pressure on the ER in the lower sides of cells of horizontally-oriented roots stimulates the release of calcium from the ER. According to this model a calcium gradient develops within individual graviperceptive cells such that a greater concentration of calcium is found in the cytoplasm along the lower cell wall. Pickard's model suggests further that an asymmetric calcium distribution across the root tip might develop in various ways. For example: **1**: Secretion of calcium might stimulate the operation of plasmalemmal calcium pumps on the underside of each gravisensitive cell which could in turn serve to expel calcium through the plasmalemma and into the apoplast. Thus, the flow of calcium would proceed in the apoplastic system eventually becoming asymmetrically concentrated in the lower side of the tips of horizontally-oriented roots. **2**: The released calcium could bind to calmodulin causing the activation of a calcium dependent kinase whose function would be to phosphorylate an auxin transport membrane protein. Auxin could then be expelled, via the phosphorylated transport molecule, through the lower side of the plasmalemma into the apoplast and would eventually accumulate in the lower side of the root tip where it could presumably

initiate growth inhibition leading to downward curvature.

Moore and Evans (1986) have proposed a theory which suggests that calcium is expelled from peripheral root tip cells into the mucilage of the root cap. It accumulates preferentially in the mucilage, on the underside of the horizontally-positioned root. They present results showing that in gravistimulated roots calcium is expelled into the region of the cell wall on the distal side of columella cells. However, the movement of calcium is symmetrical above and below the root axis. How then does the calcium gradient arise in the mucilage? These authors believe that the movement of calcium occurs in conjunction with a series of electrochemical changes which are associated with reorientation of the root from the vertical to the horizontal. In this regard, Behrens, Gradmann and Sievers (1985) have shown that the lower columella cells of gravistimulated roots of *Lepidium sativum L.* rapidly depolarize beginning after an eight-second latency period. The depolarization grows in magnitude and peaks after three minutes. The re-polarization of these statocyte cells follows quickly and is complete within sixty seconds. Subsequently, the upper statocytes slowly hyperpolarize. In addition, Behrens, Weisenseel and Sievers (1982) note a change in the pattern of current flow in the root tip following reorientation of the root from the vertical to the horizontal position. Current flows towards the tip when the root is positioned vertically downward, but within thirty seconds after the start of gravistimulation the pattern changes such that the current now flows towards the tip on the upper side of the inclined root and away from the tip on the lower side. Moore and Evans (1986) speculate that calcium is the gravitropic effector which moves downward through the apoplast of the horizontal root. They argue further that calcium then flows towards the growth zone on the under side of the root. There, it sensitizes auxin's inhibitory effect on cell elongation thus, leading to downward curvature.

Part Two: Circumnutation

1.4: Historical Introduction

Plant organs generally grow in a helical pattern around a preferred direction of growth defined in relation to the gravitational plumb line. This type of oscillatory movement, accompanying growth around a central axis, is called circumnutation or more simply nutation. The fact that plant organs oscillate as they grow has been known and studied since the middle of the last century (Dutrochet, 1843).

Charles and Francis Darwin studied the characteristics of plant organ circumnutation in The Power of Movement in Plants (1880). Charles Darwin concluded that nutational movements were primary and that geotropic responses formed a specialized subset of the more general circumnutational movements. He classified plant circumnutation as an autonomous type of motion which benefits the growing plant in some unknown way. Darwin's view held sway among plant physiologists until the 1920's when Gradmann (1921, 1927, 1928) published a series of papers promoting the idea that the helical growth pattern, described by plant organs, was derived from the lag time between the perception of a gravitropic signal at the tip of the plant organ and the response to that signal via a change in the direction of growth. This became known as the gravitational feedback model of circumnutation.

These two theories are still current in the scientific debate over the causes of nutational movements. At present, David Heathcoat is the most vigorous proponent of the autonomous oscillator model while the gravitational feedback model is supported most strongly by Anders Johnsson. Heathcote and Johnsson have engaged in the unusual practice of publishing together, in spite of disagreeing as to the motive force behind the phenomenon under study (Johnsson and Heathcote, 1973).

1.5: Internal Oscillator Model

The model assumes that cell wall expansion occurs in a saltatory or step-wise manner with alternating phases of growth and consolidation. The nutating organ is arranged in a temporal sense such that growth proceeds in a wave around the circumference of the organ. Adjacent cells, for example on the circumference of a plant stem, will be more or less in phase in terms of the timing of their extension while cells across the stem from one another will be exactly out of phase. In the simplest case, growth proceeds in a wave around the circumference of the stem such that a circular or elliptical pattern of extension occurs. The symmetry of the particular organ in question determines whether its trajectory is circular or elliptical (Johnsson and Heathcote, 1973).

According to Heathcote and Aston (1970) each cell contains an oscillator timing mechanism which controls the alternations of growth and quiescence in the cell. The oscillator timing mechanism in each cell has a period τ equal to the periodicity of nutational movement. The coordination of these waves of expansion and quiescence is controlled by information received from neighboring cells. The amount of growth which takes place during each cell's extension phase is some function of the auxin concentration in the cell at that time.

When a plant organ is tropically stimulated, i.e., by gravitation, the rotary motion of circumnutation is superposed on the gravitropically-induced curvature (Johnsson and Heathcote, 1973).

Brown and Chapman (1983) present data from an experiment conducted on Sky Lab One in which *Helianthus* hypocotyls perform oscillations during growth in the near 0 g environment of an orbiting spacecraft. This result lent strength to the proposition that nutation and gravicurvature are separate effects driven by different forces. Similar satellite experiments involving different plant organs and species must show the existence of spiral oscillations in order to generalize the conclusion that circumnutation is driven by an internally controlled mechanism.

1.6: Gravitropic Feedback Model

This model assumes that the plant stem oscillates in a vertical plane back and forth across the plumb line. Let α denote the angle between the tip of the stem and the plumb line. Since α varies with time the model specifies that $\alpha = \alpha(t)$. It follows that the angular velocity of curvature of the stem apex is $d(\alpha)/dt$. Let t_o represent the gravitropic reaction time or the time required for a gravitropically-stimulated organ to begin to alter its direction of growth. The gravitropic bending rate is equal to the sum of the gravistimuli experienced prior to $t-t_o$. The following equation holds in the simplified case in which the rate of curvature of the tip is dependent only on the gravitropic stimulation at $t-t_o$ and in which the gravitropic stimulation is equal to the sine of the angle with the plumb line:

$$\frac{d\alpha}{dt} = -k sin\alpha(t-t_o). \tag{1}$$

In the more general case the gravitropic stimulation at time t is defined by the accumulated gravitropic stimuli before $t-t_o$. The model makes the additional assumption that: $f(t')$ represents the extent to which the gravistimulation at the time $t'-t$ will affect the rate of gravicurvature at time t. The expression then becomes:

$$\frac{d\alpha}{dt} = -k\int_0^{\infty} f(t')\sin\alpha(t-t')\,dt'. \tag{2}$$

If certain conditions on the parameters in Equation 2 are satisfied, then the equation can be used to represent sustained oscillations. Oscillations in two orthogonal vertical planes can be added to produce a circular path, if the oscillations are identical; or an elliptical path, if they are not (Johnsson and Heathcote, 1973).

Johnsson relates the nutational period to the geotropic reaction time with the following relation:

$$\frac{t_o}{\tau}=\frac{(1+4m)}{4}\ , \tag{3}$$

where m=0,1,2,... .

The relation between the nutational period τ and the geotropic reaction time t_o is then given by $t_o/\tau = 1/4$. This equation is based on the following assumptions: α is restricted to small angular deviations so that $sin(\alpha)=\alpha$; $\alpha(t)$ varies sinusoidally; and $d(\alpha)/dt$ depends only on the curvature at time $t-t_o$.

Johnsson presents data from several different plant species indicating that $t_o/\tau \approx 1/5.3$ as opposed to $1/4$. According to Johnsson, the discrepancy is based on the use of the simplified Equation 1 to describe the angular deviation as a function of time, as opposed to the more realistic form of the problem stated in Equation 3. When a declining exponential term is substituted for $f(t')$, Equation 2 gives the experimentally-determined ratio of $1/5.3$ (Israelsson and Johnsson, 1967).

FIGURE 1.1

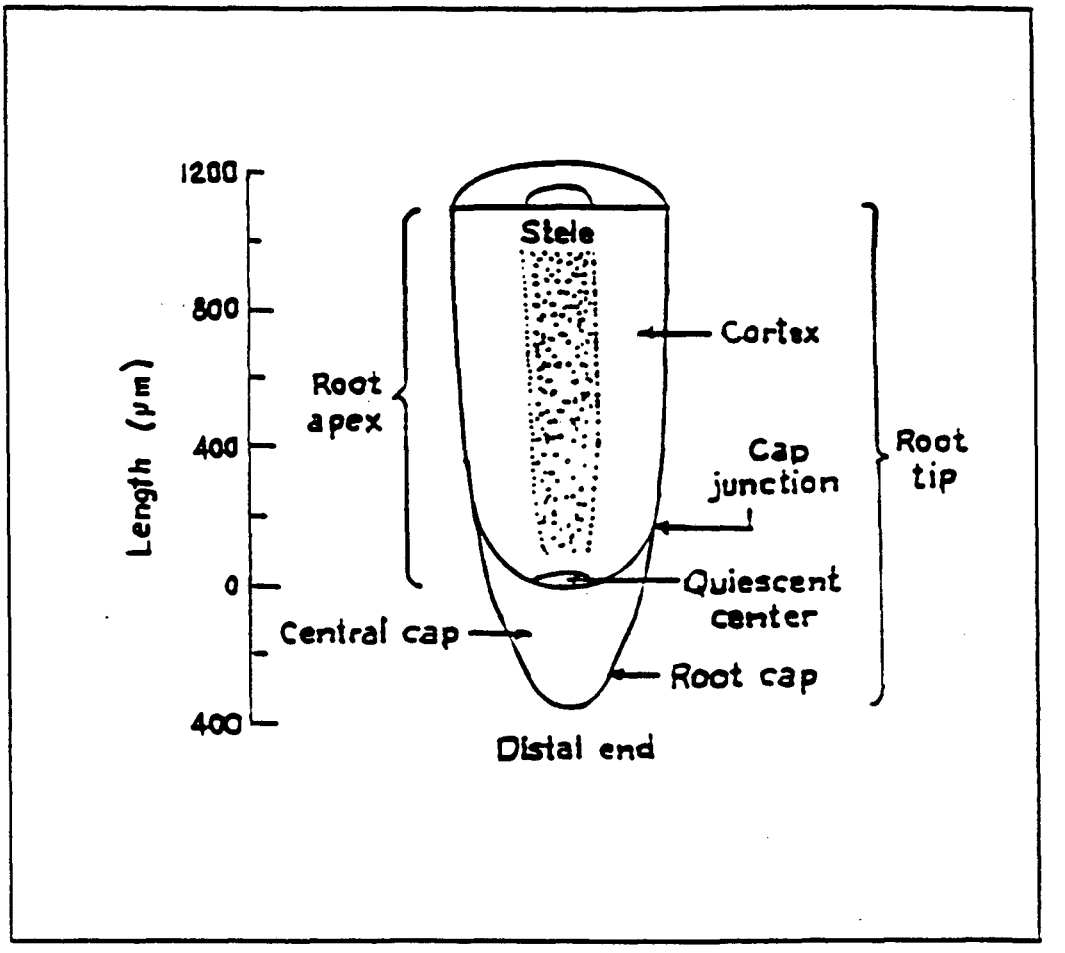
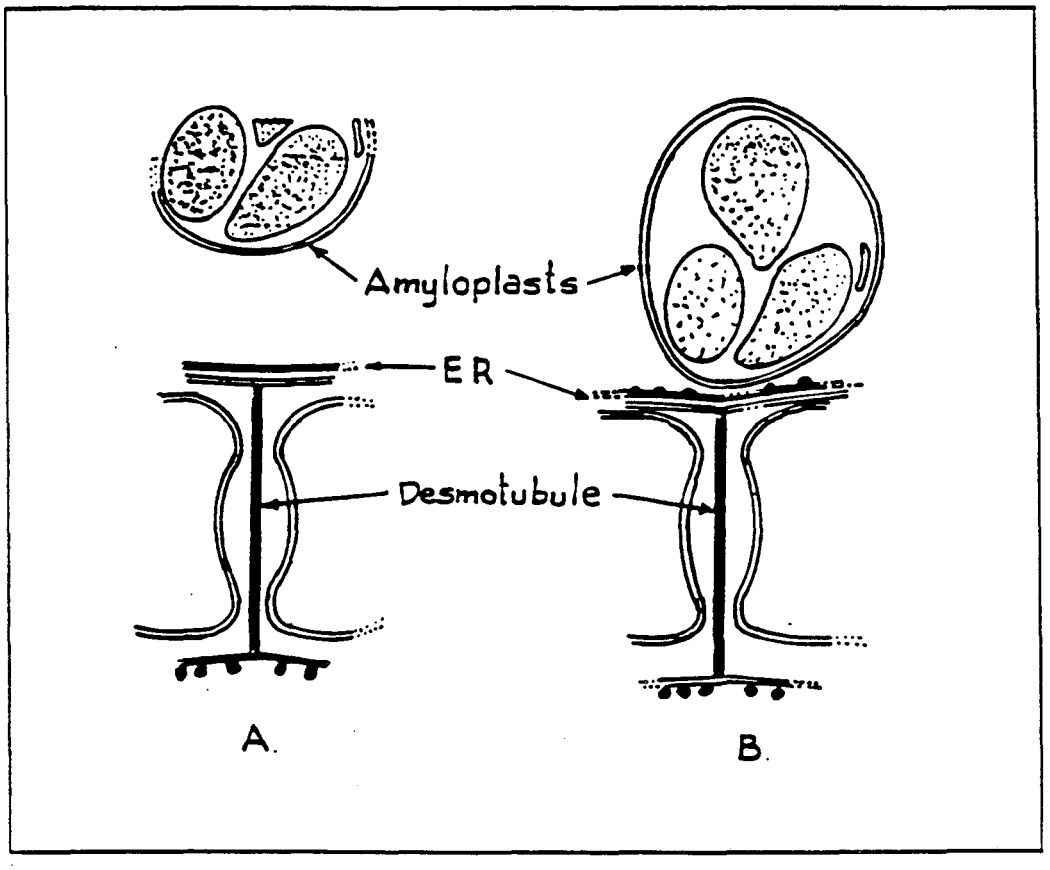


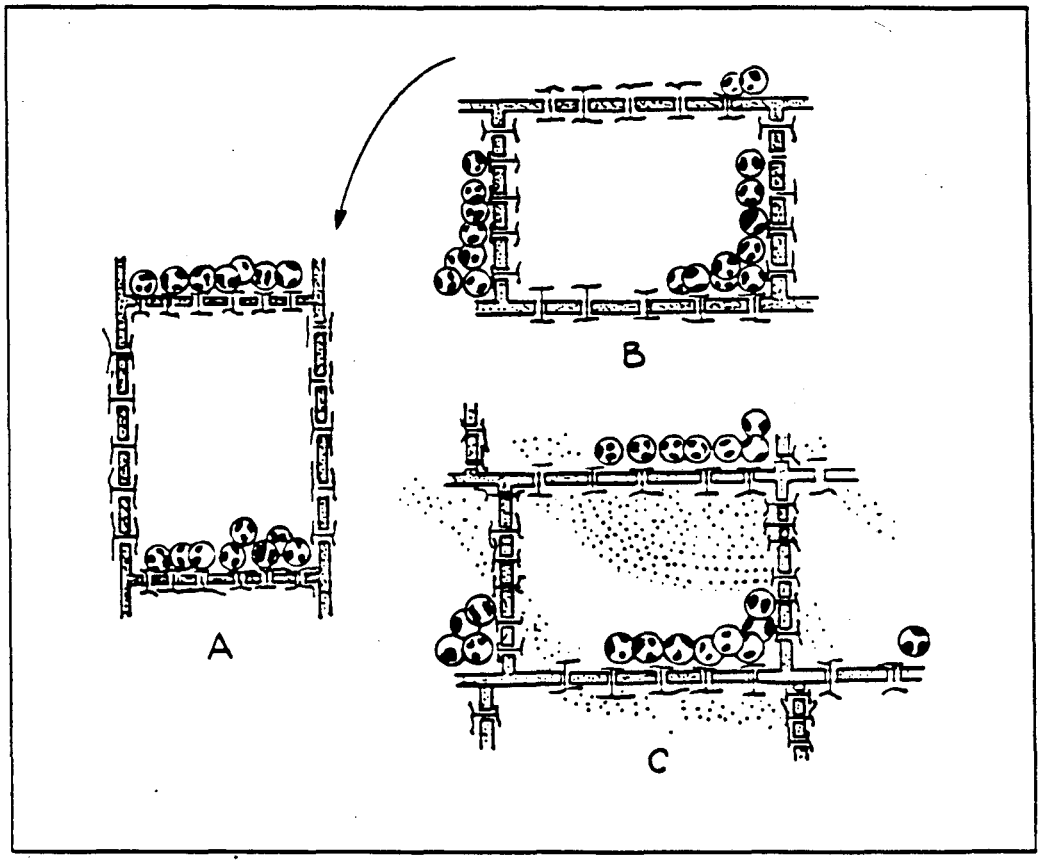
Diagram of the distal 1600 μm of a primary root of *Zea mays L.* redrawn from Jackson and Barlow (1980). The meristem is located in a region 1500 μm behind the quiescent center. The zone of maximum elongation and gravicurvature is located from 1500-2500 μm behind the quiescent center (Shen-Miller, et. al., 1978).

FIGURE 1.2



Juniper's proposed model for amyloplasts/plasmodesmatal valve.
A: Open
B: Closed.
Redrawn from Juniper (1976).

FIGURE 1.3



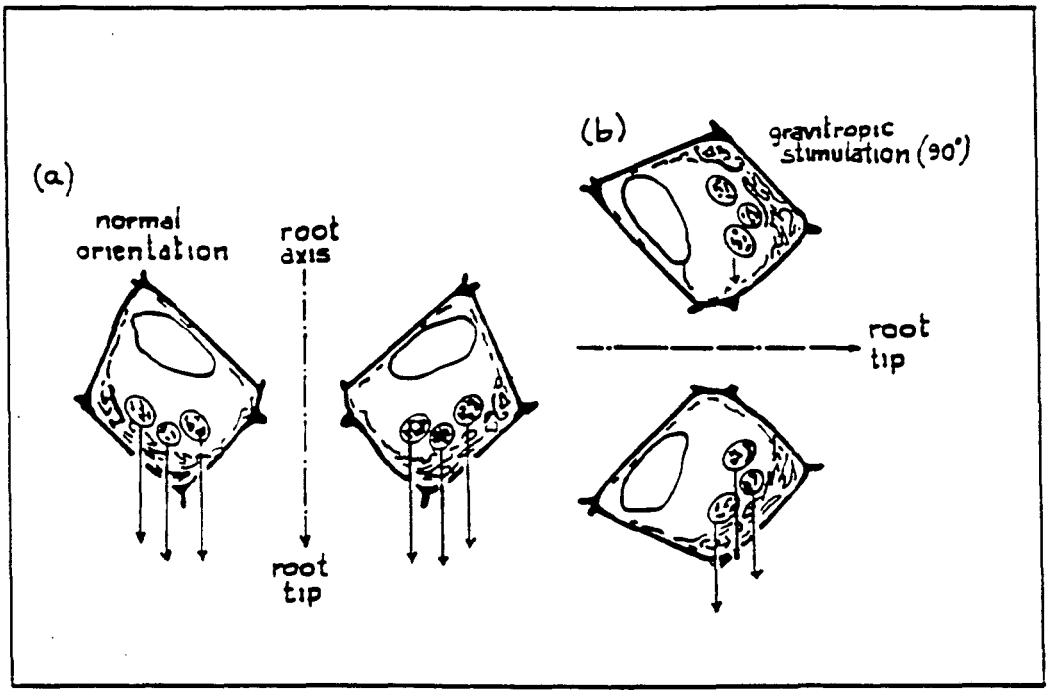
Juniper's proposed model for the amyloplast/plasmodesmatal valve system operating in the steler core of the root cap. Redrawn from Juniper (1976).

A: Cell vertical, transverse valves closed, longitudinal valves open.

B: Following reorientation into the horizontal position, the transverse valves begin to open and the longitudinal valves begin to close.

C: Prolonged period of gravistimulation, most transverse valves open. Proposed growth inhibitor (dotted lines) follows shortest path through open transverse and longitudinal valves alternately, in a step-wise fashion.

FIGURE 1.4



Pairs of symmetrically oriented peripheral statocytes in the *Lipidium* root.

A: In the normal tip-down configuration the amyloplasts exert equal pressure on the ER complex (equal arrows).

B: In the horizontal configuration the amyloplasts produce unequal pressure above and below the longitudinal root axis (unequal arrows).

Redrawn from Sievers and Volkmann (1977).

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CHAPTER 2

MATERIALS AND METHODS

2.1 General Considerations

I performed a variety of biophysical and physiological experiments which involved measuring the effects of various lighting regimens on maize root gravitropism. I also conducted research testing the nutational properties of maize primary roots.

The work was carried out in a dark room at The Lawrence Berkeley Laboratory. The room was equipped with a double light lock to allow passage into and out of the test area without disturbing the lighting program for experiments in progress. The room temperature was maintained at 24°C ±1.5°.

Lighting for experiments was provided as follows: incandescent light---500 W (General Electric); florescent light---100 W cool white (Sylvania); helium neon laser light---6328 angstroms output $.5\times10^{-3}$W (Spectraphysics Model 155); green background light---517 nm half band width 6.2 nm (Bausch and Lomb Monocrometer, Model Kl471).

Photographic lighting was provided by two 500 W incandescent lights (same as above) for optical range photography. Infrared photographs were taken with two electronic flash units (Vivitar model 3500) covered with Kodak 87c infrared filters, flash pulse duration .002 seconds and no measurable light below 800 nm. Radiant flux was measured with a United Detector Technology, model pin 10 df, radiometer calibrated at 5 nm increments from 3500 nm to 1100 nm and at 6328 angstroms (the wave length of the helium neon laser). Glass cut-off filters (Hoya Optics) and glass interference filters (Bausch and Lomb) were used in conjunction with the radiometer to permit accurate measurement of desired frequencies. For example, intensity levels for incandescent or florescent lighting used in experiments were measured in the range of

400 nm to 800 nm allowing easier comparison with other published work. Ultraviolet and infrared cut-off filters were interposed between light source and detector to accomplish the necessary band-width restriction for this purpose. Light levels were read into an operating amplifier (op amp) which converted the signal coming from the detector from DC current to DC voltage and which at the same time allowed the measurement of very low radiant flux levels from 10^{-6} to $10^{1}W/m^{2}$. Intensity levels were read on a 3 digit Beckman Multimeter, model 310.

Growing and georeacting maize primary roots were maintained in an incubator (National Appliance Company, Model 3221-13) during the experimental trials. Photographs were taken through a double-paned glass door. The glass panes were kept free from condensation by means of a series of heater strips fixed to the outer pane. These heater strips kept the glass clear but did not create a measurable temperature gradient at the seed level. This fact was established with the aid of an electronic temperature probe (Hana Company, Model 3309-50) sensitive to .1°C. The incubator temperature during trials was maintained at 27.5°±.5°C. Relative humidity of +95% was maintained during all trials. Incubator air was kept saturated with the aid of a cold water humidifier which ran continuously during trials.

During tests the seeds were held by moistened-sponge-covered clamps mounted on plexiglass columns. Each clamp was attached to a small mechanical armature which then was connected to the column via a threaded post. Post and armature were connected through a ball joint. The entire assembly swiveled allowing precise alignment of the root (Figure 2.1). The columns were carefully leveled with the aid of a precision level (Figure 2.2). Three columns (54 roots) could be accommodated in tests in which roots were oriented parallel to the ground while one column (10 roots) could be used when the root position was vertical at the start of the test period (Figure 2.3). Each column contained a metric, gridded-facing attached to two adjacent sides of the column.

Photographs were taken at specified intervals with one or two Cannon T70 35mm cameras. The cameras were equipped with various computerized functions which allowed photographs to be taken automatically on a preset schedule. Photographs were generally taken at 10-minute intervals throughout the test period. Optical range photographs were shot with Kodak kodachrome 40 ASA film while infrared photographs were taken with Kodak high intensity exposure infrared negative film.

Photographic slides were mounted and viewed under a microscope. The procedure for collecting data from the slides was as follows: Each photograph contained a profile view of the roots which were positioned approximately 1 cm in front of the gridded background. The microscope was fitted with one of two reticles allowing the measurement of root tip position or angle. Angle measurements were recorded in 2.5° increments with an error of ±2.5°, while position measurements were recorded in .02 cm increments with an error of ±.02 cm.

In general, data was taken on primary roots started in a range of ±10° from the desired beginning reference angle. At the same time, data was recorded only on roots that grew at a rate of more than .1 cm per hour during the test period with certain exceptions noted below. In addition, only those roots which measured between 1.0 cm and 1.5 cm in length at the beginning of each trial were selected. Finally, those roots with more than a 10° curvature at any point along the length of the root at the start of an experiment (as noted upon viewing of the photographs) were rejected for consideration.

In the text, tables, and in figures 0° is defined as the angle parallel to the ground and 90° denotes the angle downwards along the plumb line. The choice of reference angles is designed to accommodate both the fact that the motion of roots is generally downward and to avoid the excessive use of negative numbers.

Maize seeds, of various varieties designated below, were imbibed for two hours in distilled

water in complete darkness. Cv. Merit seeds (Asgrow Seed Company, Kalamazoo, Michigan) were used in most of the physiological experiments described. The seeds were aerated with an aquarium pump during the imbibing period. Seeds were then arranged embryo up on moistened paper towels resting inside rectangular glass trays, which were then covered with cellophane and put into a dark box for germination. Several inch-long razor cuts were made in the cellophane to allow some air circulation while still maintaining high enough humidity to maintain optimum growth conditions. The dark box was fitted with a small fan which ran continuously during seed germination in order to provide sufficient air circulation. During the subsequent germination period the primary roots emerged, in general, growing parallel to the ground keeping in contact with the moistened paper towels as they grew.

Following germination (44 hours for Merit seed and longer for other seed types used) straight primary roots were selected in dim green 517 nm light ($2.0\times10^{-4}W/m^2$) at the root level. Roots were measured and then transferred, maintaining an embryo-up configuration, onto the supporting column(s) described above. In those experiments calling for horizontal root alignment the column was set into the incubator, such that the roots were re-oriented 90° along their longitudinal axis. In this configuration the seeds were positioned such that the embryos were now pointing outward rather than upward, but the roots still remained parallel to the ground (Figure 2.4). The column was set upright allowing roots to be re-oriented from the horizontal to the vertical in those trials which required vertical alignment of roots. In all cases the re-orientation of roots, following seed selection and placement, was carried out in the dark to exclude all visible light from interfering with the effect under study. Seed selection lasted no more than 15 minutes. During this period the roots were kept moist with the aid of an ordinary plant sprayer. The equipment and protocol to test the hypothesis that, in maize, the root cap is the site of interaction with light leading to positive gravitropic curvature, were both rather involved and as a result they

will be discussed here in detail. Otherwise, variations in experimental procedures will be noted in the text as particular research issues are explored.

2.2 Laser Test Protocol

The laser was used in conjunction with an 11" focal length positive lens, a λ 653 nm interference filter and a .01 cm pin hole used to reduce stray reflections. The interference filter was needed to eliminate contaminating light produced by the laser from other frequencies (Spectra-Physics Instruction Manual, Model 155, 1983). The light intensity was adjusted by interchanging a series of Kodak neutral density filters. The laser light source and the optical components were mounted on an x-y-z table also containing a telescope which was triangulated with the light beam prior to the start of each trial (Figure 2.5).

The width of the focused laser beam was measured by translating a 7 μm pin hole across the beam and measuring light intensity as a function of distance from the central laser spot. The radius of the beam at the $1/e^2$ point was .02 cm. Dim green background light, λ 517 nm, intensity $1.71\times10^{-4}W/m^2$, was used for sighting purposes during the experimental period. Precise placement of the beam was accomplished by exposing each root to a brief low intensity laser pulse, intensity $1.73\times10^{-6}W/m^2$ or approximately .2% of the base line experimental exposure and of less than 10-second duration.

Cv. Merit seeds were germinated in complete darkness as described above. One-hundred to two-hundred seeds were germinated in each trial of which 5 seeds were selected for study. Approximately 15 minutes elapsed between irradiation of the first and last root in each trial. The green background light was required during the entire laser exposure period. In addition it was necessary to arrange seed placement with $3.05\times10^{-4}W/m^2$ intensity green background light. The selection and placement of seeds lasted about 5 minutes and was carried out before the laser

irradiations. Immediately prior to laser exposure, the roots were re-oriented 90° along the longitudinal axis such that the roots remained parallel to the ground. The laser irradiations were carried out with the laser light directed perpendicular to the root viewed in profile.

An infrared photograph was taken immediately before laser exposure and thereafter infrared photographs were taken at 10-minute intervals beginning at +20 minutes and continuing until +3 hours.

Data was recorded for those roots which grew at least .2 cm during the 3-hour experimental period, and with starting angles of +10 to −10°. At least 20 roots were studied in each test group.

2.3 Genetics Protocol

In experiments designed to determine whether a genetic component for gravitropism or circumnutation existed, roots were first tested in the lab for the particular characteristic in question and then planted and crossed as described below.

The genetics phase of the research was carried out at the University of California, College of Natural Resources, Outdoor Lab for Teaching and Research in the Agricultural Sciences (Oxford Tract). The research was conducted under the supervision of Professor Michael Freeling. Plants were grown to maturity and crosses were performed both in the field and in the greenhouse between 1985 and 1988. Plants were observed on a twice-weekly basis during the beginning of each growing season for phenotypic variation. Towards the end of each season plants were checked daily to insure that appropriate crosses were performed on schedule and with minimal risk of contamination.

The crossing procedure is as follows: At maturity each corn plant produces a tassel or male inflorescence made up of several branches which emerge from the top of the plant. The

tassels support clusters of anthers which contain the pollen. At maturity the anthers split and the pollen is "shed" onto the ears which are now ready for fertilization. Each ovule or nascent seed is connected to the outside world via a cylindrical structure known as a silk. A single pollen grain lands on the tip of a silk and migrates downward along its' interior surface to meet the egg cell. Crosses are arranged and contamination is avoided by securely covering each developing ear with a transparent ear shoot bag before any silk becomes visible. Twenty-four to forty-eight hours before a cross is made the ends of the silks are cut causing the tips of the silk to exude a substance which prevents pollination for several hours until the silk begins to grow again. From this point the silks grow at a uniform rate such that after 1 to 2 days a "brush" of " to 1" in length is formed. The ear is now ready for pollination. Meanwhile, 12 to 24 hours before the ear is ready for pollination, the tassel is covered with a collecting bag which catches the falling pollen. The cross is carried out by spreading pollen onto the ends of the silk.

2.4 Time Series Analysis and Statistics

An algorithm for the discrete Fourier transform known as the fast Fourier transform is used extensively in this paper as a means of determining periodicity in time series data (see Chapter 4). The algorithm for the transform is available as a standard function of BLSS (The Berkeley Interactive Statistics System) accessed through UNIX. Fast Fourier transforms as a class allow the rapid estimation of the Fourier transform of a function from a finite number of it's sample points.

Let us define the following terms: N consecutive sample values $h_k = h(t_k)$, $t_k = k\Delta$, with $k = 0, 1, 2...N-1$, Δ defined as the sampling interval. We seek estimates only at the discreet values $f_n = n/N$ with n between 0 and N as an alternative to determining the continuous Fourier transform H of f at all values of f between 0 and f_c. The extreme value of n corresponds to the

upper limit of the Nyquist critical frequency (see below).

Now we approximate the continuous Fourier transform by the discrete transform. The continuous function is equal to:

$$H(f_n)=\int_{-\infty}^{\infty} h(t)e^{2\pi i f_n t}dt \ ,$$

where $h(t)$ is the continuous function. We now approximate the continuous Fourier transform as:

$$\sum_{k=0}^{N-1} h_k e^{2\pi i f_n t_k}\Delta \ .$$

Δ is then factored out of the sum yielding:

$$\Delta \sum_{k=0}^{N-1} h_k e^{2\pi i k_n/N}.$$

The final summation is defined as the discrete Fourier transform of the N points h_k denoted as H_n. According to the sampling theorem the frequencies present in the transform must be restricted to frequencies less than the critical Nyquist frequency (f_c) in order to produce a true sampling of the data. The critical Nyquist frequency is defined as $f_c =$. In almost all instances the interval between data points in my own research is 10 minutes. Thus the critical Nyquist frequency is equal to 1/20 minutes. If the sampling frequency is greater than the frequency of oscillation, then the discrete transform does not drop off to 0. This gives a spurious representation of the continuous transform, a condition known as aliasing (Press, et. al., 1988). The discrete transforms of my own data always approach 0 and therefore give a true representation of the continuous transform. Thus, aliasing does not play a role in the assessment of periodic behavior in any of data sets. The Fisher test as modified by Shimshoni was used to test the statistical significance of periodogram spikes. Shimshoni's contribution was to extend the classic Fisher test to make it possible to evaluate the significance of secondary and tertiary peaks in a power spectrum.

Fisher's work applied only to the evaluation of first order peaks (Fisher, 1929; Shimshoni, 1971).

The regression method is also used in this paper to test the linearity of time series data and, where appropriate, to make statistical comparisons between sections of curves which meet the test of linearity (Dixon and Massey, 1983; also see §4.2.3). Otherwise, extensive use is made of Student's t-test for purposes of number comparison.

FIGURE 2.1

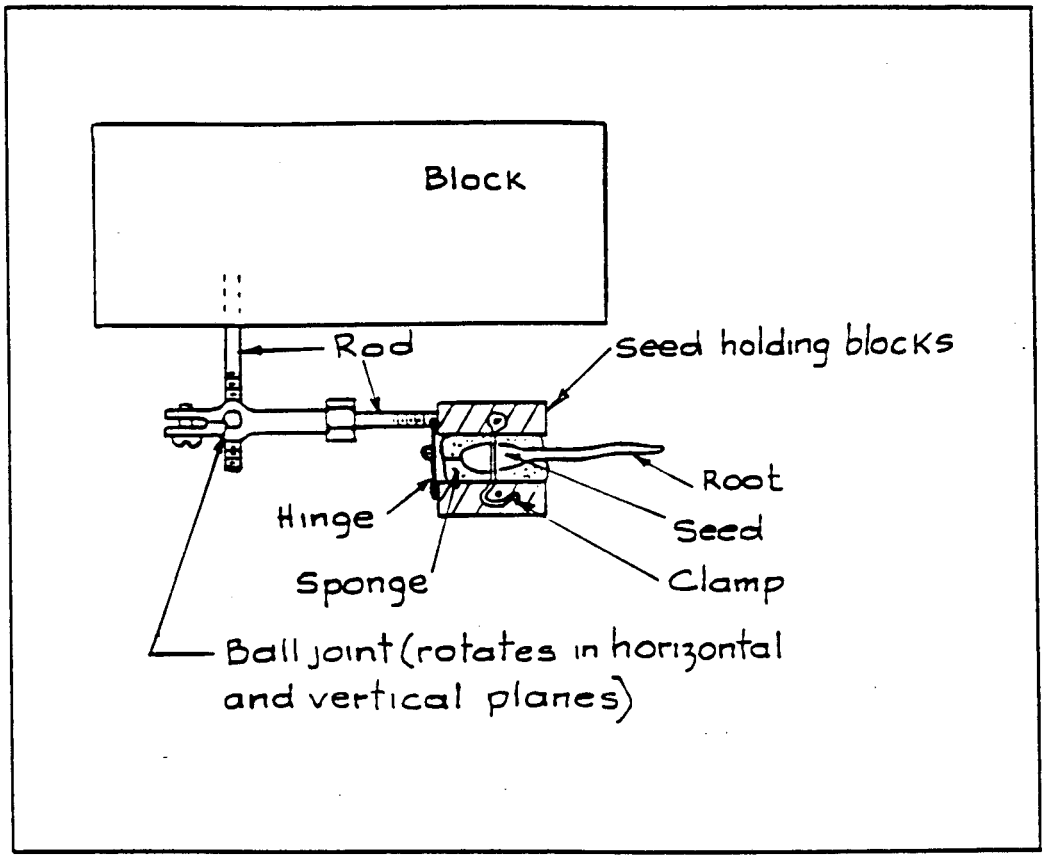
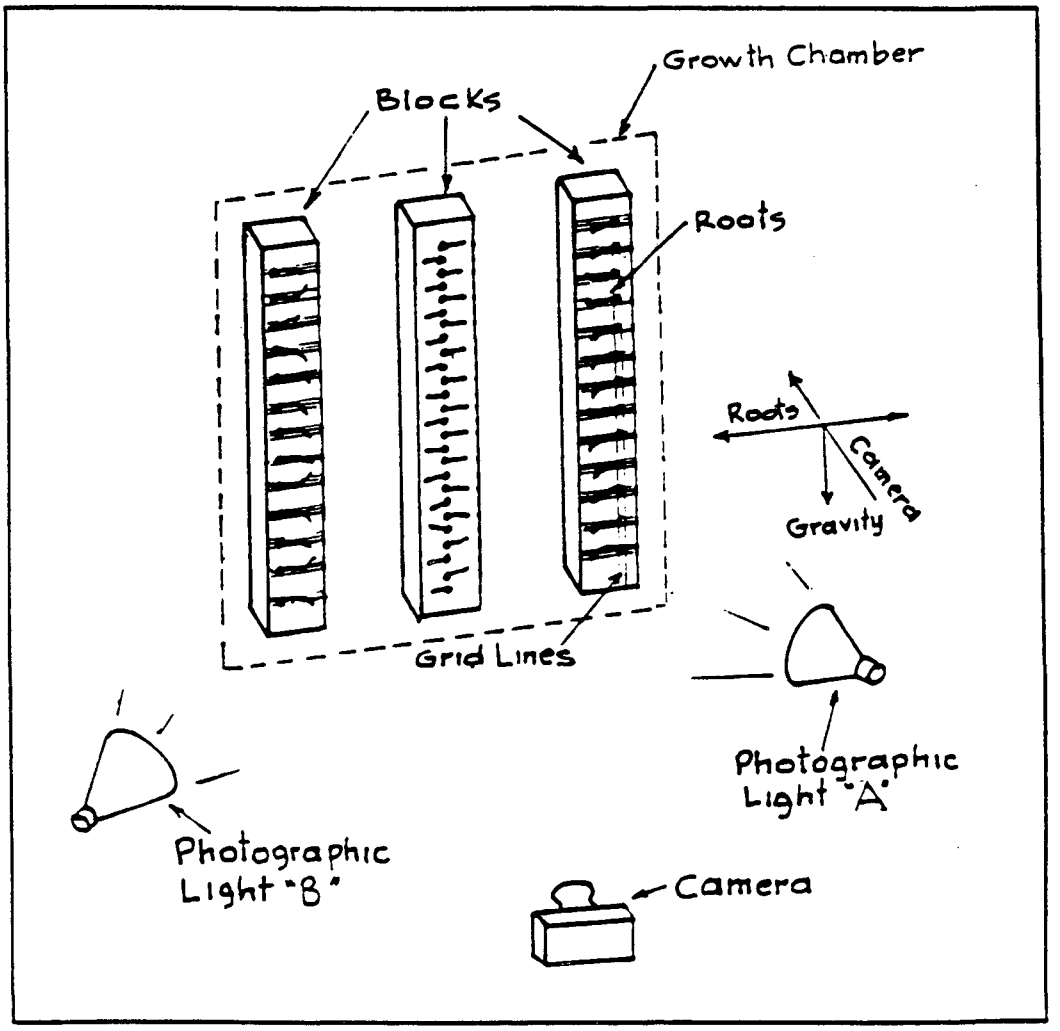


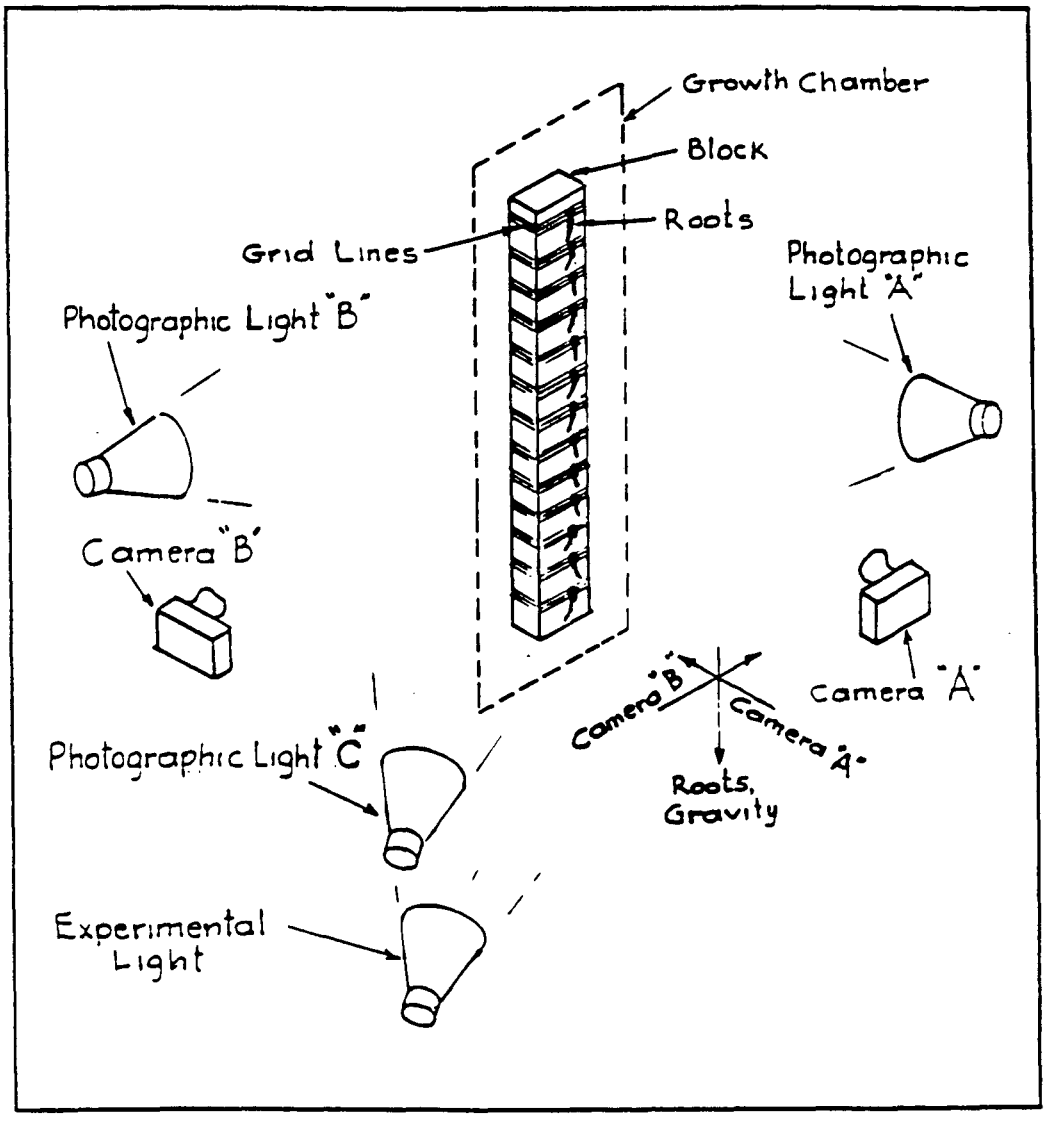
Diagram of seed mount used for precise positioning of roots in experiment.

FIGURE 2.2



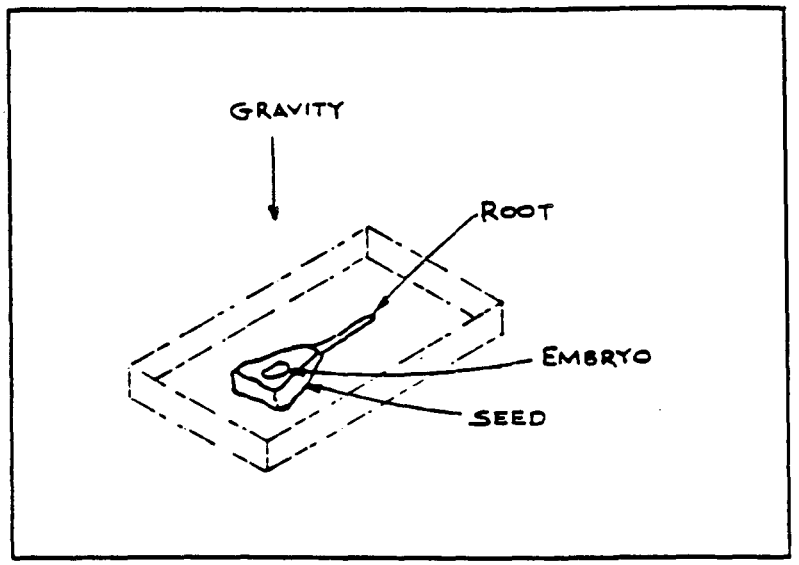
Perspective diagram indicating position of camera and photographic/experimental lighting; roots oriented horizontally at start of experiment. Details of lighting conditions for specific experiments described in text.

FIGURE 2.3

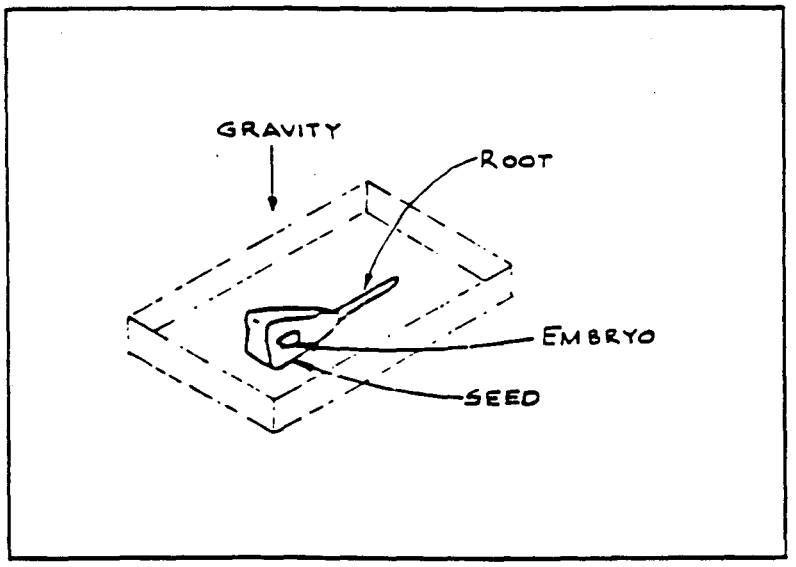


Perspective diagram showing position of cameras, photographic and experimental lighting; roots oriented vertically at start of experiment. Details of lighting conditions for specific experiment described in text.

FIGURE 2.4

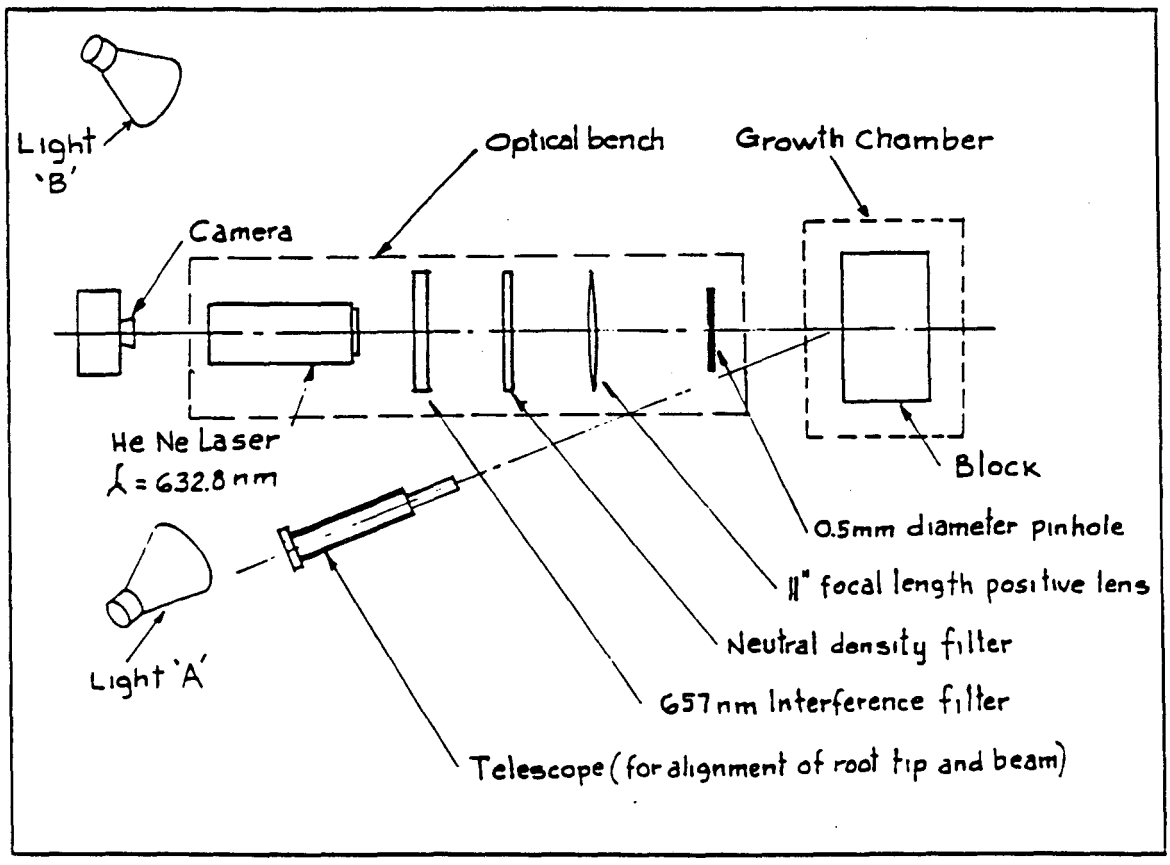


Root orientation during germination and set-up period.



Root orientation during gravitropic test period.

FIGURE 2.5



Schematic diagram depicting configuration of optical components used in laser illumination experiments. Roots oriented horizontally at start of experiments. Detail of photographic lighting for specific experiments described in text. Optical bench in place during laser exposure only.

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CHAPTER 3

LIGHT-POTENTIATED MAIZE ROOT GRAVITROPISM: IS PERCEPTION LOCALIZED IN THE ROOT CAP?

3.1 Literature Review

In recent years light has been shown to have a number of effects on root growth and development. The two findings which bear on the subject of gravitropism are light-potentiated gravitropic curvature and light-induced growth retardation.

Lake and Slack (1961) showed that light promoted georeactivity among roots of various species. Seedlings grown in light-impermeable pots produced root systems which after 23 days were oriented nearer to the horizontal than were the roots of plants grown in transparent pots whose roots became more positively gravitropic in the same time period. They reported this finding in calendula, aster, stock and tomato. However, the phenomenon was not apparent in cucumber. Studies conducted since have demonstrated the role of light as an aid to root gravitropism in lettuce and cress (Iversen, 1973; Iversen et. al. 1977) and maize (Scott and Wilkins, 1969).

What seems like a rather odd phenomenon to the casual observer (roots after all are found underground and would appear not to require light) actually does make sense from an evolutionary standpoint. Roots dry out quickly if left on the soil's surface. As a consequence it is clear that a mechanism must exist which allows roots to burrow quickly into the ground upon germination. This in turn allows the newly emerging plant to develop a secure mechanical anchorage as well as providing a source of water and inorganic nutrients. Individuals within the population which acquired the trait of rapid positive root gravitropic movement by whatever means would be at a competitive advantage and would in time become the dominant form.

Since all life has developed as much in the presence of light as gravity, it is not very surprising that light would have developed a role in many vital processes including as an ancillary factor in gravitropism. It must be stressed that light-induced gravitropic bending is not the same as photophobic curvature. Instead of directing new growth away from the incident light, roots expressing light-induced gravitropic curvature become oriented downward irrespective of the direction of the light source.

The prevailing assumption among investigators studying this effect is that light signals downward root curvature by causing the formation of a growth inhibitor. The inhibitor ultimately becomes asymmetrically distributed in the growth zone of the horizontally-positioned root causing decreased growth on the lower side. Most researchers believe that the photo-active trigger for this process is located in the root cap rather than in the extension zone itself. Specific photoreceptors have not yet been definitively identified, although it is likely that phytochrome is the pigment molecule which becomes transformed and stimulates the initial steps in the transduction pathway leading from signal perception to root curvature (Feldman and Briggs, 1987). To this point there has been no demonstration of a link between the photo-chemical stage of the process and the displacement of amyloplasts in cap cells. These two phenomena may be linked however, if the mechanism of light-induced gravitropic root curvature is similar to non-light requiring gravitropic curvature only with an additional first step which is photo-chemical in nature. The roll of the cap as the initiation point for light-induced gravitropic curvature has been demonstrated indirectly by Wilkins and Wain (1975). These investigators exchanged the caps of light- and dark-treated roots and then subjected the re-headed roots to gravitropic stimulation in the dark. The result revealed a significant increase in positive gravitropic curvature among dark-exposed roots now containing light-exposed caps as compared with light-treated roots with dark-exposed caps. Wilkins and Wain used primary roots of *Zea mays* (cv. LG 11) tested in 4 assay groups. Roots were first held

vertically in darkness or in fluorescent light ($72W/m^2$) for 18 hours. The caps were then removed and re-attached as follows: A: light-exposed caps with dark-exposed decapped roots, B: dark-exposed caps with dark-exposed decapped roots, C: dark-exposed caps with light-exposed decapped roots and D: light-exposed caps with light-exposed decapped roots. The roots were then rotated 90° into the horizontal position and allowed to develop positive gravitropic curvature in the dark for 4 hours. The average angle achieved after this period was reported to be 19.3° and 1.9° for experimental groups A and C while the means for control groups B and D were 4.9° and 19.5°. No controls were performed to establish whether the process of de-tipping reduced the magnitude of gravitropic bending. However, Wilkins and Wain rightly conclude from the data that irradiation of the cap is associated with the development of geosensitivity in the particular cultivar of maize tested. Pilet (1978) added weight to this finding by conducting decapping and re-heading experiments with two varieties of maize whose intact primary roots showed dissimilar reactions to gravitropic stimulation in the dark. He began by showing that 1.0 cm apical primary root segments of *Zea mays* (cv. Kelvedon 33) responded to gravitropic stimulation only upon exposure to light while in the dark such apical root segments remain nearly horizontal even after several hours. By contrast, apical segments of cv. Anjou 210 primary roots were shown to bend downwards quickly in the dark and only slightly more quickly upon exposure to light. Pilet followed up this finding with a series of trials in which the caps of Anjou and Kelvedon root apices were exchanged and then subjected to gravitropic stimulation in the light and in darkness. He found that the responsiveness of the root segment was determined by the source of the cap; i.e., when Anjou caps were placed on Kelvedon decapped segments gravitropic curvature was pronounced whether in darkness or in the light, while when Kelvedon caps were attached to Anjou decapped segments curvature took place only in the light.

Another line of evidence pointing to the importance of the root tip as the trigger point for

positive gravitropic curvature is the well-established fact that decapped or detipped roots, oriented perpendicular to the gravity vector, do not bend downward until the excised portion is regenerated (Barlow, 1974; Hillman and Wilkins, 1982). It may, however, be ill-advised to infer positive results from information gathered when the organism under study has been surgically tampered with. The range of compensatory responses which plants invoke as a response to wounding have begun to receive attention (Ryan, 1987). However, in general, such responses have largely been ignored by researchers too quick to overlook complications which wounding doubtless introduces. The conventional wisdom shared by investigators of gravitropic phenomena is that if the plant organ continues to grow at the same rate following excision then confounding factors derived from wounding need not be considered and the results can be taken seriously as long as proper controls are performed. There is then a body of evidence in support of the cap as the seat of light perception for gravitropic curvature. There are however, no reports in the literature in which slit lamps, lasers or optical fibers have been used to selectively irradiate regions on intact roots in order to show that the cap is indeed the initiation point for gravitropic bending. I sought to test this assumption directly using light from a helium neon laser with intact maize primary roots.

3.2: Results and Discussion

Light from a helium neon laser was used to stimulate positive gravitropic curvature when focused on the caps of horizontally-oriented cv. Merit maize primary roots. Laser light of equal intensity and duration shown on more proximal regions of primary roots had a reduced effect on the gravitropic response.

A detectable response above the dark control level was demonstrated with light directed on the root cap (exposure time: 60 seconds; intensity: $2.75\times10^{7}W/m^{2}$). Exposures of equal intensity and duration made 0.3 and 0.5 cm from the root cap, as well as on the base of the root,

produced positive gravitropic curvatures which were (like the dark controls) considerably below the response level of cap-irradiated roots. Significant differences were assessed using the t-test. Differences between cap-irradiated and non-cap-irradiated roots including dark controls, were most apparent 2.5 hours following laser irradiation. No significant differences were encountered when making pair-wise comparisons of mean curvatures for non-cap-irradiated roots, including dark controls at the 2.5-hour mark (Tables 3.1A and 3.2).

Cap-irradiated roots were also shown to develop greater positive curvatures than comparison roots when the mean of all angles recorded between 2 and 3 hours were computed. Again, there were no significant differences between dark controls and non-cap-irradiated roots when pair-wise comparisons were made of values over the same time period (Table 3.1A). These results support the contention that the root cap is the principal, if not the only, locus of interaction with light which triggers rapid positive gravitropic movement in maize primary roots.

A slightly puzzling matter emerging from the data was that, though the absolute trough of gravitropic curvature was reached at 2 hours or 2 hours 10 minutes in all groups tested, it was not possible to demonstrate significant differences of the type outlined above at these more natural time points. Still, at both of these intervals (2 hours and 2 hours 10 minutes) the mean angles of descent generally increased with greater distance from the root cap (Table 3.1B).

A point of potential confusion in the interpretation of gravitropic response data presented in this section arises from the finding by Mandoli and Briggs (1984) that light piping (internal reflection) occurs upon irradiation of plant tissue. These investigators have reported the presence of light piping at distances greater than 2.0 cm from the incident light spot on irradiated root tissues using a helium neon laser light source. According to Mandoli and Briggs, plant tissues investigated (oat mesocotyl, oat coleoptile and maize primary root tissue) transmit light in the same way as a fiber optic bundle. Total internal reflection occurs when the angle of the incident

light beam exceeds a critical angle. Mandoli and Briggs reported that in each of these tissues the measured critical angle was 47°.

In fact, I did not discover a reliable method for directly measuring the beam width on root tissue. However, simple visual inspection made clear that referred laser light caused a slight reddish glow on adjacent root tissue up to 0.5 cm from the central and intensely bright laser spot.

As reported above (§2.2) the profile of the He Ne laser beam used in my investigations showed a decline of $1/e^2$ at a distance of 0.02 cm from the center of the focused beam. From Gaussian decay alone, we note that the beam intensity declines from $2.75\times10^{7}W/m^2$ to $5.5\times10^{-15}W/m^2$ in moving from the center of the beam to a point 0.1 cm away. Therefore, it is clear that any light effect induced as far as 0.5 cm from the target could not be produced by Gaussian decay; rather it must arise from the processes leading to internal reflection. As a result a gravitropic effect could be induced in a region of the root removed from the point of incidence if, via light piping, a threshold level for gravistimulation had been exceeded at the removed site. In order to nullify any confusion arising from this effect, I attempted to demonstrate light-potentiated gravitropic curvature at the point where the effect was just detectable above the dark control level. If such a point could be established for cap-irradiated roots, and no such response found upon exposure of more proximal regions, then the primacy of the cap as the perceptive organ in maize root gravitropism would be established unequivocally. Such a point of barely detectable curvature proved difficult to demonstrate in part because of the large variability in gravitropic response levels shown in cv. Merit primary roots. To begin with, in order to find the point of barely detectible curvature, sample sizes would have to be large enough to produce a response just above the dark control level. This task proved too taxing because of the difficulty of the experimental protocol and because of the limited number of roots which could be accommodated in each trial. In spite of having abandoned, for the present, the task of establishing

an absolute threshold level for light-potentiated root gravitropism, I believe that it is safe to conclude from the data presented here that the cap is the most sensitive, if not the only, region on the Merit maize primary root important as a receiver of light in gravitropism. Any apparent rise in the amplitude of positive gravitropic curvature shown in the data describing roots irradiated 0.3 cm or 0.5 cm from the root cap likely arises from the effect of referred light which reaches the cap tissue via internal reflection.

TABLE 3.1

Cv. Merit primary roots oriented horizontally at time $t = 0$ and subjected to He Ne laser irradiation for 60 seconds at intensity 2.75×10^{7} W/m^2. Significant differences assessed according to Student's t-test.

Average curvature denoted at 2 hours 30 minutes and between 2-3 hours according to the site of irradiation.

SITE OF IRRIDATION	CURVATURE AT +2 hr. 30 min. (degrees)	AVERAGE CURVATURE +2 hr. to +3 hr. (degrees)	NUMBER OF ROOTS
ROOT CAP	30.4 (±18.1)	28.5 (±17.0)	28
3 mm. FROM CAP	20.8 (±13.1) (a)	19.9 (±12.8) (a)	35
5 mm. FROM CAP	19.9 (±14.6) (a)	18.6 (±12.2) (a)	25
BASE OF ROOT	15.4 (±14.0) (b)	14.3 (±14.0) (b)	20
DARK CONTROLS	16.2 (±13.9) (b)	16.4 (±13.8) (b)	50

(a) Significantly different from curvature of cap-irradiated roots at $P=.05$ level.
(b) Significantly different from curvature of cap-irradiated roots at $P=.01$ level.

Average curvature denoted at 2 hours and 2 hours 10 minutes according to site of irradiation.

SITE OF IRRADIATION	CURVATURE AT +2 hr. (degrees)	CURVATURE AT +2 hr. 10 min. (degrees)	NUMBER OF ROOTS
ROOT CAP	31.6 (±18.8)	33.4 (±18.8)	28
3 mm. FROM CAP	28.0 (±15.1)	27.1 (±13.9)	35
5 mm. FROM CAP	24.0 (±11.0)	23.2 (±9.9) (a)	25
BASE OF ROOT	17.8 (±18.2) (a) (b)	17.4 (±16.9) (a) (b)	20
DARK CONTROLS	20.2 (±15.6) (a) (b)	19.8 (±14.4) (a) (b)	50

(a) Significantly different from curvature of cap-irradiated roots at $P=.01$ level.
(b) Significantly different from curvature of roots irradiated 3 mm. from cap at $P=.05$

TABLE 3.2

Cv. Merit primary roots oriented horizontally at time $t = 0$ and subjected to He Ne laser irradiation for 60 seconds at intensity $2.75\times10^{7}W/m^{2}$. Average curvatures indicated between 0 and 3 hours according to site of irradiation.

TIME	AVERAGE CURVATURE (DEGREES)				
	SITE OF IRRADIATION				DARK CONTROL
	ROOT CAP	3 MM. FROM CAP	5 MM. FROM CAP	BASE OF ROOT	
0 hr.	-0.8	-1.5	-1.7	-0.3	-3.4
20 min.	2.9	2.2	1.3	4.6	2.4
30 min.	6.4	5.3	4.0	7.5	5.7
40 min.	7.5	6.7	5.1	9.1	7.7
50 min.	7.4	7.1	5.2	8.1	7.7
1 hr.	8.0	7.5	7.2	8.3	8.1
1 hr. 10 min.	10.0	11.2	10.6	11.0	10.6
1 hr. 20 min.	13.4	15.2	13.4	12.1	12.8
1 hr. 30 min.	18.6	18.5	19.0	14.4	15.4
1 hr. 40 min.	23.1	23.2	22.6	15.0	18.6
1 hr. 50 min.	28.8	26.5	23.9	15.3	19.5
2 hr.	31.8	26.0	24.0	17.8	20.2
2 hr. 10 min.	33.4	27.1	23.2	17.4	19.8
2 hr. 20 min.	33.1	24.7	20.5	17.9	18.5
2 hr. 30 min.	30.4	20.8	19.9	15.4	16.2
2 hr. 40 min.	27.3	16.7	16.3	13.3	14.9
2 hr. 50 min.	24.4	12.9	14.3	10.1	13.0
3 hr.	19.2	9.0	12.0	8.5	12.3

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CHAPTER 4

THE KINETICS OF LIGHT-POTENTIATED PRIMARY ROOT GRAVITROPISM IN MAIZE

4.1: Literature Review

4.1.1: Light-Induced Root Growth Retardation

Burstrum (1960) demonstrated, in *Triticum*, that light has the effect of reducing the rate of root extension from the dark control level. Similar findings have since been documented in connection with other species including cress, maize, pea, lentil and rice. In contrast, Shen-Miller (1974) has reported that blue light can actually increase the rate of root extension above the dark control level. It has been shown in maize that light reduces both the rate of cell elongation and cell division.

Pilet and Ney (1978) used optical fibers to selectively irradiate the caps of vertically-positioned maize primary roots with white florescent light. In this way, they demonstrated a rapid decrease in the growth rate among light-exposed roots as compared with dark controls. In contrast, these authors discovered that irradiation of the region on the primary root which elongates most rapidly did not alter the mean growth rate from the dark control level. As a result they concluded that the cap is the site of photo-receptors which stimulate growth depression as well as downward curvature as a result of gravistimulation.[1] There is no definitive evidence that the same pigment molecule in the cap controls both effects. However, it is possible that the two processes are mediated by translocation of the same compound from the cap to the extension zone where growth retardation occurs. In the case of generalized growth retardation, the translocated

[1]We demonstrated in Chapter 3 that the cap is the receiver of light which stimulates positive gravitropic curvature in maize.

compound would produce slowing of the extension rate on both sides of the root. In the case of light-induced gravitropic bending, the compound would produce growth inhibition only on the lower side of the root. Gibbons and Wilkins (1970) have provided indirect evidence that the effects may indeed be linked via transport of the same substance. They showed that removal of half of the maize root cap causes curvature towards the remaining half-cap regardless of root orientation.

The difficulty in linking the two processes through the transduction phase is that the nature of the chemical inhibitor, or inhibitors, involved in the two processes have not been determined unequivocally. For example, some reports implicate abscisic acid as the operative substance involved in light-stimulated root growth retardation but ABA is probably not the inhibitory compound transferred during gravitropic bending. Therefore, either a different substance such as calcium is translocated in both processes or the two effects are not at all mediated by the same inhibitory substance.

The relationship between the two processes at a quantitative kinetic level also shows some differences indicating that the effects indeed may be unlinked. For example, Pilet and Ney (1978) have reported that a reduction in the rate of maize root elongation occurs between 5 and 10 minutes after light-exposure when the roots are positioned vertically downward. In contrast, there is strong agreement that light-stimulated gravitropic curvature occurs after a lag time of at least 20 minutes following the onset of the light-exposure and positioning of the root transverse to the gravity vector. For purposes of discussion in this chapter, we assume that the processes of light-stimulated growth depression and light-stimulated gravitropic bending are not mediated by the same substance.

4.1.2: Light-Induced Primary Root Gravitropic Curvature In Maize

Ney and Pilet (1981) conclude that cv. LG 11 maize primary roots allowed to grow and georeact in humid air for up to 10 hours in florescent light show three distinct phases of gravicurvature. First, a lag time of about 20 minutes occurs following re-orientation of the root from a vertical to a horizontal position before the start of gravicurvature. Next, a steep decline phase begins, lasting about 3 hours 30 minutes during which the root tip approaches a 70° angle. Finally, a feedback phase begins during which the root tip oscillates with an amplitude of 5° to 20° with a period of 1 hour.

Hillman and Wilkins (1982) used intact Kelvedon 33 maize primary roots oriented initially in a horizontal position and exposed them to incandescent light (intensity: $1.34 W/m^2$). They report that the profile of gravitropic curvature is characterized by certain common features. The process begins with a one-hour lag time followed by a period of rapid downward curvature which persists for 4 hours. Most roots attain angles of approximately 90° by the end of the steep decline phase. Then, a negative curvature phase begins, lasting 2 to 3 hours, until angles of approximately 45° have been reached. These authors also report the occurrence of subsequent cycles of curvature centered on angles of less than 90° with a 6-hour period. Such cycles continue on for at least 24 hours.[2]

In both papers cited, some suggestive data and curves are presented. However, there were no carefully documented and objectively-based statistics for other investigators to examine. As a result, an evaluation of conclusions presented must be based on the reader's perception of the investigator's scientific intuition rather than directly on the data. A scientist's intuition focused

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[2]The maize cultivars used by Hillman and Wilkins (1982) and by Ney and Pilet (1981) use light to enhance gravitropic bending. By contrast, other maize cultivars have been examined such as Anjou and Kiowa which develop positive gravicurvature irrespective of the presence or absence of light. A light-requiring cultivar (cv. Merit) and a non-light-requiring cultivar (cv. Pioneer) are examined in terms of possible allelic variations in Chapter 6 below.

on a particular problem for many years may well be very highly tuned. However, in a research area with so much admitted variability it is essential that statistical evidence be available to examine. Otherwise, it is impossible for the reader to evaluate the accuracy of the conclusions.

In general, what is missing in the literature is a coherent theory or firm biophysical treatment that joins together the various stages of root movement following gravistimulation in the presence of light. Here I attempt to clarify and extend some of the known results with data obtained from my own work. I then propose a biophysical model which links together the stages of root movement that follow gravistimulation under the influence of light.

4.2: Results

4.2.1: Introduction

In §4.2.2 through §4.2.6 I consider the profile of light-stimulated maize root gravicurvature in detail on the basis of my own observations and analysis for one particular cultivar of maize (cv. Merit). Although the investigation and results range over a number of topics that appear somewhat disconnected, the findings are brought together and explained in a speculative model given in §4.2.7.

I begin with an overview of the first cycle of root movement after light treatment. The latency period, the first positive curvature, and the first negative curvature are examined. Next, the data for a cyclic pattern of gravicurvature is explored. Finally, I investigate the possibility of entraining a pattern of cyclic gravicurvature by means of sequential light treatments.

In §4.2.7 a fluid-mechanical model for light-induced cyclic gravicurvature is presented. Some early iterations of this model are discussed in §4.2.8. (An alternate model is presented in Appendix A.) In §4.3 I then turn to a discussion of the findings of the model. The section concludes with a program for work to further test the validity of the theory. The chapter ends

§4.4 with an exploration of two issues which have a relative bearing on root gravitropism. These are the question of light-potentiated root gravitropism---as a threshold or a graded response, and the effect of green light pre-treatment on root gravitropism.

4.2.2: Overview of First Cycle of Gravicurvature in Maize Roots

The profile of gravitropic curvature was assessed for cv. Merit primary roots oriented horizontally at time zero, and exposed to incandescent light treatments as follows: two periodic light assays were performed with light pulsed on a 6 sec./10 min. schedule at intensities 1.9W/m^2 and 18.8W/m^2. Two continuous light assays were conducted at the same intensities. In addition, a dark control was carried out. The duration of the trials was 6 hours (Figures 4.1A--4.1E).

The broad result was that each test group showed a typical pattern of curvature which included a lag phase followed first by a steep decline period, and then by a less steep but pronounced rise. The mean time for the trough of the descent phase varied from 2 hours 30 minutes to 2 hours 50 minutes. The peak of the rise varied from 3 hours 50 minutes to 5 hours 40 minutes. The dark control group revealed a similar pattern (Table 4.1).

4.2.3: Latency Period and First Positive Curvature

All four test groups experienced a latency period ranging from 20 to 60 minutes at the start of gravistimulation. During this phase the roots remained unchanged from their initial horizontal positions. The latency period was followed by a brief ramp up to a period of fixed positive curvature (Table 4.2). All four test groups underwent periods of linear decline during the descent phase. The period of linear decline ranged from 50 to 90 minutes (Dixon and Massey,

1983).[3] The variation in the rate of decline for individual experimental groups ranged from .43° to .66° per minute. The dark control group was also characterized by a period of linear decline. The slope of the decline (.20° per minute) is considerably below the value for any of the non-control groups (Table 4.2).

The mean maximum amplitude of descent ranged between 42.7° and 66.8°. The dark control served as a base line with a mean maximum amplitude of decline of 17.6°. There is another characteristic of the first phase of positive curvature. That is, the amplitude of descent increases as a function of light intensity. (This relationship is explored further in §4.4.2.)

4.2.4: First Negative Curvature Phase

Data presented here shows that there is a rise in angle following the first decline phase. The rise occurs irrespective of the light regime. An examination of the four groups discussed above shows a mean negative curvature of 21° after the initial descent. The mean amplitudes of negative curvature fell in a range from 16.4° to 28.5° (Table 4.1).[4]

Following the initial decline, a small rise also occurred in the absence of all light. The mean amplitude of the rise was 3.8° for the dark control. Although small, this rise proved to be statistically significant at the 5% level.

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[3]This decline was determined by a statistical test of linearity which specifies that a regression is linear at a given confidence level.

[4]There was also a rise which occurred following the descent phase in gravistimulated roots exposed to helium neon laser light. The duration of the rise was at least 40 to 50 minutes. It is likely that this rising trend would have continued beyond 3 hours, but the experiment was concluded at this point (Table 4.8). The rise in angle following the first decline was even present in two trials where the roots were started below the horizontal at 31.3° and 40.6°. In these tests the magnitude of upward curvature was 19.5° and 16.4°, respectively (Figure 4.2).

4.2.5: Existence of Cyclic Gravicurvature

I next investigated the possible existence of a cyclic pattern of curvature which may persist beyond the end of the first negative phase. I use the discrete Fourier transform method applied to data from test groups subjected to an initial gravitropic stimulus in trials of 6-hour duration.[5] The mean power spectra for individual trial groups did not show the presence of any spikes at meaningful frequencies and thus no general pattern of oscillatory activity could be perceived. The single exception was the average transform for the dark control group. However, the spike present at 2 hours 15 minutes was not quite significant at the 5% level according to Shimshoni's extension of the Fisher test (Table 4.3).

Some individual roots did exhibit a pattern of oscillatory activity based on their Fourier transforms but these cases never constituted more than a third of the roots in any of the 6-hour trials. However, a striking similarity did exist among those roots whose transforms indicated the presence of cyclic behavior. When the power spectra for these oscillatory roots were examined 91% of roots (62 of 68) showed spikes of period length 2 hours 15 minutes. The totals included both light-exposed and dark control roots. This apparent uniformity points to the possibility that 2 hours 15 minutes may be near to the native period of oscillation among those gravistimulated roots which show a tendency to oscillate (Table 4.3).

The amplitude of curvature among roots showing cyclic behavior varied considerably. The amplitude ranged from 4.4° among cyclic dark controls to 26.3° among oscillatory roots

[5]The procedure followed in computing all of the discrete Fourier transforms of roots (exposed to a maximal gravitropic stimulus, at time zero) involved omitting the first 10 values in each data set. The reason was that the first stage of the initial decline is clearly not repeated. It was a somewhat arbitrary decision to eliminate exactly 10 data points but, for many roots, this equalized the amplitude of curvature represented by the new first point with the amplitude of the rise following the first decline (see below). In general, when the beginning values were included the test data sets did not exhibit significant spikes at meaningful frequencies. Instead the transforms declined monotonically indicating the lack of oscillatory activity.

exposed to continuous illumination at $1.9W/m^2$. [6]

I next explored the question of whether the cyclic pattern of curvature discerned in some roots continues in longer duration trials. I carried out a 12-hour pulsed incandescent light trial (frequency: 6 sec./10 min., intensity: $18.8W/m^2$) with roots started parallel to the ground (Figure 4.3A). A 12-hour dark control was also performed (Figure 4.3F).

The graph of averages for the pulsed light experiment revealed that the rise following the initial decline was succeeded by an apparent flattening of the curve. The angles ranged between 30° and 40° and persisted to the end of the trial.

I considered the following questions in connection with the above result. Does the flattening of the mean curve in the second half of the test period imply the actual existence of a liminal angle? Secondly, does a pattern of oscillatory activity continue among individual roots masked by a phase drift as the individuals become increasingly uncoupled from one another during the last 6 or 7 hours of the trial? In examining the graphs of individual roots it was clear that some roots showed a pattern of cyclic behavior which continued to the end of the test period. This result is similar to the case for shorter duration trials.

The discrete Fourier transforms of individual roots showed that 16 of 49 roots in the pulsed-light trial showed significant oscillatory peaks. The 12-hour dark control also suggested the presence of some oscillatory behavior. Here, 5 out of 18 roots showed a cyclic pattern of curvature (Table 4.4).[7]

I next conducted a 12-hour trial with light pulsed on a 3 min./hr. schedule. In this case

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[6]The amplitude of curvature we define here as half the number of degrees between the average of the local minima and the average of the local maxima. Note that this is not equivalent to the maximum ordinate found in the periodogram, because of the presence of several strong modes.

[7]The mean amplitude of curvature among cyclic roots in the 12-hour trials was 5.9° for dark controls and 10.48° among light-exposed roots.

the graph of averages closely resembles the trial with light pulsed at a frequency of 6 sec./10 min. (compare Figures 4.3B with 4.3A). In the 3 min./hr. trial, the first cycle is clearly demonstrable. Thereafter, the graph of averages becomes relatively flat ranging from 36.5° to 43.9° in the interval from 3 hours 40 minutes to 12 hours. In both trials only one third of the roots display power spectrum spikes while the transform of the graph of averages did not indicate oscillatory activity (Table 4.4).

4.2.6: Entrainment of Cyclic Gravicurvature

I next investigated the possibility of entraining the pattern of cyclic gravicurvature by pulsing incandescent light on a preset schedule in a 12-hour trial. Four hours was chosen as the time between light pulses because it is the approximate interval between the beginning of gravitropic stimulation and the end of the first rising phase of curvature (Figure 4.3D). I reasoned that the first cycle would be completed after 4 hours and therefore the roots would be optimally prepared to transform another light pulse into another cycle of oscillatory curvature. This hypothesis was confirmed.[8]

Three clear cycles of curvature were demonstrated with remarkable consistency. The pattern was born out in the discrete transform data (Table 4.4) and in the time average of the raw data (Figure 4.3D). The mean value of the discrete Fourier transforms indicates a substantial peak at a period of 3 hours 30 minutes. (This is the sampling period nearest to 4 hours in the power spectrum.) In addition, analysis of the individual power spectra showed spikes at 3 hours 30 minutes in 21 of 28 roots.

In a follow-up trial I pulsed incandescent light every 2 hours and continued for 16 hours,

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[8]At the same time, if two hours were the approximate period of the fundamental wave then a schedule of pulses equivalent to an integral multiple of this period should also produce entrainment.

skipping the pulse at 2 hours (Figure 4.3C). This trial was conducted to test the theory that 2 hours between light pulses represented the shortest interval for the entrainment of cyclic gravicurvature.

The graph of averages for the 2-hour trials presented difficulties in interpretation. On the one hand, the graph shows the presence of cyclic activity during the first 11 hours. However, it seems unlikely that a true resonance effect was achieved as a result of the 2-hour spacing between light pulses. Examination of the graph of averages shows that the light pulse schedule does not appear to be quite in phase with a natural period of oscillation. I carried out a total of four discrete Fourier transforms over different intervals of the data in an effort to determine the actual effect of the light pulses on the cycling rate.[9] Examination of the power spectra for each of the intervals indicated that a minimum of 26 out of 35 roots showed at least one significant periodogram spike. However, analysis of the power spectrum data also produced an ambiguous result. The transforms of the graphs of averages display spikes at periods ranging from 5 hours 15 minutes to 2 hours depending on the interval examined (Table 4.5). In addition, there is considerable variation in peak periods represented among individual transforms for each interval. For example, in the interval from 4 to 10 hours, 14 roots showed power spectrum peaks at 2 hours, and 12 showed peaks at other periods.

Finally, I conducted a trial to determine the result of giving one 3-minute light pulse at the start of gravistimulation and a second at 2 hours 10 minutes (Figure 4.3E). The second pulse was timed to coincide with the trough of the descent period.[10] The result was that the roots bent

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[9]The time intervals were 1 hour 40 minutes to 8 hours, 1 hour 40 minutes to 10 hours, 1 hour 40 minutes to 12 hours and 4 hours to 10 hours.

[10]Here, the intention was to introduce the second light pulse as close to the absolute trough of the descent phase as possible. Since this point was reached at +2 hours 10 minutes in Experiment 4.11 (Figure 4.3D)---comparable to the trial in question---the second light pulse was given at the same point in Experiment 4.12 (Figure 4.3E).

upwards but after a delay of about 1 hour. In this context then, there is a lag which appears to result from the timing of the second light pulse. Evidently, the second pulse does not have the power to initiate further downward bending. However, it does serve to postpone rather than to eliminate the negative phase of the gravitropic cycle.[11]

Another apparent feature of cyclic gravicurvature is that the amplitude of successive waves diminishes with time.[12] For example, in Experiment 4.11, the three waves which occur in the mean time series data decline in their amplitude from 16.0° to 6.0° to 3.55° (Figure 4.3D). A similar result emerges from an examination of the mean data for cyclic roots in all of the 6-hour test groups. In each of the nine groups studied, the graph of averages reveals that the amplitude of the first wave is greater than that of the second wave. The ratios range from 2.8:1 to 1.21:1. (The experiments referred to include those depicted in Figures 4.1 and 4.2 as well as Experiments 4.17 and 4.18 from Figure 4.10B.)

What then do we know about the phenomenon of cyclic gravicurvature in maize primary roots? The effect is characterized by a persistent wave-like pattern of curvature which is excited by the combined influences of light and gravity. The data from all of the trials examined shows that the first wave is consistently longer in duration than subsequent cycles. The effect is stimulated by light but it can also occur in the dark. However, the wave amplitude is considerably

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[11]The supporting evidence on this point is as follows: in Experiment 4.12, one hour following the second light pulse, the mean angle of curvature had only risen by 2.1°. By contrast, the minimum rise in mean angle was 10.9° over a comparable period taking into account all of the test groups evaluated.

[12]I used the following method to measure the amplitude of successive waves in the graphs of averages for individual trials. Generally, the cycle length of interest was dictated by the highest-order statistically-significant peak in the power spectrum in each instance. For example, if n = 3 was the peak value of the power spectrum, then the data set would be divided into 3 equal intervals with each interval representing the period length of a single wave. The angle of curvature at the midpoint of each period was then subtracted from the average of the angles at the beginning and end of the period to give a value equal to twice the amplitude of the wave.

reduced in the dark. In 12-hour- and 16-hour-long trials the periodic light treatments (6 sec./10 min. and 3 min./hr.) induced the effect in about a third of the roots. When the frequency of light pulses was diminished to 3 min./2 hr. there was a substantial rise in the proportion of roots which exhibited cyclic gravicurvature. However, the power spectra for individual roots indicated a mixture of significant peaks suggesting that the proposed resonance effect was still not achieved. By reducing the frequency to 3 min./4 hr. the percentage of roots which manifested cyclic gravicurvature rose again. In this case, 24 of 28, or 86%, of roots exhibited the effect. Of these, nearly all showed power spectrum spikes close to 4 hours.

Finally, if a light pulse is timed to correspond to the trough of the first wave it does not eliminate the subsequent negative curvature phase. However, such a timed pulse does prolong the onset of the rising phase of the curve.

4.2.7: A Model of Light-Induced Cyclic Gravicurvature

Preliminary Considerations:

The fundamental change which takes place in the root during gravitropic bending is differential growth above and below the central root axis. Although the change in root tip angle with respect to time is the descriptive measure of gravicurvature used by most researchers, the effect is merely the consequence of a process of asymmetrical growth. The growth process is complicated by the fact that the root cap serves as a sensor of the root's position with respect to both light and gravity (see Chapter 3). A further complicating factor is that the differential growth response occurs principally in an area between .2 and .4 cm behind the root cap. Therefore, there must be communication between the root cap which is the organ of perception and the growth zone which is the organ of response. In addition, there must also be a biochemical linkage at the level of the root tip between the absorption of light and the sensation of gravitational position.

With the above considerations and descriptions in mind, I propose the following model beginning with the process of asymmetrical growth.

Assumption 1: The root is deflected entirely by the growth length difference between the top and bottom of the growth zone. The location of the growth zone is constant in relation to the root cap[13] while the growth accumulates on the proximal side of the growth zone. Thus, the angle which the root tip forms with the horizontal is directly related to the difference in the rate of elongation above and below the central root axis.

Assumption 2: All growth in the growth zone occurs through the elongation of existing cells rather than through cell division. Growth is retarded by the presence of an inhibitor produced at the root tip and transported to the growth zone. The effect of the inhibitor is assumed to be felt immediately. The retardation of growth at the bottom and top of the growth zone is proportional to the respective concentrations of inhibitor present in those regions.

Assumption 3: Inhibitor is produced in the root tip and transported to the growth zone along the bottom of the root entirely by diffusion.[14]

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[13]This is a simplifying assumption in the sense that there is not actually a discrete point on the root which constitutes the growth zone. The zone of maximum elongation in maize primary roots has been shown to be from .2 cm to .4 cm behind the root cap. Interestingly, Buff, Baake and Sievers (1987) have shown that growth can occur along the entire length of the primary root. However, their data indicates that the rate of elongation declines sharply in moving away from the zone of maximum elongation.

[14]Basipetally-directed vascular flow occurs in the xylem rather than in the cortex of the root. Because we model the flow of the inhibitor along the perimeter of the root we assume that diffusion is the primary transport process. In addition, the assumption of non-convective flow is supported numerically by the fact that the diffusion-based model captures the essential features of the simulation curves (Figures 4.7 and 4.8).

It is of course, possible that the axial movement of inhibitor from tip to growth zone occurs via facilitated diffusion rather than through simple diffusion. For example, when calcium ion is exposed to an electrical force field it can alter the properties of membranes in such a way as to regulate the uptake and/or expulsion of specific materials. At least two laboratories have presented data supporting a role for calcium in root gravitropism as was discussed in §1.3.3. However, without a more concrete understanding of the biochemical details of the process and of the role for calcium, we propose that the transport of inhibitor is by diffusion.

Assumption 4: Upon reaching the growth zone the inhibitor is carried in a circumferential flow around the central root axis.

Assumption 5: The concentration of inhibitor at all points is reduced exponentially in time by the processes leading to chemical disappearance.

Mathematical Treatment:

The goal of the model is to calculate $\alpha(t)$, the angle subtended between the growth vector and the horizontal. Mathematically, the problem can be formulated as follows:

Let ℓ_1 and ℓ_2 be the lengths of the growth zone at the bottom and top, respectively. Let d be the diameter of the root at the growth zone. Then, as can be seen in Figure 4.4, $\alpha(t)$ can be expressed by:

$$\tan(\alpha(t)) = \frac{\ell_2(t) - \ell_1(t)}{d}. \tag{1}$$

Let C_1 and C_2 be the concentrations of inhibitor at the bottom and top of the growth zone. Then, by assumption 2, we have:

$$\frac{d\ell_1(t)}{dt} = R - \kappa C_1(t), \qquad \frac{d\ell_2(t)}{dt} = R - \kappa C_2(t), \tag{2}$$

where κ is the constant of proportionality in units of growth rate/concentration and R is the rate of growth if no inhibitor is present.

Then taking the time derivative of Equation 1 and using Equation 2, we see that:

$$\frac{d(\tan\alpha)}{dt} = \frac{\kappa}{d}(C_1(t) - C_2(t)). \tag{3}$$

Thus, the difference in inhibitor concentration across the growth zone determines the elongation

pattern above and below the root axis which in turn controls the deflection of the root tip.

Assumption 3 above proposes one-dimensional diffusion of the inhibitor along the bottom of the root. Let $c_{diff}(x,t)$ be the concentration of inhibitor along the root as a function of time t and distance x from the root tip. The diffusion of this concentration is governed by the equation:

$$\frac{\partial c_{diff}}{\partial t} - D\frac{\partial^2 c_{diff}}{\partial x^2} = f(t), \tag{4}$$

where D is the diffusion constant[15] and $f(t)$ is the source term at $x=0$.[16]

This source term corresponds to production of inhibitor at the root tip. As will be seen later, the source term for this model is:

$$f(t) = \begin{cases} \cos(\alpha(t)) & \text{for } t_0 < t < t_{cutoff}, \\ 0 & \text{otherwise}. \end{cases} \tag{5}$$

Here t_{cutoff} is the point at which inhibitor is no longer produced.

In assumption 4 we propose a circumferential flow of inhibitor at the growth zone as a mechanism for transport of the inhibitor between the bottom and top of the root. Let $c_{rot}(\theta,t)$ be the concentration of inhibitor around the circumference of the growth zone, oriented so that

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[15]For calculation purposes we use a value for D of $.5\times10^{-9}\text{m}^2/\text{sec}$. Because the specific chemical identity of the inhibitor is unknown we select an intermediate value for D based on the tabulated value for sucrose (Nobel, 1983). This value is near to the molecular weight of a small plant hormone like auxin or abscissic acid and it is, therefore, probably in the correct range.

[16] For a unit source at time $t = 0$, we know the concentration is simply the Green's function of the diffusion operator so that:

$$c(x,t) = \frac{1}{\sqrt{4Dt}} e^{-x^2/4Dt}.$$

Figure 4.6 shows the value of the concentration at the location of the growth zone as a function of time, for a unit source at $t = 0$. Thus, the solution at any time t is given by a convolution of the source term with the Green's function:

$$c(x,t) = \int_0^t \frac{1}{\sqrt{4Dt'}} e^{-x^2/4Dt'} f(t-t')dt'.$$

$\theta = 0$ is at the bottom of the root, $\theta = \pi$ is at the top, and the flow is in the positive θ direction. Then after each time increment Δt we define:

$$c_{rot}(0, t + \Delta t) = c_{rot}(0, t) + c_{diff}(x_g, t + \Delta t) , \tag{6}$$

i.e., the concentration at the bottom of the growth zone at the new time is the concentration which has diffused to that point from the root tip added to the concentration already in the circumferential flow. Here, x_g is the location of the growth zone along the x-axis. All concentrations are set to zero at $t = 0$.

Within the growth zone we see that:

$$c_{rot}(\pi, t) = \begin{cases} 0 & for \quad t < t_f , \\ c_{rot}(0, t - t_{rot}) & for \quad t \geq t_f , \end{cases} \tag{7}$$

where t_f is the time of the first arrival of inhibitor at the top, and t_{rot} is the time between inhibitor leaving the bottom and reaching the top. For a uniform angular speed ω, $t_{rot} = \pi/\omega$.[17]

We define here:

$$C_1(t) = c_{rot}(0, t) \quad , \quad C_2(t) = c_{rot}(\pi, t) . \tag{8}$$

Assumption 5 presents the process of chemical disappearance of the inhibitor. This is modeled by multiplying $c_{diff}(x,t)$ and $c_{rot}(\theta,t)$ at each time step by $e^{-k\Delta t}$, where $k = (\ln 2)/T_{1/2}$. Here $T_{1/2}$ is the half-life of the inhibitor, i.e., the time in which the concentration is reduced to half of its previous level.

Numerically the computation is broken into two stages. First, the diffusion equation

[17] For a nonuniform angular speed $\omega(t)$, $t_{rot}(t)$ is defined by:

$$\int_{t-t_{rot}(t)}^{t} \omega(t') \, dt' = \pi .$$

(Equation 4) is solved using the second-order accurate Crank-Nicholson method (Sod, 1986).[18] The solution c_{diff} is multiplied by the exponential factor mentioned in Assumption 5, and then is evaluated at $x = x_g$, and this value is added to $c_{rot}(0,t)$ according to Equation 6. The concentrations $C_1(t)$ and $C_2(t)$ are defined according to Equation 8, after the values of c_{rot} have been diminished by the exponential factor. (The model-generated curves are plotted with the graphs of averages of the data for purposes of comparison in Figures 4.7 and 4.8. The parameters used in the model are listed in Table 4.6.)

Details of Inhibitor Production:

Molecular sedimentation of geosensor molecules G occurs in the columella cells of the root cap under the influence of gravity. We assume these molecules are heavier than the surrounding liquid medium, and thus sink under the influence of gravity. Hence, the effective force on the particles is proportional to $cos(\alpha)$, so that the rate of sedimentation is also proportional to $cos(\alpha)$.[19] It is this fact which causes the source term $f(t)$ to have the form it does in Equation 5.

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[18]The Crank-Nicholson method for the equation $\frac{\partial u}{\partial t} = \mathcal{L}u$ (in our model, $\mathcal{L}u = \frac{\partial^2 u}{\partial x^2} + f(t)$) is written:

$$u^{n+1} = u^n + \frac{\Delta t}{2}(\mathcal{L}u^n + \mathcal{L}u^{n+1}) \quad ,$$

where u^n is the solution at time $n\Delta t$, u^{n+1} is the solution at $(n+1)\Delta t$.

[19]From a modeling standpoint this is equivalent to the situation in which the molecules are lighter than the surrounding medium. In this case G would undergo flotation and trigger a process leading to advancement of the elongation rate along the upper surface of the root.

The sedimentation of G triggers the formation or activation of inhibitor hormone I.[20] A small quantity of I is produced when the root is gravistimulated in the dark. In the light however, a greater quantity of I is generated as follows. The absorption of a light quantum causes a pigment molecule to become converted into an active form (conversion of P* to P) which in turn interacts with a precursor molecule to transform this molecule into the active form of the inhibitor (conversion of I* to I).[21] Even in the presence of light, however, only a finite supply of precursor substrate I* is available at one time. Thus in our model, we assume that the inhibitor I is produced over a period of 1 hour. The pool of I* can be replenished if sufficient time elapses between stimulating light pulses. If the interval between light pulses is not sufficiently long then the pool of I* is not replenished and the elaboration of growth-controller I from the tip ceases.[22] The inhibitor is then expelled through the lower membrane of columella cells where it enters the apoplast. The steps described here comprise t_0, the time delay in gravitropism. From $t = 0$ to t_0, the elongation rates at the top and bottom of the growth zone remain equal in the absence of inhibitor and as a result the root remains undeflected during this period.

Details of Rotation:

In our model the stream of inhibitor moves, by convection, in a circumferential direction

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[20]Pickard (1985) suggests that the process leading to hormone activation might involve the sedimentation of amyloplast-geosensors onto the rough ER. The ER is then stimulated to secrete calcium into the cytoplasm near the lower plasmalemma of the geostimulated cells.

[21]It is possible that phytochrome is the pigment molecule involved in this phase of the process. Feldman and Briggs (1987) have shown that the photo-molecular conversion of phytochrome plays an integral, although as yet undefined, role in the process of positive maize root gravitropism.

[22]From §4.2.6 above it appears that a dark period of 1 to 2 hours between stimulating light pulses is required to restore a sufficient concentration of precursor substrate to permit the production of more inhibitor.

around the growth zone.[23] The movement of inhibitor is assumed to begin at time t_0 after the first gravity/light impulse. The flow rate of the inhibitor stream accelerates from zero to a constant angular velocity which is reached near the end of the first rotation. Thus, the first rotation is assumed to have a longer period than subsequent rotations. In turn, this results in a first cycle of gravicurvature which is longer than subsequent cycles. (As noted, this is a consistent feature of the data.)[24]

Another feature of the model is that there is a finite pool of inhibitor produced as a result of the first gravity/light stimulus. Thus, a point is reached at which the concentration of inhibitor

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[23]There are some general considerations which support the idea of a circumferential flow of inhibitor. In particular, it is widely accepted that plant organs grow in a helical pattern around a preferred direction of growth defined in relation to the plumb line. Such a pattern when applied to roots should involve the coordinated extension of cells on the perimeter of the growth zone. The pattern of extension develops in such a way that adjacent cells are more or less in phase in terms of the timing of their elongation while cells which are across the root from one another are exactly out of phase in terms of the timing of elongation. Our model predicts such a pattern of coordinated growth. It achieves this effect by assuming that inhibitor molecules retard the elongation rate of cells in their path as they are transported, in one direction, around the perimeter of the growth zone.

In reality, however, we are not entirely satisfied with this stage of the model because of the requirement for convective flow of inhibitor around the growth zone. Convection implies that the transported material is pumped as in a vascular flow. It also presupposes the existence of channels through which the pumped material would be carried. The direction of vascular flow is primarily axial in the root and therefore a specialized region of convective flow has to be introduced in order to accommodate this feature of the model.

One must also be concerned about the energetics of inhibitor flow in the growth zone. We might speculate that the absorption of light quanta at the root tip leads to the production of ATP which in turn helps to power the flow of inhibitor.

[24]Here, we might propose an alternative to a somewhat arbitrary speed-up of inhibitor flow around the periphery of the growth zone. Assume for the sake of argument that the rate of circumferential inhibitor transport in the growth zone is dependant on the sine of the angle between the root tip and the horizontal. In this way the rate of inhibitor transport would increase as the root bends downward and would settle at a nearly constant rate of movement at the approach of the liminal angle. Preliminary testing of a modified model which incorporates this assumption indicates general agreement with the experimental data.

At the same time we might consider the possibility that convective flow in the growth zone is not merely limited to the transport of inhibitor. Instead there could be a general circumferential flow of material in which the inhibitor participates. Such an assumption does not alter the mathematical formulation of the problem.

at the bottom of the growth zone begins to decrease. As the flow of inhibitor around the growth zone continues the concentration of inhibitor at the top of the growth zone eventually exceeds that at the bottom. As a result the root tip begins to bend back upwards. Even so, the maximum amplitude of the negative bending phase is less than that seen in the initial descent phase. The damped effect is the result of the exponential decline in inhibitor concentration, as well as the residual concentration at the bottom of the growth zone. For this stage in the model we ignore the diffusion of inhibitor during cell rotation.

Secondary Cycles of Gravicurvature:

At the end of one cycle of gravicurvature (equivalent to the movement of inhibitor around the growth zone) and assuming no new inhibitor flow from the tip, the old flow continues to circulate in the growth zone. The result is successive, damped cycles of downward followed by upward movement of the root which persist until the inhibitor disappears through the processes leading to chemical dissipation.[25] Finally, when the supply of inhibitor is exhausted the root adopts a liminal angle.

If a second effective light pulse is given, for example, at the end of the first cycle of inhibitor flow in the growth zone a new flow of inhibitor is initiated which adds to the residual flow from the first cycle. The pool of new inhibitor is smaller because of the changed root tip angle (see Equation 5 above). The additional inhibitor produces a second cycle of curvature still damped in relation to the first but larger than what would have been expected without any new inhibitor flow.

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[25]The forces responsible for the exponential loss of inhibitor might include chemical degradation as a result of friction as well as chemical conversion into other species due to the involvement of the inhibitor in biochemical pathways.

4.2.8: Model Development

At this point we digress a bit to discuss the development of the model and to contrast the simulated results from the final model with results taken from some early formulations of the problem.

In the original formulation of the model we assumed that cells themselves rotate within the growth zone, rather than the inhibitor itself convecting around the circumference. By numerical testing and further analysis, it was ascertained that this formulation cannot give results which are consistent with the data, for the following reason. Consider the model as explained in the previous section, but with the cells in the growth zone rotating. This means that cells which are initially at the bottom of the root receive inhibitor starting at $t = t_0$. These cells rotate once inhibitor is present, and thus each cell receives only a limited amount of inhibitor before moving on in the rotation. Once a cell has inhibitor, its growth rate is retarded proportionally to the amount of inhibitor present. The maximum angle of decline is reached at the peak of the diffusion curve (Figure 4.6), assuming that this peak occurs before the first cell from the bottom has reached the top. Now consider the time when the cell which was at the bottom at $t = t_0$ reaches the top. This cell has contained inhibitor during its entire half-rotation, thus its growth has been retarded for that time. In contrast, the cell now reaching the bottom (which was at the top at $t = t_0$) has received no inhibitor, and thus has been growing at its full rate for the entire half-rotation. The root now rises dramatically, because the cell reaching the top is so much shorter than the cell now reaching the bottom, and this difference in length is greater than the difference in length at the maximum decline, precisely because the cell reaching the top has contained inhibitor for so much longer.

Note that this result is independent of the duration of inhibitor production and the rate of chemical disappearance; it is entirely an effect of the fact that the cells which determine the

growth are rotating.

The fact that with this model the rise after a half-rotation is greater than the initial decline is entirely inconsistent with the data, which shows a rise smaller than the initial decline. Thus, for modeling purposes, we changed the assumption, and allowed only inhibitor to rotate, while the elongating cells remain in place.

With this assumption in mind, we next considered different rates and durations of production of inhibitor. Figure 4.9 displays simulations from two early iterations of the model. In the model's first iteration, inhibitor was produced by the tip at a constant rate continuously throughout the trial. In the second iteration, there was continuous production of inhibitor at a rate varying with the cosine of the angle between the root tip and the horizontal.

Also shown in this figure is the data from the pulsed light trial with light frequency 6 sec./10 min. The general shape of the simulation curves reflects some key features of the data, i.e., a steep decline phase followed by a small rise and a leveling off at a liminal angle. But the simulations shown here do not capture the full rise. We observe that the simulation including the gravitational feedback term is more consistent with the data than the simulation without. As a result we tried a limited flow of inhibitor, and saw that the version of the model containing finite inhibitor and gravitational feedback best fit the data. This is consistent with the physical explanation that there is a limited supply of inhibitor precursor I*, and thus a limited flow of inhibitor, either for continuous or rapidly-pulsed light.

In Figures 4.7C and 4.7D (data) we observe later oscillations which are not seen in Figures 4.7A and 4.7B (data). We model this by having these more widely spaced pulses of light generate new flows of inhibitor, of the same duration as the original pulse. When the pulses are 4 hours apart (Figure 4.7D), the model best fits the data if the production of inhibitor due to later pulses is the same as the production of inhibitor due to the original pulse. When the pulses are

only 2 hours apart, however (Figure 4.7C), the best fit occurs with later flows scaled to be .8 times the magnitude of the previous flow. We conclude therefore, that I* would have been fully regenerated in a time between 2 and 4 hours following a light pulse.

As a final comment on model development we note that there are other ways of conceptualizing the problem which could also lead to a close fit between data and theory. We have carried out preliminary numerical testing on one such model. This model features transport of two different inhibitors from the root tip to the growth zone, rather than depending on circumferential flow of a single inhibitor at the growth zone. The model is presented in Appendix A.

4.3: Discussion

In light of the biophysical model just described, various aspects of the experimental data (§4.2.2--§4.2.6) come into sharper focus and begin to link together. In the next several pages we will re-cover some of this ground and point out its connection and variance with the model. In addition, a program of experimental work required to test the validity of the general theory will be proposed.

1. The 20- to 60-minute latency period (§4.2.3) is not entirely accounted for in the t_0 term of the model. In general, the lag time prior to significant gravitropic bending is composed of two phases:

a. The formal t_0 period. That is, the time between gravistimulation and the beginning of diffusion.

b. The transport time for a sufficient number of inhibitor molecules to diffuse from tip to growth zone such that positive curvature just becomes detectible.

The model and data agree that gravitropic curvature $d\alpha/dt = 0$ (Equation 3) during the first ten minutes. However, the model provides that curvature begins immediately after t_0. At this stage of the model the first molecules are assumed to defuse from tip to growth zone virtually instantaneously where their effect on growth retardation is also assumed to be immediate. Analysis of the data actually gives a somewhat different picture. The data indicates that positive curvature is not detectible for some minutes after t_0. This accounts for the 20- to 60-minute lag time given in §4.2.3.

2. The fact that it was possible to make a straight line fit with the steepest portion of the decline phase for several of the data sets (Table 4.2) is justified by the model in a qualitative sense. Figure 4.6 shows the concentration at the growth zone due to diffusion of inhibitor produced at the tip over a period of 1 hour. This curve rises very slowly for about 20 minutes, then steepens to look nearly linear and finally tails off. The middle portion of the diffusion curve is comparable in timing and general appearance to the linear period of descent seen in several of the experimental trials. Such a correlation between simulation and experiment supports the idea of a diffusion-based process.

3. The similarity between the experimental curves in the first cycle of curvature (0 - 4 hours) dictated some of the parameters of the model. For example, it is necessary to account for the existence and timing of a rising phase in the data (§4.2.4). It is also necessary to account for the finding that the rising phase is smaller in amplitude than the initial descent period. The model incorporates the presence of a rising phase whose duration is shorter than the descent period by providing that the growth zone rotate around the central root axis at a speed which increases linearly during the first revolution. The linear increase in speed shortens the duration of the

second half of the rotation in relation to the first half. This makes it possible to develop a simulation with a rising phase roughly between 2.5 and 4 hours. The simulation matches the experimental result in this respect.

The finding that the rising phase was smaller in amplitude than the descent phase is satisfied by the model in two ways. First, the exponential decline in inhibitor concentration ensures that the magnitude of C_2 during the rising phase is less than the magnitude of C_1 during the descent period. Second, the residual presence of inhibitor at the bottom of the growth zone during the latter stages of the first rotational cycle served to reduce the effect of C_2 on upward curvature. The fact that these experimental features could be reproduced by the simulations supports the model.

4. In §4.2.3, we noted the presence of a positive correlation between light intensity and downward curvature. The model incorporates this result by scaling the constant in Equation 2 to the trough angle of the descent phase. Therefore, the constant is a direct numerical measure of the concentration of inhibitor produced by the tip. (The constants for various trials in which light intensity was varied are presented in Table 4.7.)

5. In §4.2.5, we noted that as many as a third of gravistimulated roots showed signs of persistent cyclic motion even in the absence of a pattern of entraining light pulses. This finding applied to light-exposed roots as well as to the dark controls (Table 4.3 and Table 4.4---Experiments 4.8, 4.9 and 4.13). This result is interpreted in light of the model as follows. The model predicts that cyclic root motion will continue until the inhibitor disappears completely from the growth zone. When the amount of inhibitor produced at the tip is modeled to shut off completely after a finite time period the first cycle of curvature is very pronounced. However, there is an indication of

a low amplitude second wave as well (Figures 4.7A and 4.7B). As noted the cyclic pattern of curvature displayed by some of the roots indeed appears to reflect the predicted behavior of the model.

6. In all of the test groups examined, a general characteristic of the data is a high degree of scatter around the mean. The standard deviation in the graphs of averages is often as large as 15 degrees. In Chapter 6, I explore the trait of graviresponsiveness in maize roots from a genetic stand point. I conclude that there is no simple one- or two-gene system governing the extent of graviresponsiveness in the two varieties of maize roots tested.

The model presented above may offer some insight into what then appears to be a trait controlled by physiological variation at the level of the individual plant. We have observed that the model is quite sensitive to several of the parameters given in Table 4.6. Variations in these parameters could easily be due to the physiological differences among plants. Thus the magnitude of variation we could reasonably expect between plants may be enough to produce the large scatter in the observed data.

7. In §4.2.6 we discussed the result of a trial with light pulsed at the beginning of gravistimulation, and then again at 2 hours 10 minutes (Figure 4.7E--data). The result of the second pulse was to delay the onset of the rising phase of curvature. The pattern of inhibitor flow predicted by the model accounts for this delay in upward curvature in the following way. The second light pulse is timed to coincide with the conclusion of the initial descent period. This corresponds to the time when the concentration of inhibitor at the top and bottom of the growth zone have become equal. Without the new flow of inhibitor, the growth zone would continue to rotate producing a period of asymmetrical inhibitor concentration in favor of the top. Given the

new flow of inhibitor however, the inflow at the bottom would, for a time, overwhelm the concentration of inhibitor at the top of the growth zone. This has the effect of postponing the period of upward bending. This effect is seen clearly in both simulation and data (Figure 4.7E).

8. As noted in §4.2.6 the graph of averages for the trial with light pulsed on a 3 min./4 hr. schedule strongly resembles a damped sine wave (Figure 4.7D--data). However, the proposed 4-hour resonance frequency can not be directly related to the modeled rotation speed of the growth zone. The maximum rotation speed of 1 revolution per 2 hours is reached approximately 3.5 hours after the original gravity-light impulse. In fact, it is not intuitively clear why a 4-hour interval between light pulses produces a resonance effect. Some of the factors involved include: the characteristic speed of diffusion of the inhibitor; the shape of the diffusion curve; the speed of rotation of the growth zone; the concentration difference from top to bottom of the growth zone over time; and the time to restore the inhibitor precursor I* molecule. Given this complex of dynamic processes, it is my view that computer-based numerical integration is the only viable method to establish a connection between experiment and theory in this case.

9. Finally, we note that the partial entrainment of cyclic gravicurvature achieved with light pulsed at 2-hour intervals is lost completely after about 11 hours. The graph of averages becomes relatively flat from 11 to 16 hours even though the train of light pulses continues. This trend is confirmed in the simulation (Figure 4.7C). The abrupt cut-off of oscillatory activity suggests that the pool of inhibitor precursor I* is depleted to the point where further cyclic activity is prevented. In the model, progressive depletion of I* is accomplished by stepping down its production rate in the tip to 80% of the previous release for each effective light pulse. Following this line of reasoning the inhibitor present in the growth zone will eventually disappear and the root will

assume a liminal angle.

A number of theory-based predictions can be drawn from the model as a means of testing its basic validity:

1. How would a phytochrome-lacking mutant respond to light stimulation in the presence of transverse gravity? We assume that the primary root of such a mutant would behave like a dark-exposed gravistimulated root. In other words, it would undergo limited downward curvature followed by a slight rise. Such a result would add weight to the finding of Feldman and Briggs (1987) that phytochrome is the pigment molecule involved in light-stimulated maize root gravicurvature.

2. What would be the effect of positioning the straight root below the horizontal at the start of gravistimulation in the presence of light? According to the model (§4.2.7), a diffusion gradient draws the inhibitor from tip to growth zone. In the horizontal root, once downward bending begins, the transport of inhibitor as a result of diffusion would be countered by the oppositely-directed gravitational force.[26] The resistive effect of gravity would be more dramatic in the case of the straight root positioned below the horizontal at the beginning of the test period. Here, we might expect both the reduction in the speed of diffusion of the inhibitor as well as a decline in the quantity of inhibitor released at the tip.

The reduction in the speed of inhibitor movement would be seen as an increased lag time prior to the start of noticeable downward gravitropic bending. In fact, this longer latency period is

[26]The countervailing effect of gravitation on the diffusion of inhibitor is not included in the mathematical model for the sake of simplicity. It is logical, however, that such an effect should exist.

observed in Figures 4.2A and 4.2B.

The model also predicts a decline in the quantity of inhibitor released by the tip. The source term from Equation 5 provides that the quantity of inhibitor produced depends on $cos(\alpha(t))$. As a result, a straight root positioned below the horizontal would produce a smaller quantity of inhibitor than a straight horizontal root. From this, we might expect a smaller net curvature from the straight root tipped below the horizontal. A reduction in net curvature can be seen when comparing either Figure 4.2A or Figure 4.2B with Figure 4.1B.

3. What does the theory predict about the growth direction of the root positioned vertically downward? Both the primary model in §4.2.7 and the alternate model in Appendix A provide for inhibitor movement towards the outer margin of the gravistimulated root under the influence of a sinking and/or a buoyant geosensor. In the vertically-oriented root the direction of movement of the geosensor would be axial, and thus no inhibitor would be produced at the perimeter. The result would be continued straight growth downward along the plumb line. However, if the root were knocked off course, it is logical that the processes leading to differential growth across the growth zone would be initiated. The result would be deflection of the root back toward the vertical and perhaps the instigation of a pattern of periodic curvature.

4. When a gravistimulated root undergoes oscillatory curvature is the pattern of movement helical, or is the movement confined to a vertical plane? The principal model provides that the inhibitor travels in one direction around the circumference of the growth zone. As the inhibitor moves, it is assumed to retard the rate of elongation of every cell in its path. The result is a coordinated, helical pattern of growth. The alternate model predicts that the inhibitor will come into contact with only those cells positioned above and below the root axis. Therefore, the motion

of the root will be confined to a plane. In principal, it should be a straightforward matter to examine the three dimensional growth pattern which gravistimulated roots undergo. Such an investigation would serve to test the basic validity of the two models.

5. It is somewhat difficult to predict whether there would be a temperature effect on the underlying process. The diffusion constant of a molecule in a liquid medium is related to the temperature by the equation: $D = URT$, where U is defined as the inverse of the viscosity of the supporting medium, R is the gas constant and T is the absolute temperature. The viscosity of the liquid medium supporting the inhibitor would remain nearly constant in a normal physiological range for maize (10° to 40°C). Therefore, we can assume that D is linearly related to T. Over a 30°C range, D would change by a factor of approximately .1. This would have practically no effect on the rate of diffusion of the inhibitor. It is however, possible that there would be a temperature effect expressed in the conversion of P to P* or from I to I* since temperature is known to have a strong effect on the rate of molecular conversion.

There are a great many basic facts, interrelations and pathways which must be unearthed and explored in order to begin to satisfy our curiosity about root movement in relation to gravity and light. Some basic issues requiring further investigation are stated below.

1. What is the nature of the interaction between light quanta, geosensor movement and growth inhibitor?
2. What is the inhibitor molecule, and by what pathway is it translocated from tip to growth zone, i.e., apoplast or ion-specific channel?
3. Does a pattern of inhibitor flow around the growth zone actually exist? If there is

inhibitor flow without cell movement, then by what mechanism does the transport of inhibitor occur, i.e., convection or diffusion? What are the energetics of inhibitor transport in the growth zone?

4. What is the nature of the process governing growth inhibition in root cells? Stretch receptors are believed to exist which promote cell elongation. Is there an analogous inhibition receptor? How does it function?

4.4: Some Related Issues

4.4.1: Introduction

As a concluding section, I take up the question of whether root gravitropism is a threshold or graded response. This question represents a division in the field of root tropism studies. Therefore, it seems appropriate to place my data and model in their appropriate camp. And finally, throughout the literature green light is considered to be equivalent to complete darkness. Are roots insensitive to green light? It seems a worthwhile question to answer.

4.4.2 Light-Potentiated Root Gravitropism: A Threshold or a Graded Response?

Suzuki and Fujii (1978) and Shen-Miller (1973, 1978) present lines of evidence leading to different conclusions on the question of whether light-induced primary root gravitropism is a graded or a threshold response. Suzuki demonstrates a sharp increase in positive gravitropic curvature following 26 seconds of light at 663 nm with intensity 2.43×10^{-10} einsteins/cm^2/sec. He interprets this pronounced increase in curvature which does not grow with increasing time as an all-or-nothing (or threshold) type of response. By contrast, Shen-Miller demonstrates a log linear enhancement of positive gravitropic maize root curvature in the range of 10^2 to 10^6 ergs/cm^2 at 660 nm, with the exposure time held constant.

Using the technique outlined in §2.2, I used a helium neon laser to irradiate maize root caps, with roots held perpendicular to the gravity vector. I thereby established the presence of at least two levels of light-enhanced positive root gravitropic activity. A 60-second exposure (intensity: $2.75\times10^7 W/m^2$) produced a significant rise in root curvature as compared to the dark control between 1 hour 40 minutes and 2 hours 50 minutes. An increase in the light intensity by one order of magnitude (2.75×10^7 to $2.75\times10^8 W/m^2$), holding the time constant, produced another significant rise in root curvature between approximately 1 and 3 hours (Table 4.8).

I developed an additional line of evidence which also supports the existence of a graded, rather than a threshold, response. Four groups of cv. Merit roots were subjected to pulsed incandescent light (6 sec./10 min.) at intensities 1.9, 9.4, 14.1 and $18.8 W/m^2$. Roots were started horizontally and allowed to georeact for 6 hours.

The greatest separation exists between the four groups at 3 hours indicating a positive relationship between light intensity and downward curvature. However, the 3-hour point is the only time during the test period that mean curvature values are clearly ordered in alignment with light intensity. (The separation between the four groups is significant here at the 5% level.) The reason is that between 0 and 2 hours 40 minutes, the $9.4 W/m^2$ test group descends more quickly than the $1.9 W/m^2$ group. In spite of this ambiguity, a comparison of the 1.9, 14.1 and the $18.8 W/m^2$ intensity groups indicates a simple pattern of curvature. The pattern is that positive curvature increases as a function of light intensity throughout most of the test period. A comparison of the 9.4, 14.1 and the $18.8 W/m^2$ groups produces a similar pattern between 40 minutes and 3 hours 10 minutes (Figures 4.10A and 4.10B). Finally, after the first 40 minutes, the $18.8 W/m^2$ group is always more positively gravitropic than any of the other assay groups.

In summary, we are left with a three-step gradient. First, a comparison between the dark control and any of the four groups produces, by any measure, significantly less curvature

throughout the test period. The second step occurs between 1.9 or 9.4 to 14.1W/m^2. The last step occurs between 14.1 and 18.8W/m^2. Such a three-stage incremental rise in gravitropic curvature is clearly not consistent with a threshold model of gravitropism. [27]

4.4.3: The Effect of Green Light Pre-Treatment on Root Gravitropism

I carried out a series of dark controls in order to test the assumption of most investigators that dim green background light has no effect on the amplitude of positive maize root gravitropic activity. Researchers in the field generally regard dim green light as indistinguishable from complete darkness, in that neither set of conditions is thought to stimulate positive root curvature. Therefore, most research is conducted using dim green illumination as a "safe" work light required as an aid to root selection and seed manipulation. I performed a series of dark controls in order to test the validity of this assumption (Table 4.9).

In the first control, infrared light was used as background illumination for infrared photographs taken at 10-minute intervals during the 6-hour period of gravistimulation. The pulse duration was .002 seconds with no measurable light below 800 nm.

A second control was conducted in identical fashion with one exception. In the second test only 2 infrared pulses were administered as compared to 37 in the first control.[28] As a result, only two photographs were taken; one at the beginning and one at the end of the test period.

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[27]Evidence is also presented here indicating that the positive relationship between light intensity and downward curvature may be reversed at very high fluence levels (Figures 4.1C and 4.1D). The mean trough of gravitropic curvature rose from 66.8° to 54.0° when comparing trials of intensity 1.9 and 18.8W/m^2. In addition, the values recorded from +30 minutes to +4 hours 30 minutes were, in all cases, greater in amplitude for the lower light intensity test group.

[28]The final pulse at +6 hours, of course, does not contribute to the development of gravicurvature.

In the third control, dim green light was used to select and transfer seeds onto the viewing apparatus. Approximately 10 minutes of 517 nm light was required for seed selection and placement at an intensity level of $2.0\times10^{-4}W/m^2$. The background monochromatic illumination was unilaterally directed from a source approximately 20° above the roots. The green background light was switched off as the roots were re-oriented 90° around the longitudinal axis so that the roots would be in the correct experimental configuration in the absence of all visible light (Figure 2.4).[29] As in the first control, infrared photographs were taken at 10-minute intervals during the test period.

From the controls outlined above, the most noteworthy result is that cv. Merit maize primary roots are less gravitropically responsive in the dark than they are in dim green light in the period from 2 to 3 hours after the start of gravitropic stimulation. The trough of light-induced gravitropic curvature occurs during the 2- to 3-hour period irrespective of the type of illumination. Otherwise, the data do not differ significantly.

It is particularly noteworthy that there are no significant differences among the 3 treatments described above when comparing average root angles at +6 hours. This implies that the positive gravitropic effect of green light exhibited between 2 and 3 hours dissipates as the time increases from the point of light exposure.

The findings demonstrate that dim green light, at least in these conditions, potentiates root gravitropism above the dark background level. The differences are far greater however, when comparing the dim green light controls with broad spectrum white light or monochromatic light. Investigators should be aware of the residual green light effect for several reasons. First, it calls attention to the fact that dim green light cannot be used with absolute impunity as a safe work

[29]This third control describes the real extent of etiolation among the dark controls referred to earlier in Chapter 4 (Figures 4.1E and 4.3F).

light in studies of maize root gravitropism. It also serves to establish a sort of baseline for root sensitivity in a region of the spectrum generally considered to be without effect on root gravitropic curvature. Finally, it is significant that in the green light control the only visible light was given before the roots were re-oriented and prior to the beginning of the 6-hour experimental period. Therefore, the pigment molecules converted in the presence of the green background light must have continued to exert a positive effect on root curvature even though the roots were re-oriented after the end of the light treatment.

APPENDIX A

Recall from Footnote 18 that a geosensor heavier than the surrounding medium triggering inhibitor at the bottom of the root was, from a modeling standpoint, equivalent to a geosensor lighter than the surrounding medium triggering a growth enhancer at the top. In this alternate model we assume that two geosensors are present; one which sinks and one which floats; and that both trigger the formation of inhibitor. The inhibitor triggered by the sinking geosensor diffuses along the bottom of the root, as in the primary model; while the inhibitor triggered by the buoyant geosensor will diffuse along the top. Since we assume that the inhibitors are different molecules, we allow them to have different diffusion constants and different strengths. Numerical studies (Figures A1A-F, pp. 126-28) show that the best fit with the data is achieved with a diffusion ratio of 1.4, where the inhibitor on top diffuses more slowly,[30] and a strength ratio of approximately 1.1, where the top inhibitor is more potent.

We assume that Assumptions 1, 2, 3 and 5 from §4.2.7 are still valid, so that the angle of deflection of the root tip is still governed by the differential growth at the top and bottom of the growth zone. Equation 1 through Equation 5 from §4.2.7 also apply, but in a modified form:

$$\tan(\alpha(t)) = \frac{\ell_2(t) - \ell_1(t)}{d}, \tag{1'}$$

$$\frac{d\ell_1(t)}{dt} = R - \kappa_1 C_1(t) \qquad \frac{d\ell_2(t)}{dt} = R - \kappa_2 C_2(t), \tag{2'}$$

[30]Note that since the diffusion constant varies approximately as the square root of the molecular mass, this corresponds to the bottom inhibitor having approximately twice the mass of the top inhibitor.

$$\frac{d(\tan\alpha)}{dt} = \frac{1}{d}\left(\kappa_1 C_1(t) - \kappa_2 C_2(t)\right), \tag{3'}$$

$$\frac{\partial c_{diff_1}}{\partial t} - D_1 \frac{\partial^2 c_{diff_1}}{\partial x^2} = f(t) \qquad \frac{\partial c_{diff_2}}{\partial t} - D_2 \frac{\partial^2 c_{diff_2}}{\partial x^2} = f(t), \tag{4'}$$

$$f(t) = \begin{cases} \cos(\alpha\ (t)) & \textit{for}\ \ t_0 < t < t_{cutoff}, \\ 0 & \textit{otherwise}. \end{cases} \tag{5'}$$

Note that Equation 1′ here is exactly Equation 1 from §4.2.7; Equations 2′ and 3′ here are equivalent to Equations 2 and 3, but with different strength constants κ_1 and κ_2 allowed for bottom and top. Equation 4′ reflects the fact that we now allow diffusion along the bottom and top of the root, but with different inhibitors and thus different diffusion constants D_1 and D_2. Equation 5′ is just Equation 5. We see here that the source function is the same for top and bottom. This follows because the same reasoning which gave us the $cos(\alpha)$ dependence in §4.2.7 for the sinking geosensor applies also to the buoyant geosensor. Also, any difference in quantity of inhibitor produced is captured in the constants κ_1 and κ_2. We do assume that the inhibitors are produced for the same period of time, thus t_{cutoff} is the same for both.

Equations 6 through 8 from §4.2.7 no longer apply, since we no longer assume rotation at the growth zone; instead, $C_1(t)$ and $C_2(t)$ are defined as:

$$C_1(t) = c_{diff_1}(x_g, t) \qquad C_2(t) = c_{diff_2}(x_g, t). \tag{6'}$$

Note that there is no buildup of inhibitor at either top or bottom of the growth zone; the inhibitor simply diffuses past. Chemical disappearance is also present in this model. See Table A1 (p. 106) for a list of parameters used in the model.

Notice in Figures A1A-F that this model is able to capture the initial decline and

subsequent smaller rise. It can also model the secondary cycles of gravicurvature stimulated by later light pulses. However, the simulations do not reflect the fact that the first cycle of gravicurvature is longer than subsequent cycles.

TABLE 4.1

Cv. Merit primary roots oriented horizontally at time $t = 0$ and subjected to programs of incandescent illumination as specified. Each assay group shows a characteristic pattern of curvature. First, an initial descent phase which terminates at the trough of the positive phase. Next, this is followed by a period of negative curvature which ends at the peak of the negative phase. Difference in amplitude between trough of negative phase and peak of positive phase assessed by use of Students t-test for each assay group.

	EXP. 4.1 PERIODIC LIGHT 6 sec./10 min.	EXP. 4.2 PERIODIC LIGHT 6 sec./10 min.	EXP. 4.3 CONTINUOUS LIGHT	EXP. 4.4 CONTINUOUS LIGHT	EXP. 4.5 DARK CONTROL
I(W/m$_2$)	1.90	18.80	1.90	18.80	0.0
NUMBER OF ROOTS	52	116	18	36	88
TROUGH (MIN) POSITIVE PHASE	150	160	160	170	110
PEAK (MIN) NEGATIVE PHASE	230	340	270	240	190
DURATION (MIN) NEGATIVE PHASE	80	180	110	70	80
TROUGH (DEG) POSITIVE PHASE	42.7 (±14.2)	54.0 (±16.3)	66.8 (±15.9)	54.0 (±15.8)	17.6 (±15.7)
PEAK (DEG) NEGATIVE PHASE	25.2 (±17.8)	32.6 (±20.7)	38.3 (±21.2)	37.6 (±23.4)	12.8 (±14.6)
AMPLITUDE(DEG) NEGATIVE PHASE	17.5	21.4	28.5	16.4	4.8
STATISTICAL SIGNIFICANCE	.01	.01	.01	.01	.05

TABLE 4.2

Span and slope of linear descent phase for specified assay groups.

	EXP. 4.1 PERIODIC LIGHT 6 sec./10 min.	EXP. 4.2 PERIODIC LIGHT 6 sec./10 min.	EXP. 4.3 CONTINUOUS LIGHT	EXP. 4.4 CONTINUOUS LIGHT	EXP. 4.5 DARK CONTROL
I(W/m^2)	1.90	18.80	1.90	18.80	0
NUMBER OF ROOTS	52	116	18	36	88
REGRESSION (MIN) BEGINNING	50	30	100	90	30
REGRESSION (MIN) END	120	120	150	140	140
REGRESSION (MIN) DURATION	70	90	50	50	110
SLOPE (DEG/MIN)	.43 (±.03)	.49 (±.04)	.53 (±.11)	.66 (±.13)	.20 (±.05)

TABLE 4.3

Average power spectra for cv. Merit roots oriented horizontally at time $t = 0$, and subjected to 6 hours incandescent lighting as specified. The average power spectrum for all roots in each test group and for the cyclic roots in each group are considered. An average power spectrum is the mean of the power spectra computed for all roots in a particular test group as opposed to the power spectrum for the data corresponding to the graph of averages for the group. The significance of periodogram spikes is assessed according to the Shimshoni-modified Fisher test.

TIME (MIN)	Exp. 4.1 PERIODIC LIGHT 6 sec./10 min. I = 1.9W/m^2 N = 52 ALL	Exp. 4.2 PERIODIC LIGHT 6 sec./10 min. I = 18.8W/m^2 N = 116 ALL	Exp. 4.3 CONTINUOUS LIGHT 6 sec./10 min. I = 1.9W/m^2 N = 18 ALL	Exp. 4.4 CONTINUOUS LIGHT I = 18.8W/m^2 N = 36 ALL	Exp. 4.5 DARK CONTROL I = 0.0W/m^2 N = 88 ALL
270.0	143.96	150.63	207.20	178.18	66.07
135.0	110.83	86.64	118.38	173.33	76.58
90.0	47.31	40.52	54.81	61.53	38.17
67.5	32.22	29.45	41.11	41.03	28.23
54.0	22.09	19.07	26.68	27.04	19.27
45.0	16.89	17.65	21.49	22.89	17.80
36.6	14.26	14.40	19.35	20.36	16.13
33.8	13.73	14.03	19.33	18.22	11.83
30.0	13.04	12.28	20.71	17.32	12.09
27.0	13.33	11.40	13.85	15.03	11.73
	N = 11 Cyclic	N = 15 Cyclic	N = 2 Cyclic	N = 11 Cyclic	N = 29 Cyclic
270.0	117.04	124.34	96.28	139.25	60.71
135.0	241.88*	192.48*	328.47*	298.85*	131.30*
90.0	59.01	62.04	70.30	78.21	47.16
67.5	33.95	46.17	60.31	42.89	26.49
54.0	26.92	25.16	19.36	26.75	22.63
45.0	18.09	23.64	9.25	20.02	19.39
36.6	18.28	19.45	13.87	21.24	16.48
33.8	18.32	16.88	20.42	16.86	12.62
30.0	14.60	15.98	22.07	18.78	13.66
27.0	16.04	13.21	9.16	14.72	13.01

* Significant at the $P = .01$ level.

TABLE 4.4

Average power spectra for cv. Merit roots oriented horizontally at time $t = 0$, and subjected to 12 hours incandescent lighting as specified. The average power spectrum for all roots in each test group and for the cyclic roots in each group are considered. The significance of periodogram spikes is assessed according to the Shimshoni-modified Fisher test.

TIME (MIN)	Exp. 4.8 PERIODIC LIGHT 6 sec./10 min. I = 18.8W/m² N = 49 ALL	Exp. 4.9 PERIODIC LIGHT 3 min./1 hr. I = 18.8W/m² N = 38 ALL	Exp. 4.10 PERIODIC LIGHT 3 min./2 hr. I = 18.8W/m² N = 36 ALL	Exp. 4.11 PERIODIC LIGHT 3 min./4 hr. I = 18.8W/m² N = 28 ALL	Exp. 4.13 DARK CONTROL I = 0.0W/m² N = 19 ALL
630.0	276.57	100.46	137.40	201.76	140.51
315.0	174.11	70.57	148.65*	240.10	69.52
201.0	132.03	54.70	101.91	319.72*	77.14
157.5	122.78	56.18	94.13	252.41	94.42
126.0	147.22	42.0	108.39	127.54	74.60
105.0	75.04	49.06	69.47	166.52	65.37
90.0	53.71	30.16	53.53	75.71	42.69
78.8	41.28	35.53	41.98	79.60	39.19
70.0	40.14	25.75	39.18	84.47	36.13
63.0	36.57	28.83	34.0	71.47	26.02
	N = 16 Cyclic	N = 12 Cyclic	N = 30 Cyclic	N = 24 Cyclic	N = 5 Cyclic
630.0	214.83	57.69	142.07	179.52	116.68
315.0	204.90	86.82	171.35*	242.74	66.98
201.0	187.95	72.04	146.09	326.08*	120.29
157.5	185.75	72.63	98.97	256.31	190.87*
126.0	250.87*	48.67	119.70	136.39	137.59
105.0	81.79	84.67	71.67	159.72	99.54
90.0	58.31	33.77	53.84	76.40	48.29
78.8	40.19	39.61	46.93	75.50	33.96
70.0	44.65	33.62	33.21	87.60	37.87
63.0	34.79	30.52	38.16	73.0	29.54

* Significant at $P = .01$ level.

TABLE 4.5

Average power spectra for four specified intervals from Experiment 4.10. Periodic incandescent illumination pulsed at 3 min./2 hr. except at +2 hours (see Figure 4.3C). Period units are given in minutes.

INTERVAL:	1 hr.40 min. to 8 hr.	INTERVAL:	1 hr.40 min. to 10 hr.	INTERVAL:	1 hr.40 min. to 12 hr.	INTERVAL:	4 hr. to 10 hr.
PERIOD (MIN)	VALUE	PERIOD (MIN)	VALUE	PERIOD (MIN)	VALUE	PERIOD (MIN)	VALUE
380	138.09	500	149.08	630	137.40	360	91.00
190	159.92*	250	169.35*	315	148.65*	180	81.44
127	92.61	167	147.50	210	101.91	120	93.80*
95	103.62	125	106.32	158	94.13	90	82.47
76	60.16	100	117.70	126	108.39	72	52.87
63	48.37	83	65.77	105	69.47	60	41.34
54	38.74	74	70.97	90	53.53	51	32.27
48	29.00	63	54.95	79	41.98	45	26.21
47	30.07	56	42.18	70	39.18	40	28.43
38	28.42	50	38.22	63	34.00	36	22.06

*Significant at $P = .01$ level.

TABLE 4.6

MODEL PARAMETERS

DESCRIPTION	VALUES
1. Distance of growth zone from tip	.3 cm
2. Diffusion constant D	$.5\times10^{-9}$ m^2/sec
3. Half-life of chemical disappearance	37 minutes
4. Duration of inhibitor production per pulse	60 minutes
5. Time for full rotation	120 minutes
6. Time to reach full rotation speed	200 minutes
7. Lag time t_0	10 minutes

TABLE 4.7

MODEL SCALING CONSTANTS κ BY EXPERIMENT

Roots oriented horizontally at time $t = 0$, and subjected to incandescent illumination as specified. The constant is scaled to the trough angle in each trial. Recall that,

$$\frac{d\ell_1(t)}{dt} = R - \kappa C_1(t), \quad \frac{d\ell_2(t)}{dt} = R - \kappa C_2(t),$$

is Equation 2 from §4.2.7 in the text.

EXPERIMENT #	DESCRIPTION	DURATION	INTENSITY	CONSTANT κ
Exp. 4.1	PERIODIC LIGHT 6 sec./10 min.	6 hr.	1.9W/m^2	.00039
Exp. 4.17	PERIODIC LIGHT 6 sec./10 min.	6 hr.	9.4W/m^2	.00042
Exp. 4.18	PERIODIC LIGHT 6 sec./10 min.	6 hr.	14.1W/m^2	.00052
Exp. 4.2	PERIODIC LIGHT 6 sec./10 min.	6 hr.	18.8W/m^2	.00063
Exp. 4.3	CONTINUOUS LIGHT	6 hr.	1.9W/m^2	.00100
Exp. 4.4	CONTINUOUS LIGHT	6 hr.	18.8W/m^2	.00060
Exp. 4.5	DARK CONTROL	6 hr.	0.0	.00013
Exp. 4.8	PERIODIC LIGHT 6 sec./10 min.	12 hr.	18.8W/m^2	.00054
Exp. 4.9	PERIODIC LIGHT 3 min./hr.	12 hr.	18.8W/m^2	.00054
Exp. 4.10	PERIODIC LIGHT 3 min./2 hr.	12 hr.	18.8W/m^2	.00054
Exp. 4.11	PERIODIC LIGHT 3 min./4 hr.	12 hr.	18.8W/m^2	.00054
Exp. 4.12	1 pulse 3 min. at *t=0 min.* & *t=130 min.*	4 hr. 10 min.	18.8W/m^2	.00054
Exp. 4.13	DARK CONTROL	12 hr.	0.0	.00017

TABLE 4.8

Gravitropic response data of cv. Merit roots oriented horizontally at time $t = 0$, and subjected to He-Ne laser light. Exposure time 60 seconds at beginning of each trial. Pairwise comparisons of curvature are made between the two test groups and the dark control. Significant differences assessed according to Student's t-test.

TIME (MIN)	Exp. 4.14 I= $2.75\times10^7 W/m^2$ N = 26	Exp. 4.15 I= $2.75\times10^8 W/m^2$ N = 20	Exp. 4.16 DARK CONTROL N = 50	$\frac{DARK}{2.75\times10^7 W/m^2}$ Significance	$\frac{DARK}{2.75\times10^8 W/m^2}$ Significance	$\frac{2.75\times10^7}{2.75\times10^8} W/m^2$ Significance
0	-0.8	-1.1	-3.4	n.s.	n.s.	n.s.
10	-	-	-	-	-	-
20	2.9	3.9	2.4	n.s.	n.s.	n.s.
30	6.4	8.0	5.7	n.s.	n.s.	n.s.
40	7.5	8.9	7.7	n.s.	n.s.	n.s.
50	7.4	11.5	7.7	n.s.	n.s.	n.s.
60	8.0	15.8	8.1	n.s.	0.1	0.1
70	10.0	20.8	10.6	n.s.	0.1	0.1
80	13.4	26.6	12.8	n.s.	0.1	0.1
90	16.6	34.6	15.4	n.s.	0.1	0.1
100	23.1	40.4	18.6	n.s.	0.1	0.1
110	28.8	45.6	19.5	.05	0.1	0.1
120	31.6	48.0	20.2	.01	.01	.01
130	33.4	49.1	19.8	.01	.01	.01
140	33.1	47.6	18.5	.01	.01	.01
150	30.4	44.6	16.2	.01	.01	.01
160	27.3	41.1	14.9	.01	.01	.01
170	24.4	38.1	13.0	.01	.01	.01
180	19.2	35.8	12.3	n.s.	.01	.01

TABLE 4.9

Comparison of gravitropic response data for three groups of cv. Merit primary roots oriented horizontally at time $t = 0$. Roots exposed to monochromatic green light and/or pulses of infrared light as indicated. The data represents point-by-point comparisons of curvature at 10-minute intervals between 2 and 3 hours. The mean curvature is also compared between Experiment 4.20 and Experiment 4.19 over the same period. In addition, comparisons of curvature are made among the three test groups at 6 hours. Significant differences are assessed according to Student's t-test.

TIME (MIN)	Exp. 4.20 1 IR PULSE at $t = 0$ N = 24	Exp. 4.19 36 IR PULSES 1 pulse/10 min. N = 28	Exp. 4.5 GREEN LIGHT Pre-treatment[1] 15 min. N = 88	SIGNIFICANCE
120		10.8	16.1	n.s.
130		9.4	16.4	.05
140		9.5	16.4	.01
150		9.0	16.1	.01
160		9.8	15.7	.05
170		9.0	15.6	.01
180		9.2	15.6	.05
360	10.1	13.1	14.8	n.s.
120-180 min. average		9.5	16.0	.01

[1] Monochromatic green light pre-exposure 517 nm, $2.0 \times 10^{-4} W/m^2$.

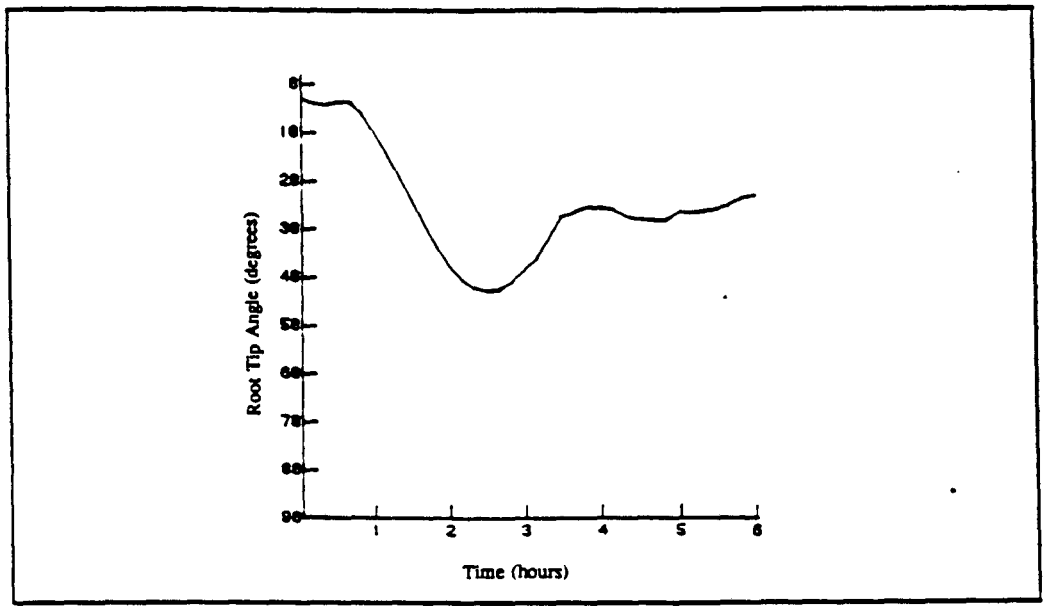
TABLE A-1

MODEL PARAMETERS

DESCRIPTION	VALUES
1. Distance of growth zone from tip	.3 cm
2. Diffusion constant D_1 (bottom)	$.50\times10^{-9}$ m^2/sec.
3. Diffusion constant D_2 (top)	$.35\times10^{-9}$ m^2/sec.
4. Half-life of chemical disappearance (bottom and top)	60 minutes
5. Duration of inhibitor production per pulse (bottom and top)	60 minutes
6. Lag time t_0 (bottom and top)	10 minutes

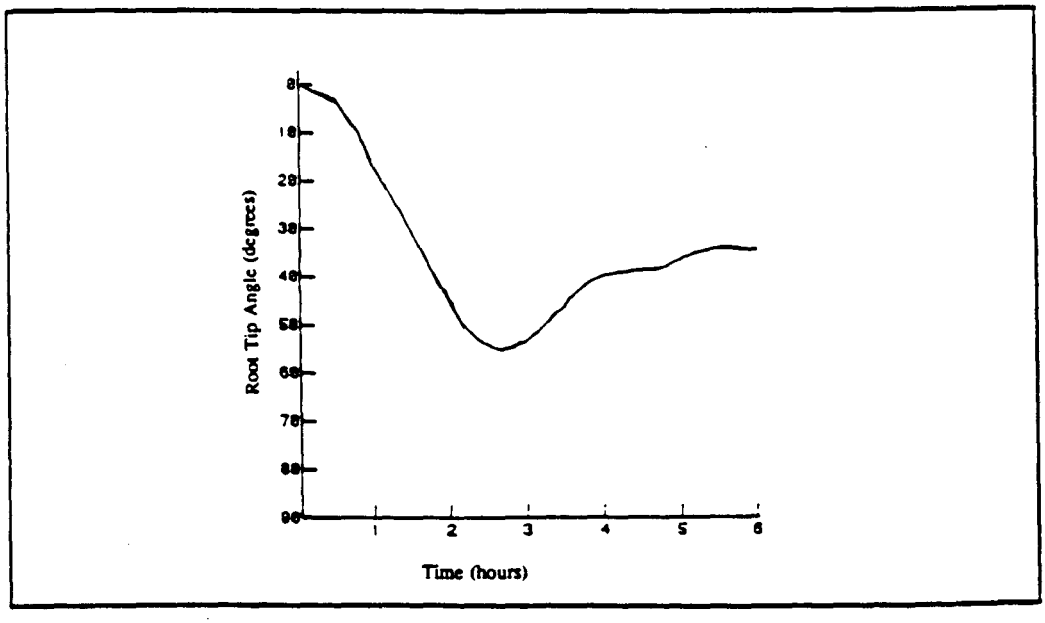
EXPERIMENT #	κ_1 (BOTTOM)	κ_2 (TOP)
4.8	.0032	.00365
4.9	.0032	.00365
4.10	.0032	.00365
4.11	.0032	.00365
4.12	.0032	.00365
4.13	.001	.0011

FIGURE 4.1A



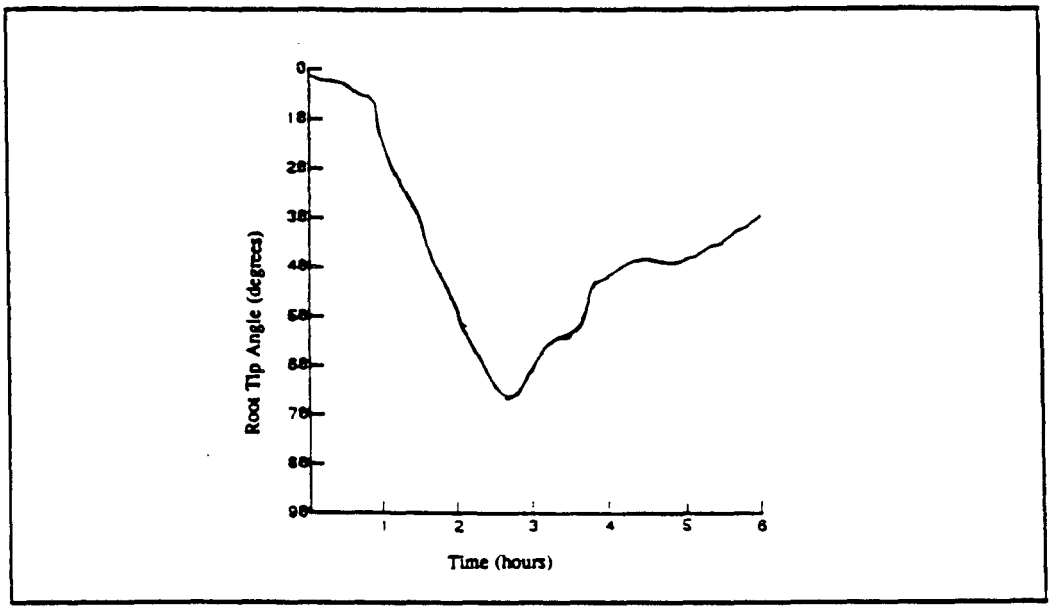
Mean georeaction curve. Periodic incandescent illumination frequency 6 sec./10 min., intensity 1.9W/m^2. Experiment 4.1

FIGURE 4.1B



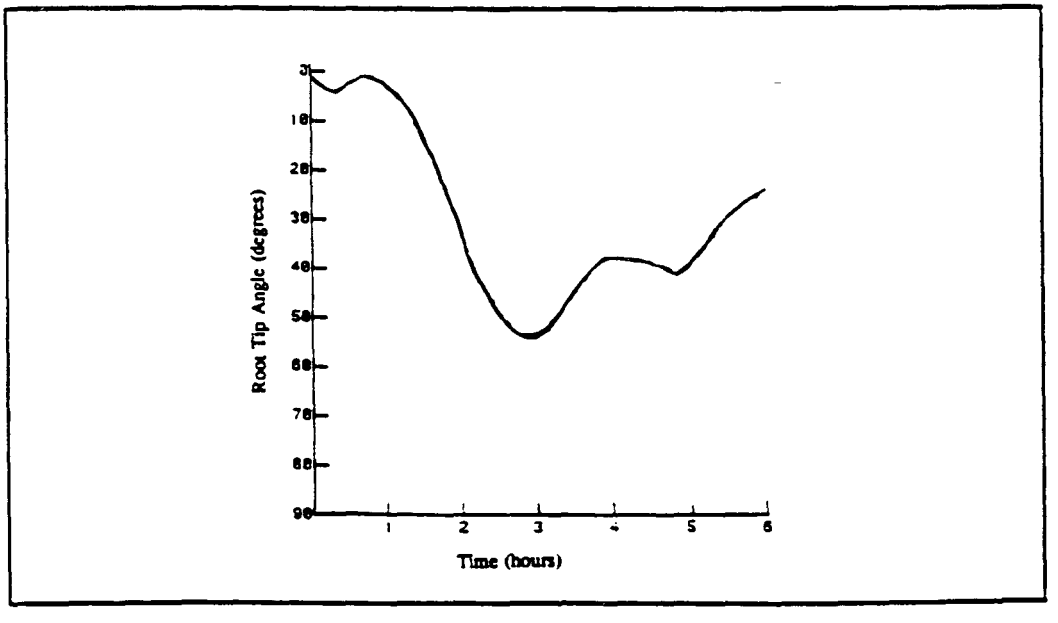
Mean georeaction curve. Periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Experiment 4.2.

FIGURE 4.1C



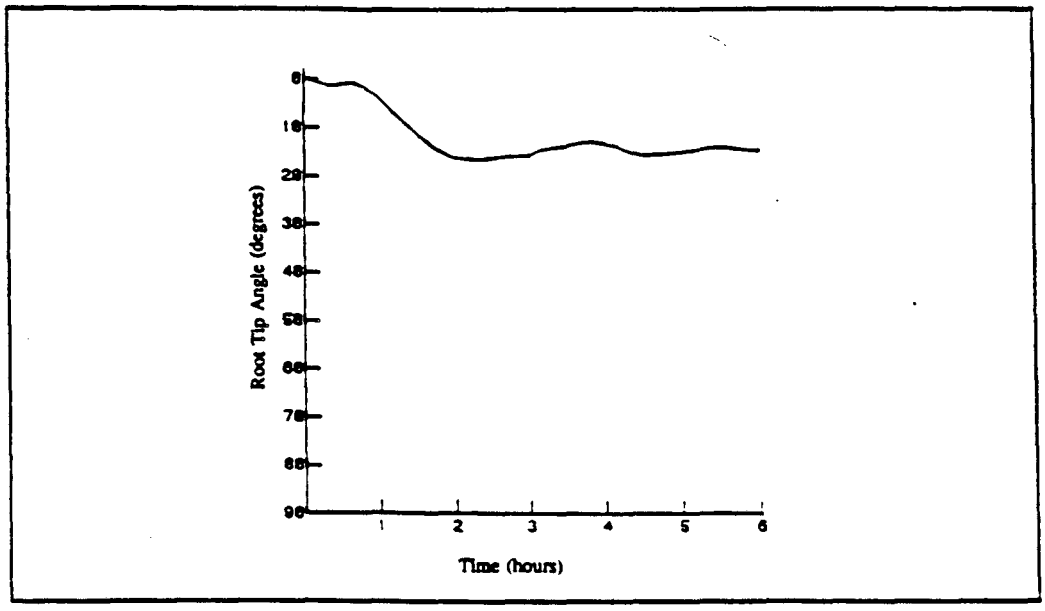
Mean georeaction curve. Continuous incandescent illumination frequency 6 sec./10 min., intensity 1.9W/m^2. Experiment 4.3.

FIGURE 4.1D



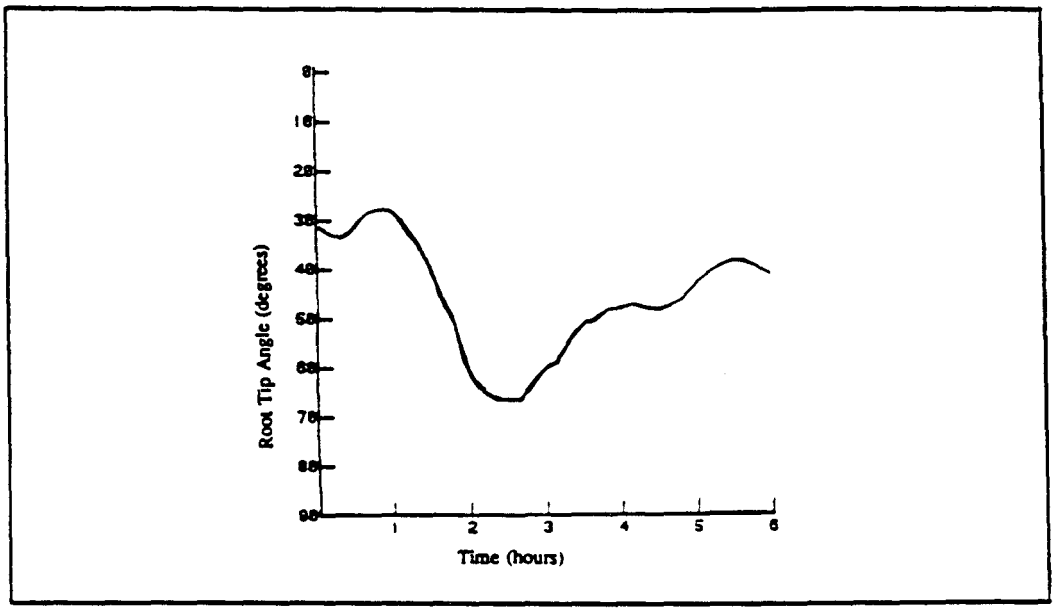
Mean georeaction curve. Continuous incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Experiment 4.4.

FIGURE 4.1E



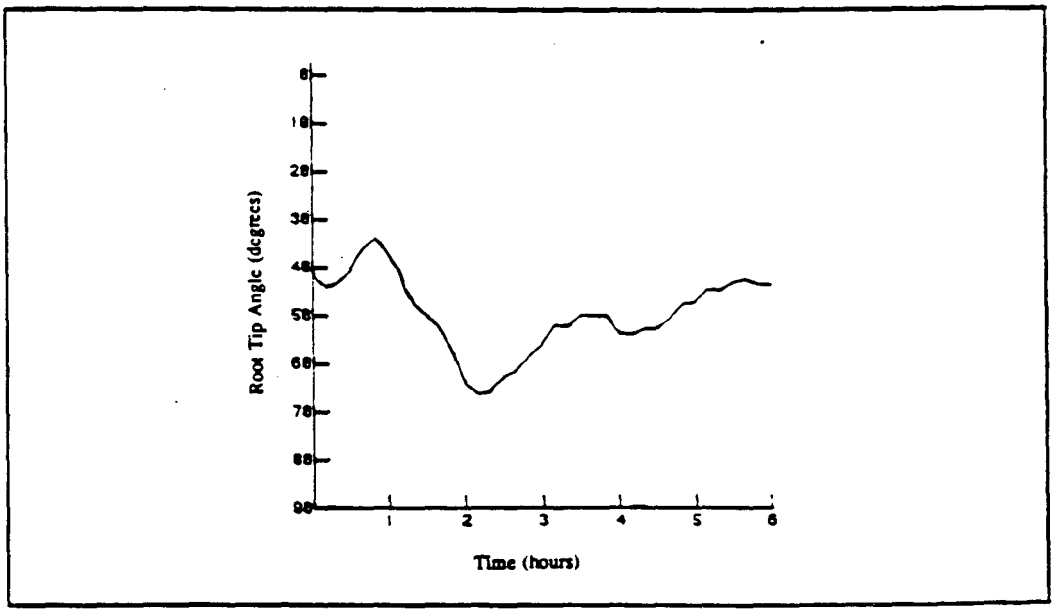
Mean georeaction curve for dark control. Experiment 4.5.

FIGURE 4.2A



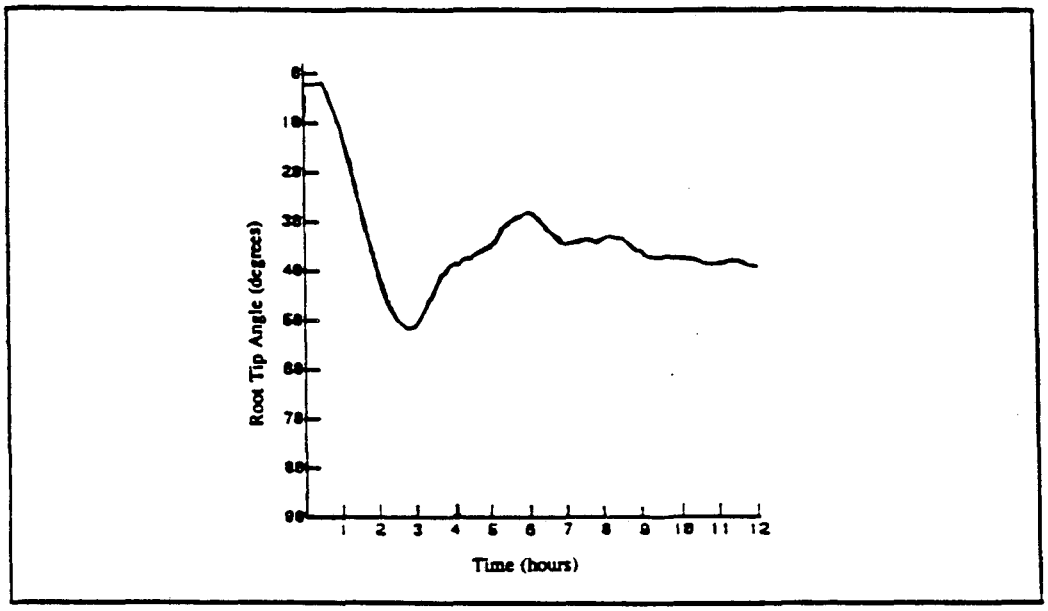
Mean georeaction curve. Periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Beginning mean root orientation = 31.6°. Experiment 4.6.

FIGURE 4.2B



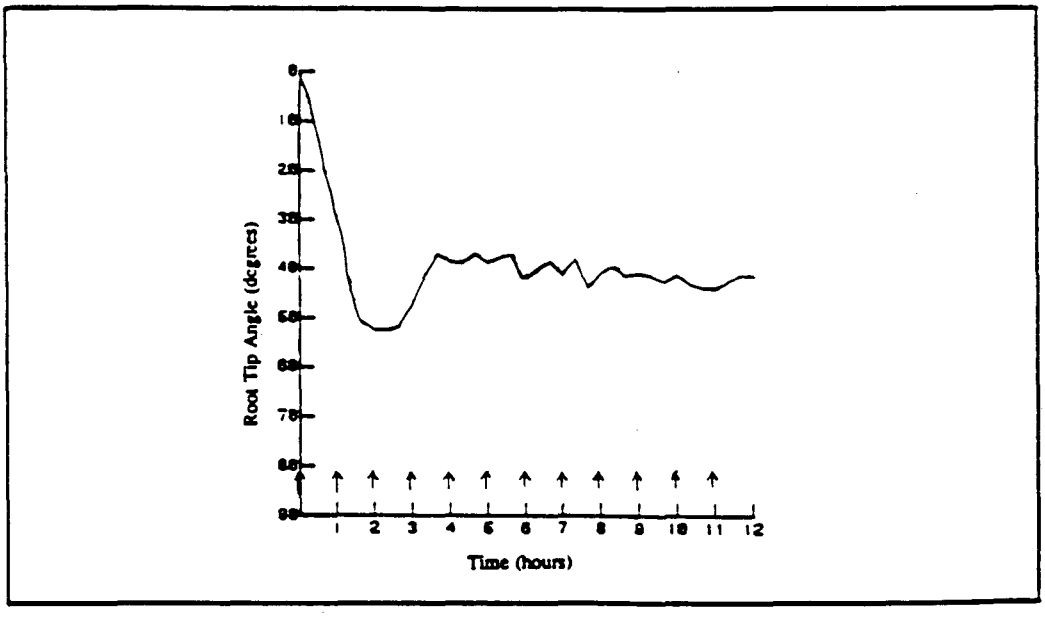
Mean georeaction curve. Periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Beginning mean root orientation = 40.6°. Experiment 4.7.

FIGURE 4.3A



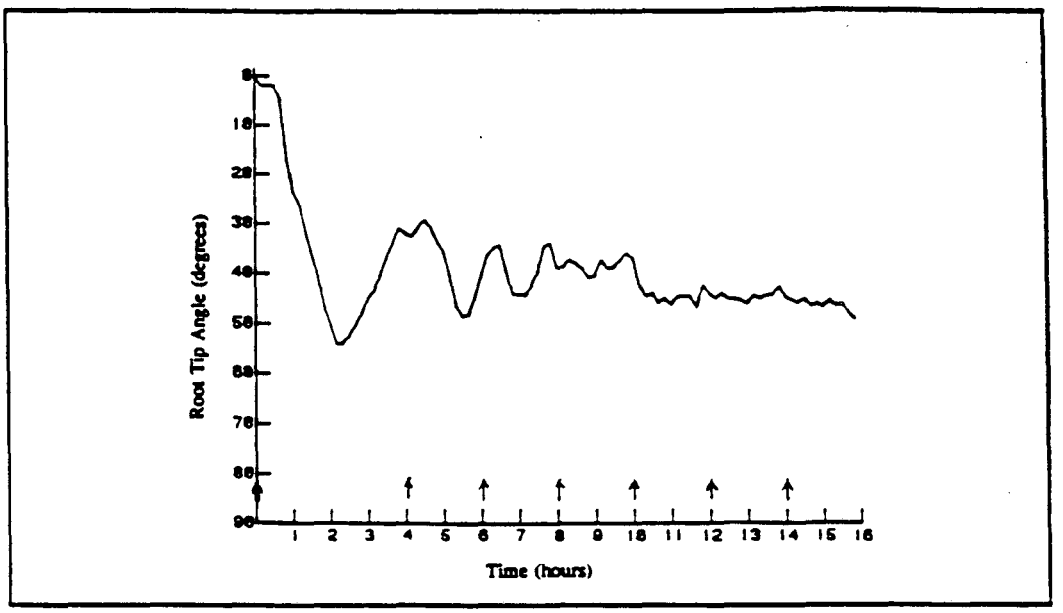
Mean georeaction curve. Periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Experiment 4.8.

FIGURE 4.3B



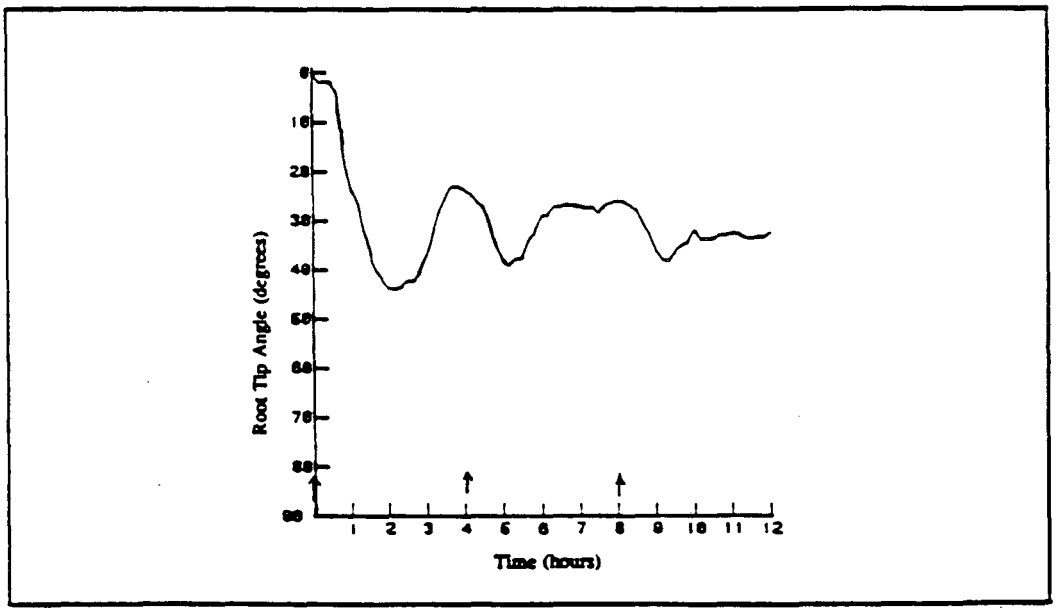
Mean georeaction curve. Periodic incandescent illumination frequency 3 min./1 hr., intensity 18.8W/m^2. Arrows represent time of each new light pulse. Experiment 4.9.

FIGURE 4.3C



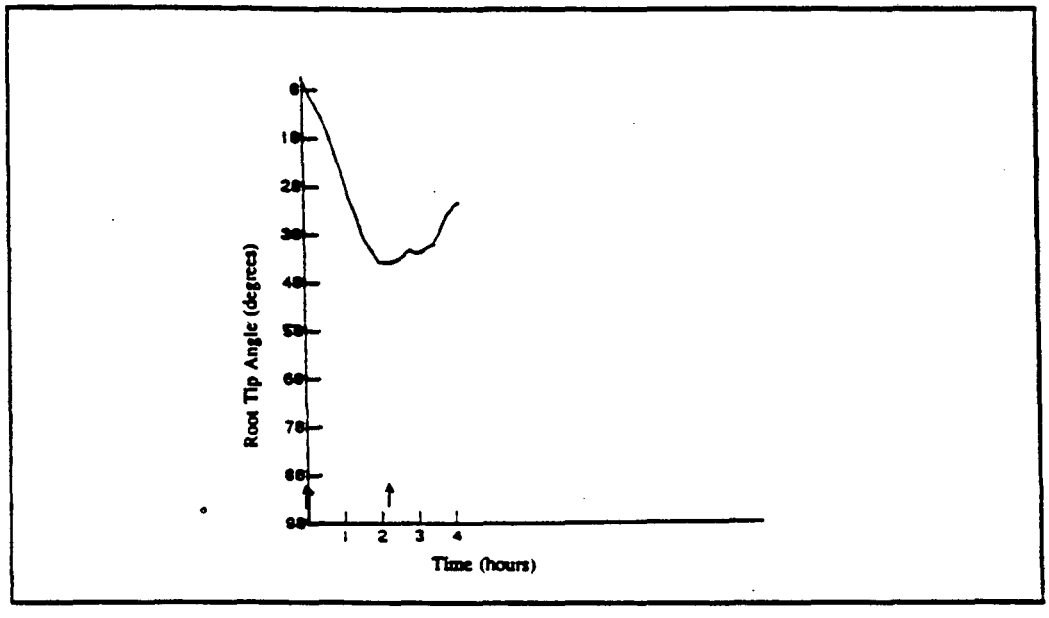
Mean georeaction curve. Periodic incandescent illumination frequency 3 min./2 hr., except at 2 hours; intensity 18.8W/m^2. Arrows represent time of each light pulse. Experiment 4.10.

FIGURE 4.3D



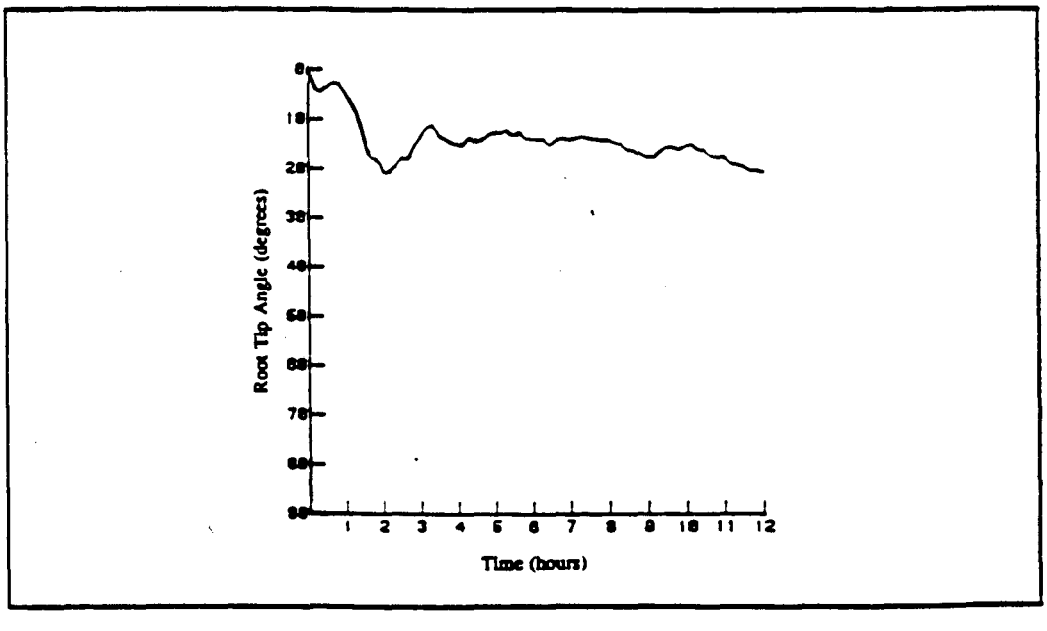
Mean georeaction curve. Periodic incandescent illumination frequency 3 min./4 hr., intensity 18.8W/m^2. Arrows represent time of each new light pulse. Experiment 4.11.

FIGURE 4.3E



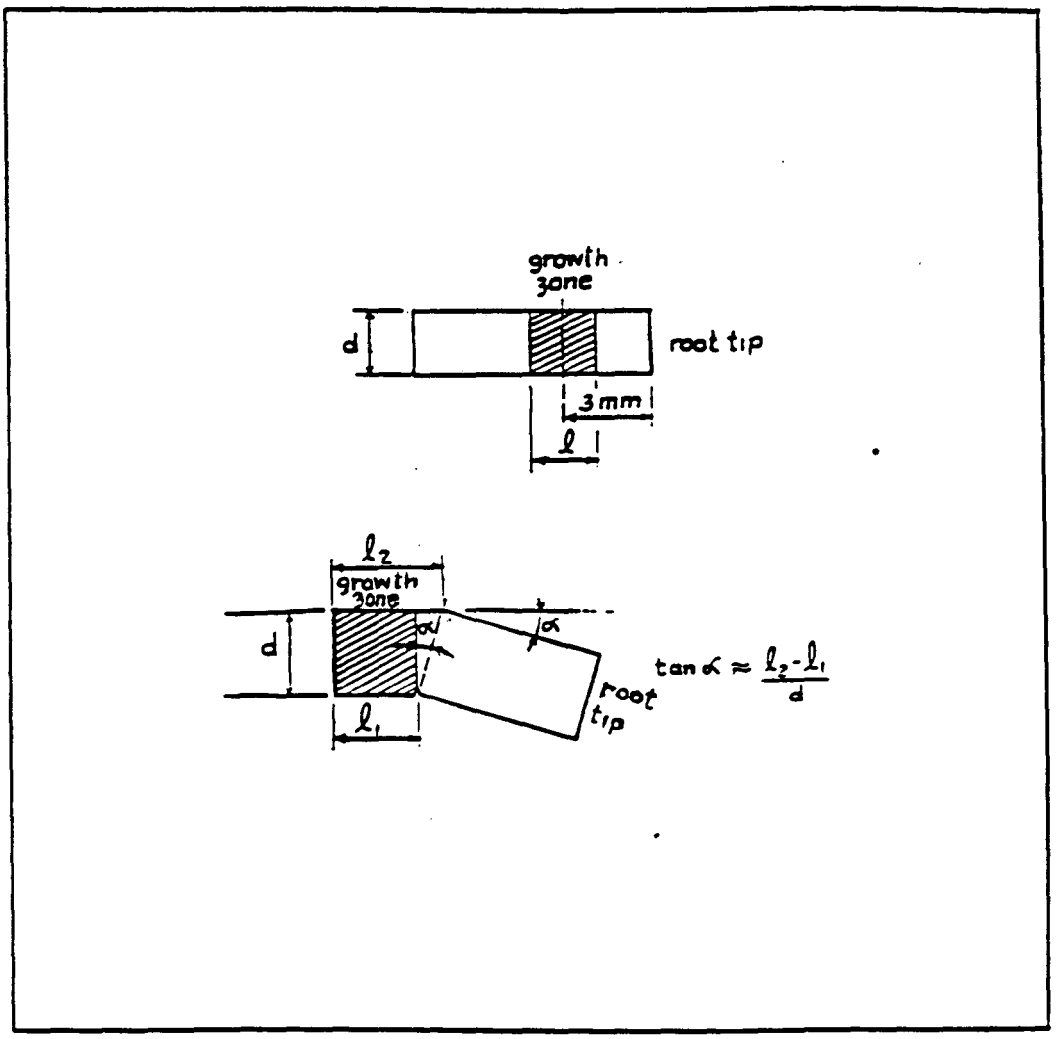
Mean georeaction curve with light pulses at time $t = 0$ and $t = 130$ minutes, intensity 18.8W/m^2. Arrows represent time of each new light pulse. Experiment 4.12.

FIGURE 4.3F



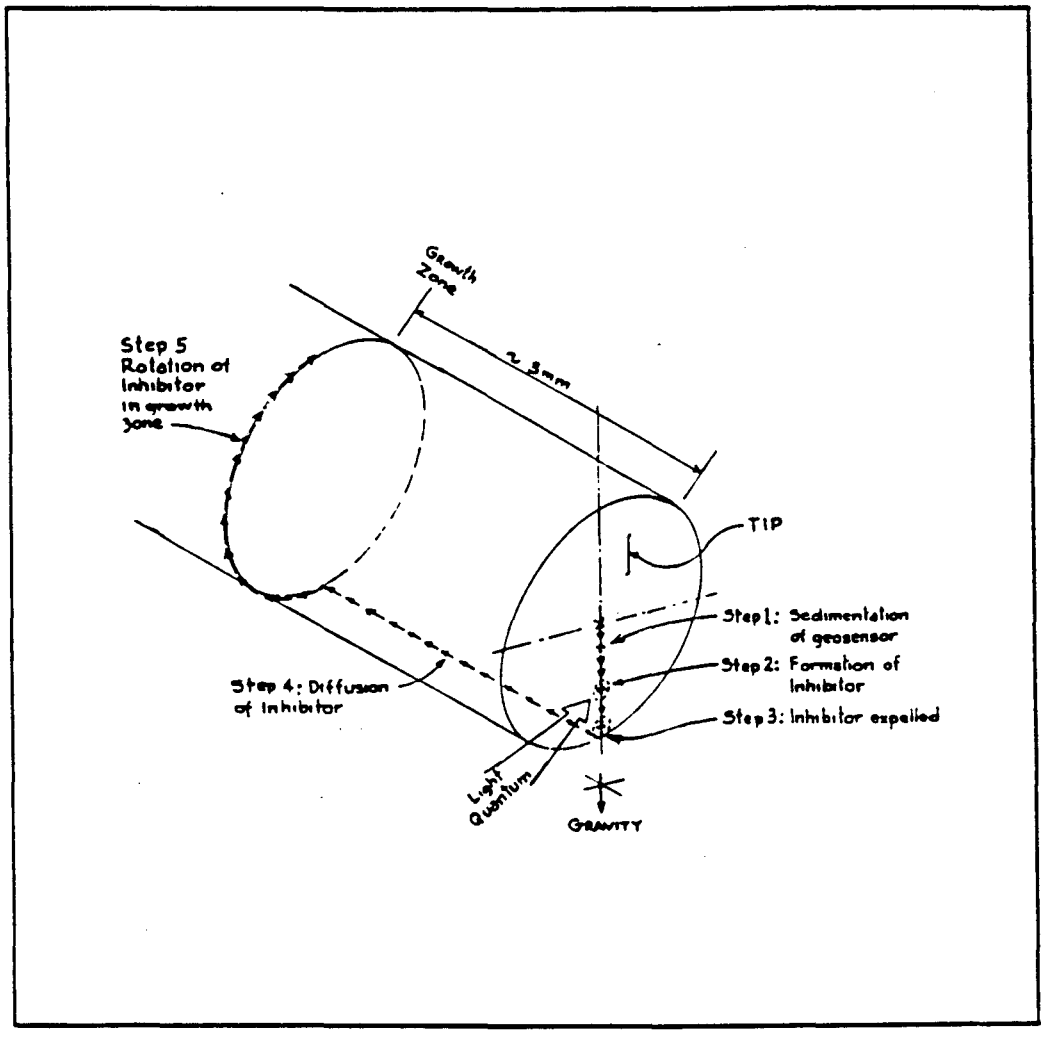
Mean georeaction curve for dark control. Experiment 4.13.

FIGURE 4.4



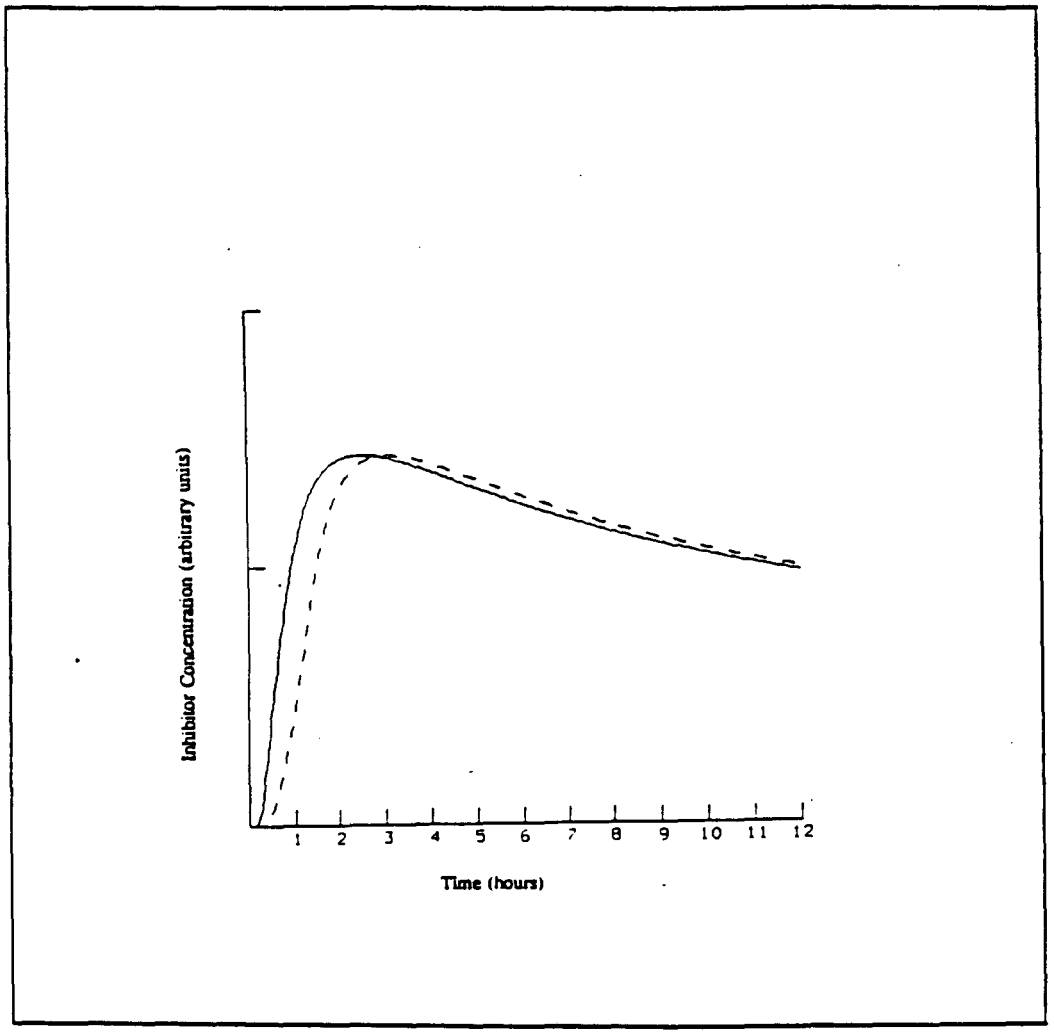
Deflection angle of root tip as a result of elongation difference between top and bottom of growth zone. ℓ_1 = length of bottom of growth zone at time t; ℓ_2 = length of top of growth zone at time t; d = diameter of root; ℓ = length of top and bottom of growth zone prior to flow of inhibitor; α = root deflection angle at time t.

FIGURE 4.5



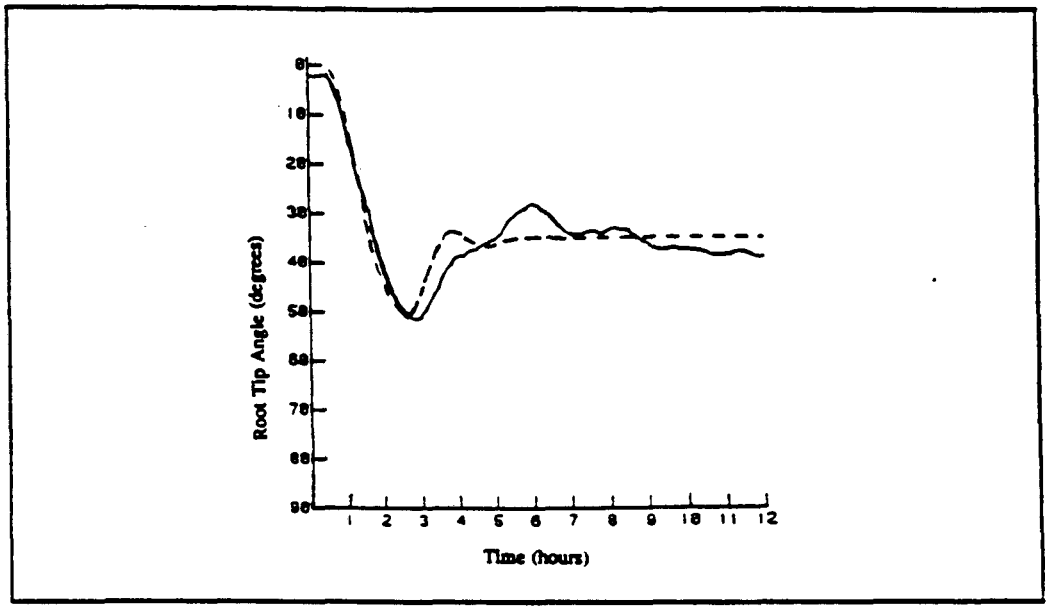
Stages in the model of light-stimulated maize root gravicurvature (as presented in §4.2.7).

FIGURE 4.6



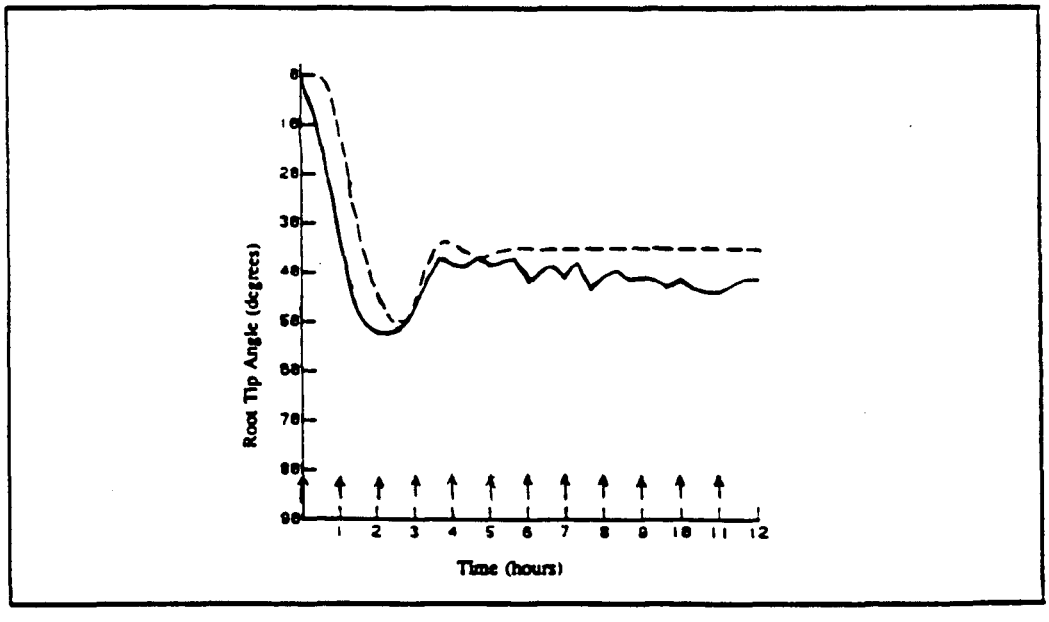
Concentration of inhibitor at growth zone due to unit pulse at time $t = 0$ (solid) and hour-long pulse with same total release of inhibitor (dashed).

FIGURE 4.7A



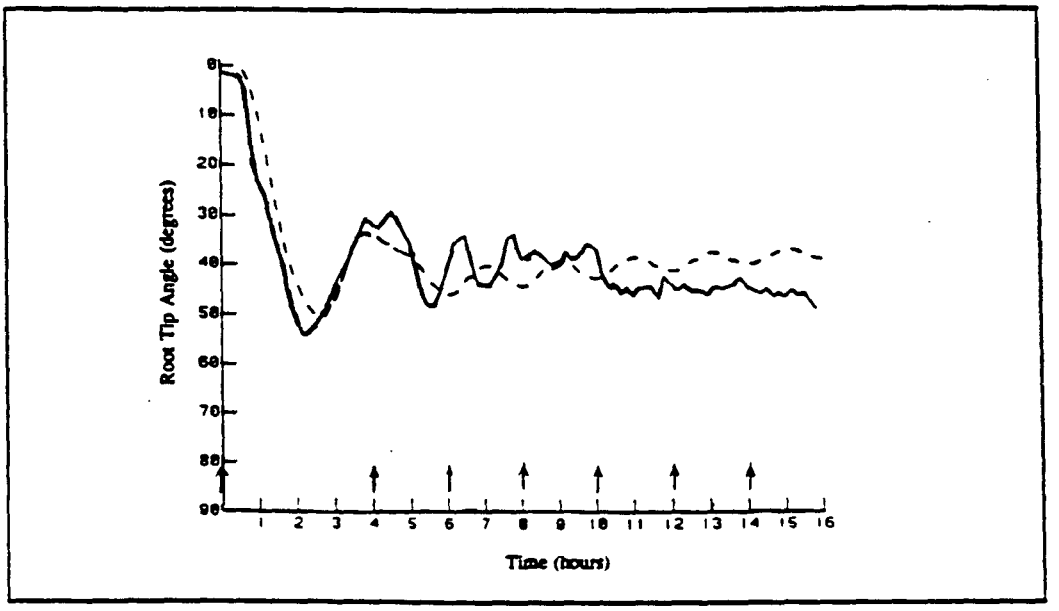
Mean georeaction curves of data (solid) and simulated (dashed). Data represents periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Simulation models a single flow of inhibitor released at time $t = t_0$. Experiment 4.8.

FIGURE 4.7B



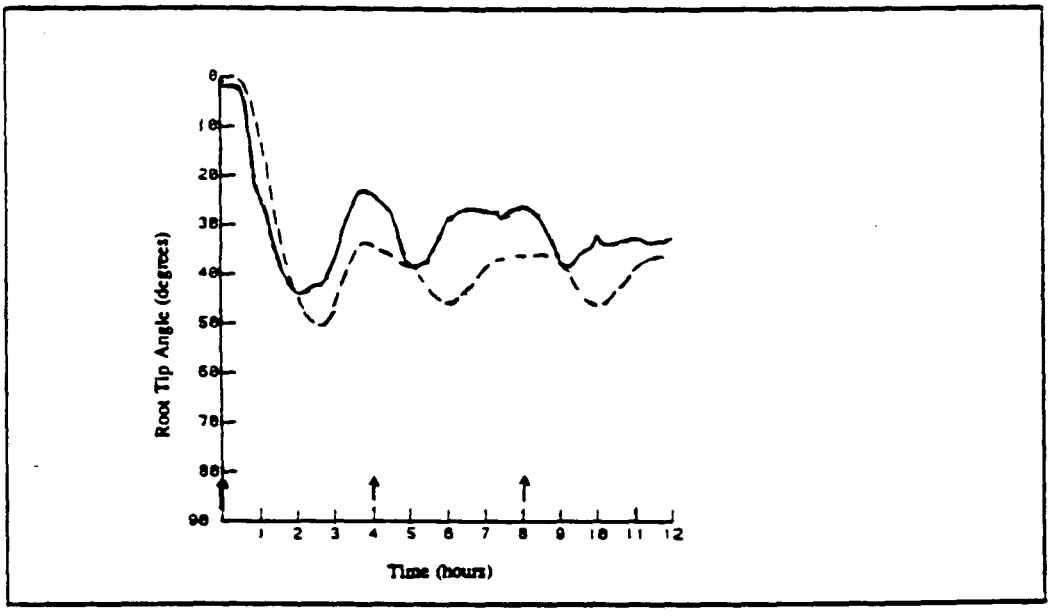
Mean georeaction curves of data (solid) and simulated (dashed). Data represents periodic incandescent illumination frequency 3 min./ 1h., intensity 18.8W/m^2. Simulation models a single flow of inhibitor released at time $t = t_0$. Arrows represent time of each new light pulse. Experiment 4.9.

FIGURE 4.7C



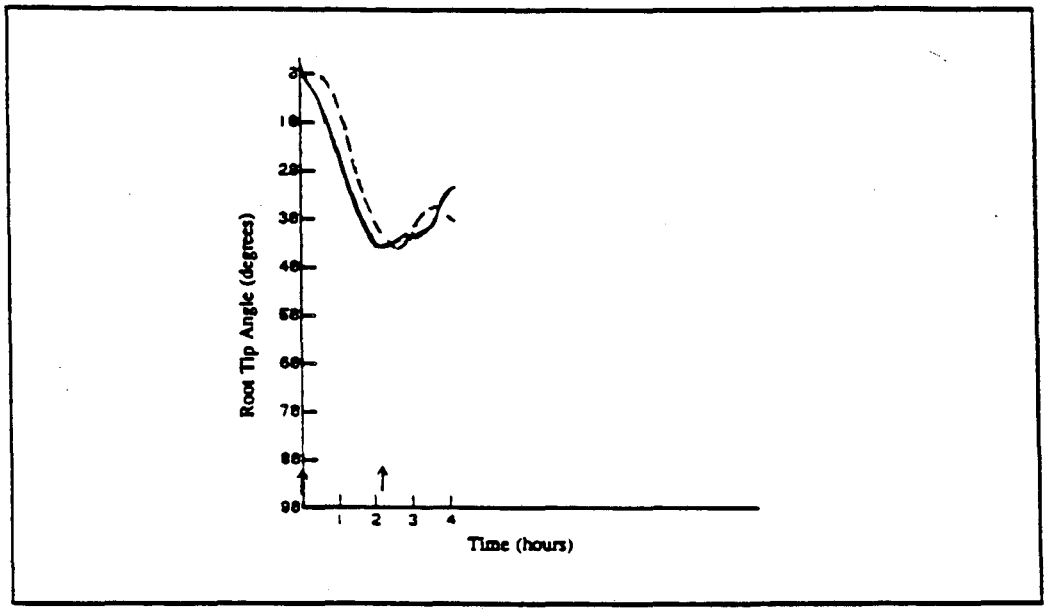
Mean georeaction curves of data (solid) and simulated (dashed). Data represents periodic incandescent illumination frequency 3 min./2 hr., except at +2 hours: intensity 18.8W/m^2. Simulation models new flow of inhibitor, of .8 times the previous flow, following each light pulse, except for the pulse at +4 hours which is at full strength. Arrows represent time of each new light pulse. Experiment 4.10.

FIGURE 4.7D



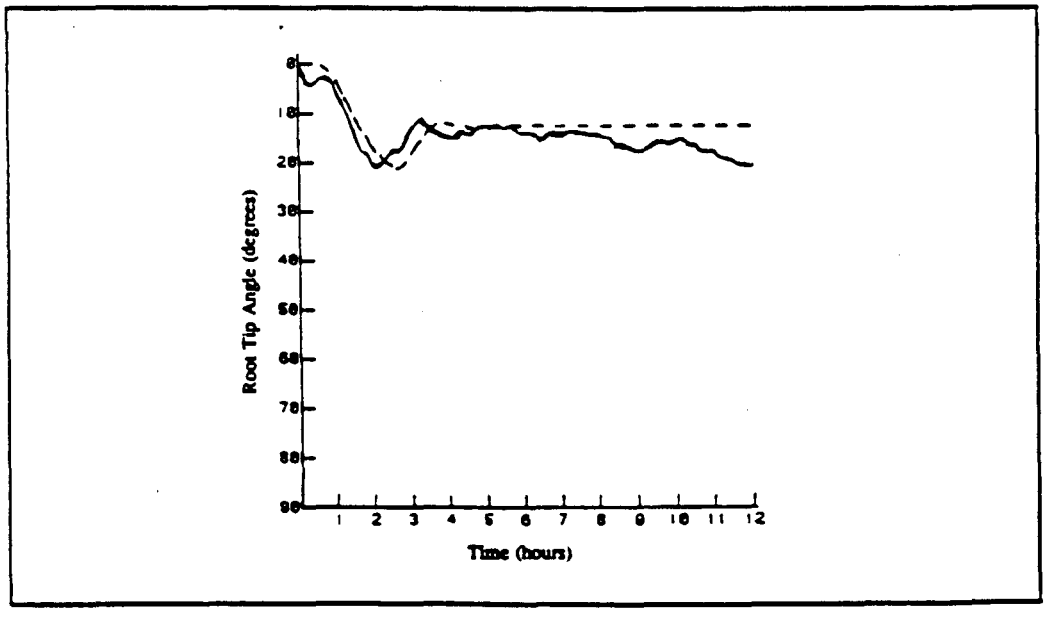
Mean georeaction curves of data (solid) and simulated (dashed). Data represents periodic incandescent illumination frequency 3 min./4 hr., intensity 18.8W/m^2. Simulation models new flow of inhibitor following each light pulse of the same magnitude as the original flow. Arrows represent time of each new light pulse. Experiment 4.11.

FIGURE 4.7E



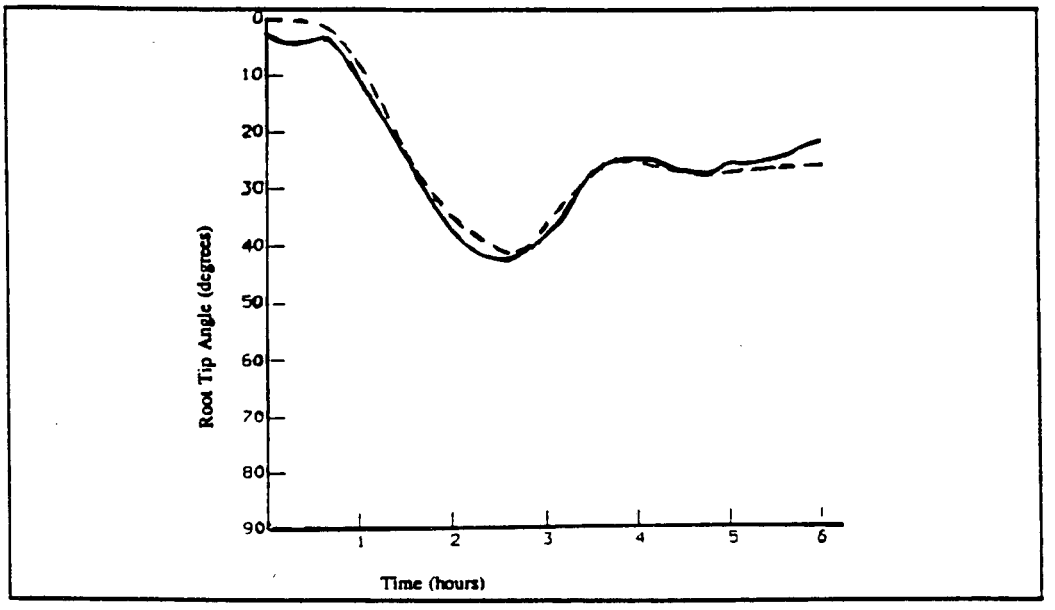
Mean georeaction curves of data (solid) and simulated (dashed). Data represents light pulses at time $t = 0$ and $t = 130$ minutes, intensity $18.8 W/m^2$. Simulation models second flow of inhibitor at .8 times the magnitude of the first flow. Arrows represent time of each light pulse. Experiment 4.12.

FIGURE 4.7F



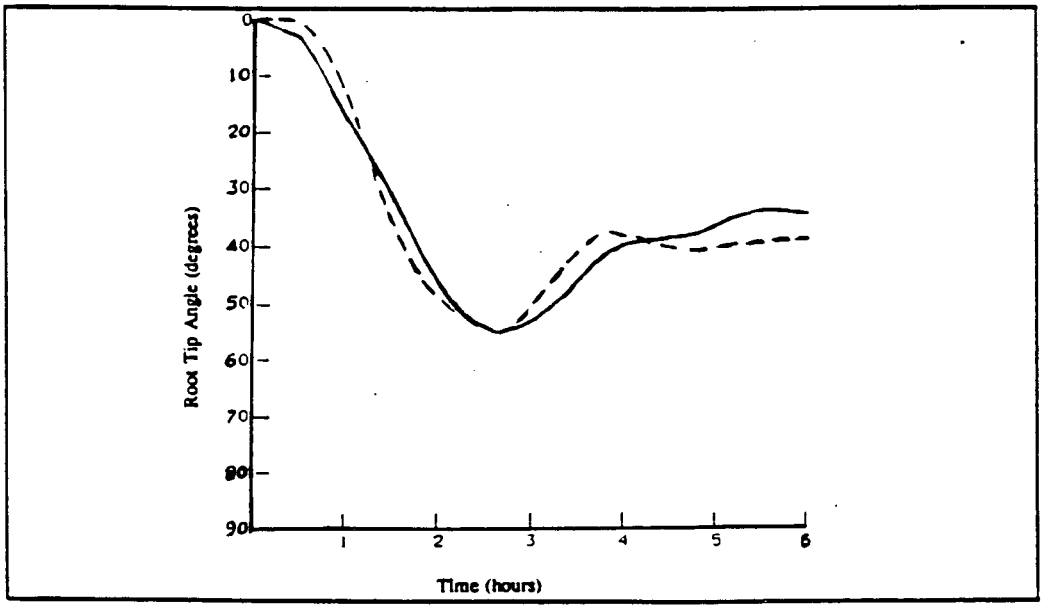
Mean georeaction curves of data (solid) and simulated (dashed). Data represents dark control. Simulation models a single flow of inhibitor at time $t = t_0$. Experiment 4.13.

FIGURE 4.8A



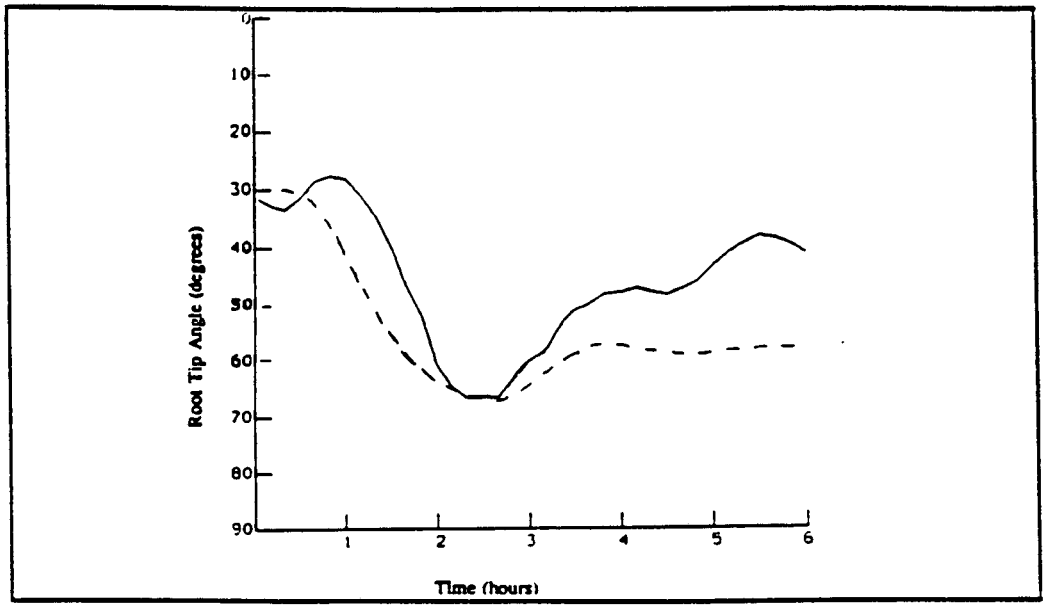
Mean georeaction curves of data (solid) and simulated (dashed). Data represents periodic incandescent illumination frequency 6 sec./10 min., intensity 1.9W/m^2. Simulation models a single flow of inhibitor released at time $t = t_0$. Experiment 4.1.

FIGURE 4.8B



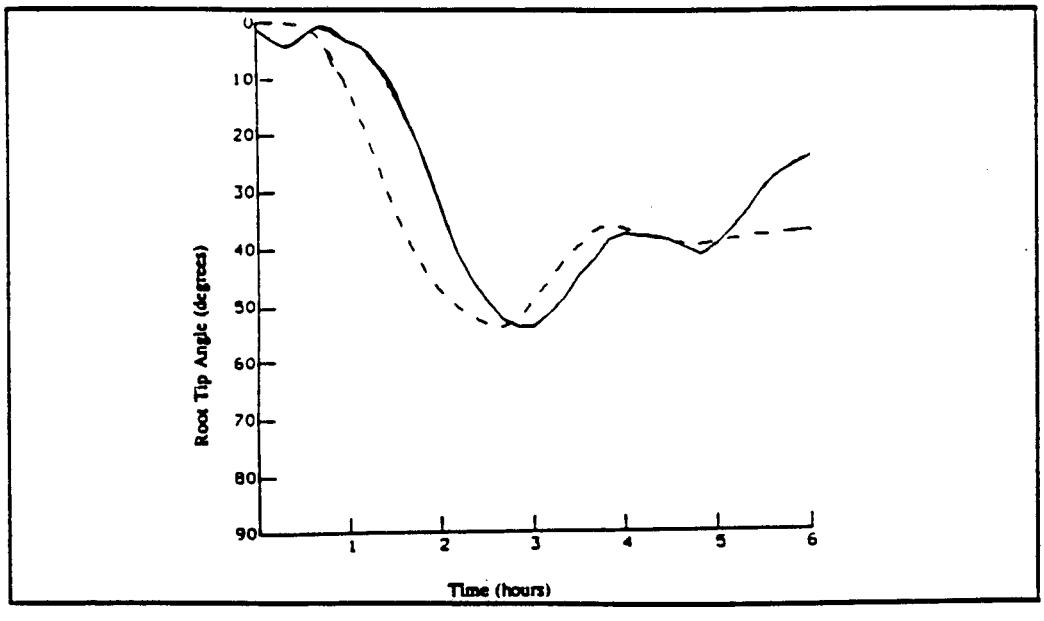
Mean georeaction curves of data (solid) and simulated (dashed). Data represents periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Simulation models a single flow of inhibitor released at time $t = t_0$. Experiment 4.2.

FIGURE 4.8C



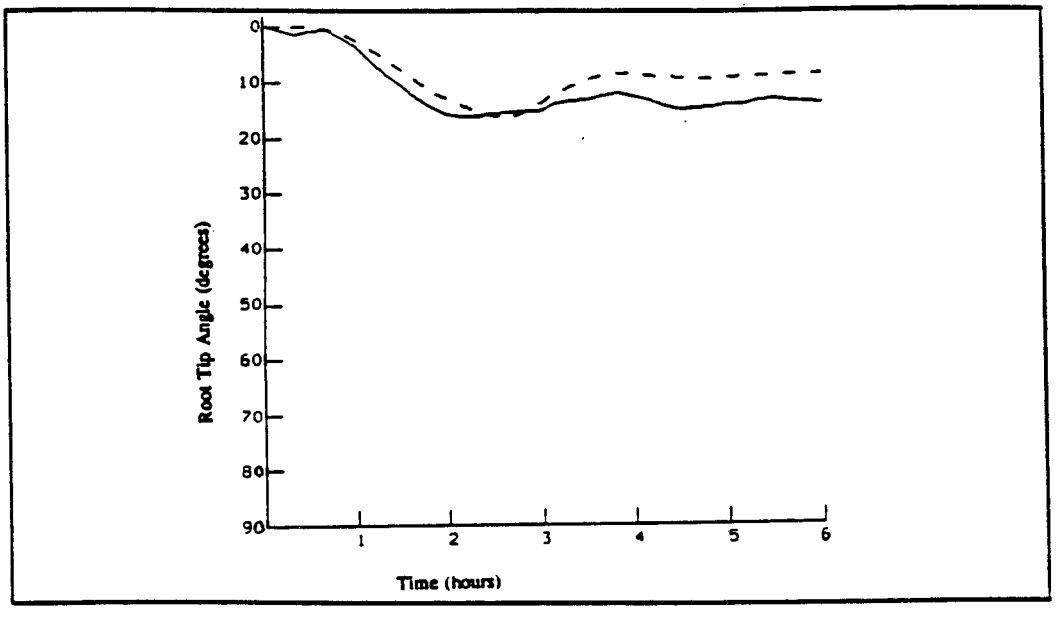
Mean georeaction curves of data (solid) and simulated (dashed). Data represents continuous incandescent illumination frequency 6 sec./10 min., intensity 1.9W/m^2. Simulation models a single flow of inhibitor released at time $t = t_0$. Experiment 4.3.

FIGURE 4.8D



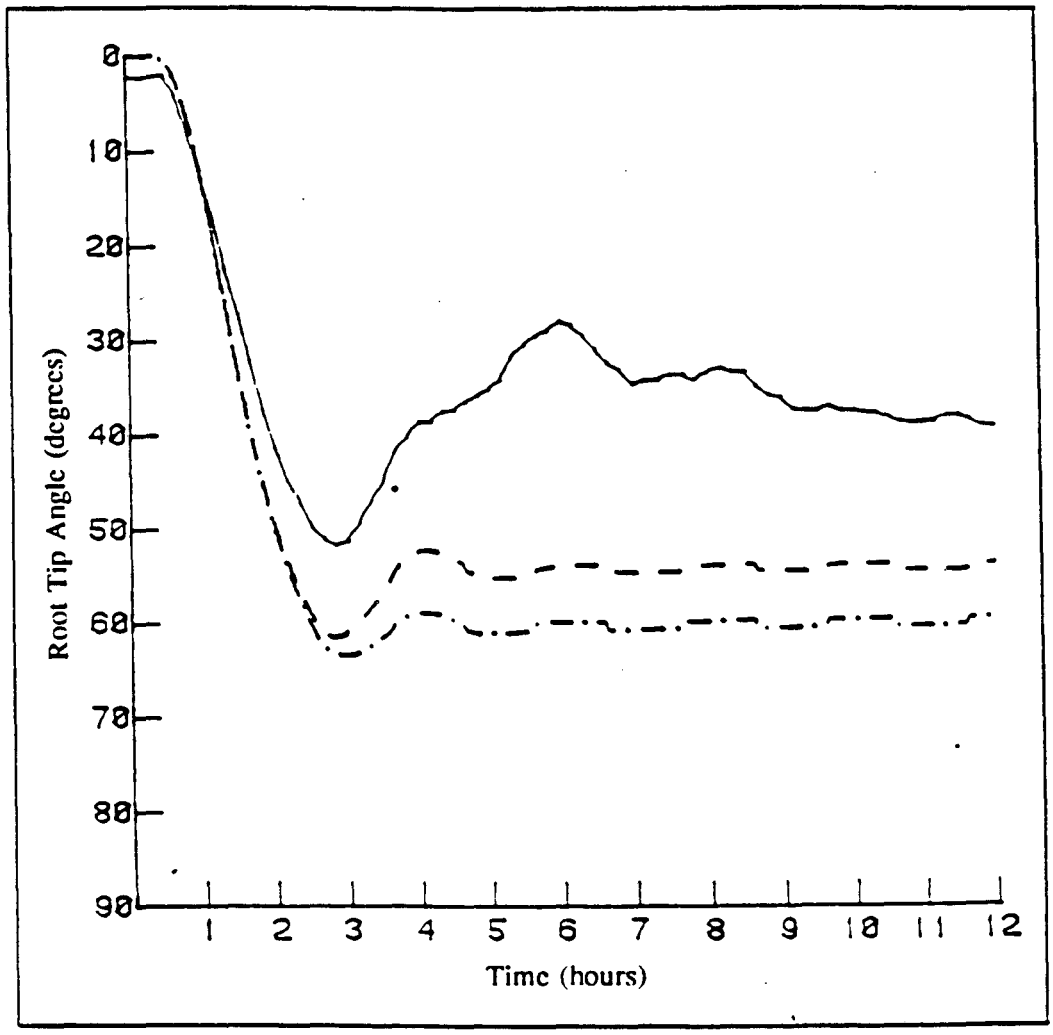
Mean georeaction curves of data (solid) and simulated (dashed). Data represents continuous incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Simulation models a single flow of inhibitor released at time $t = t_0$. Experiment 4.4.

FIGURE 4.8E



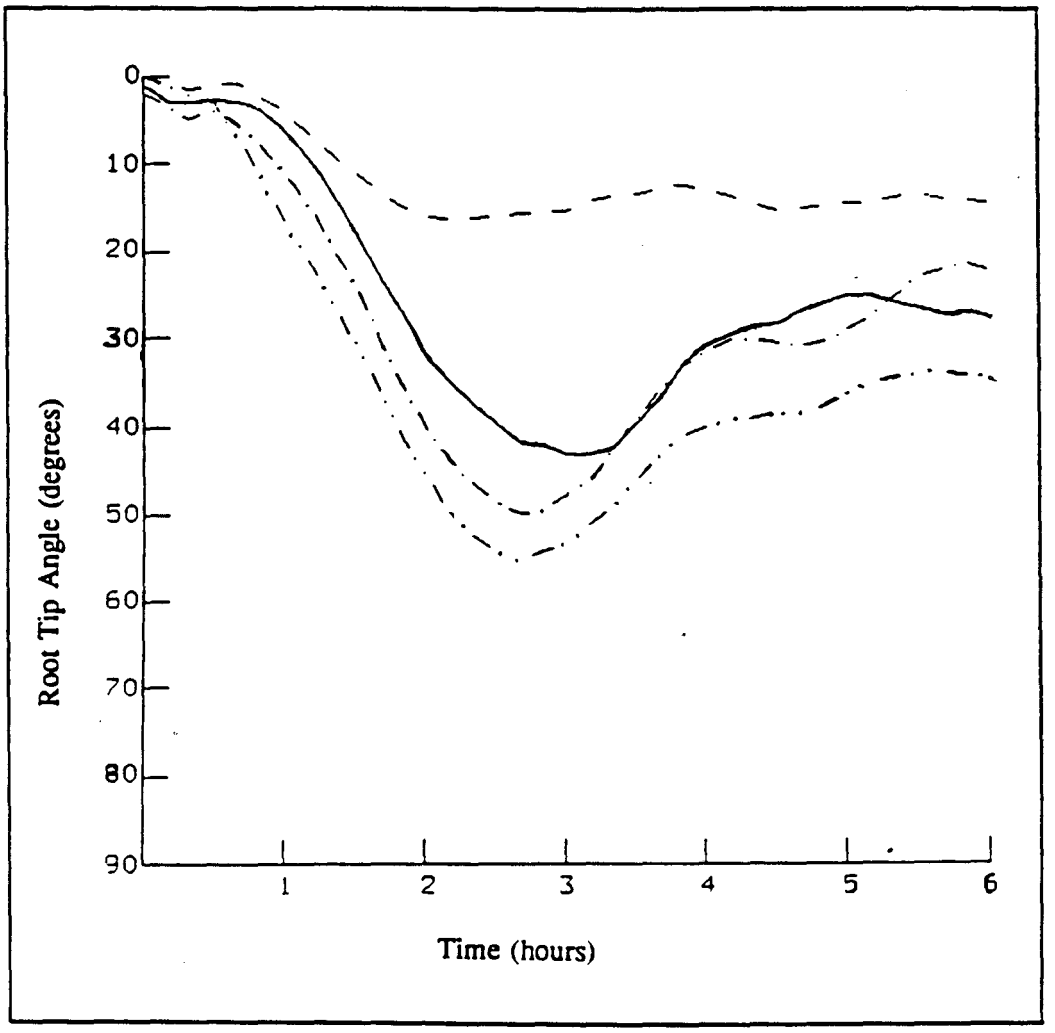
Mean georeaction curves of data (solid) and simulation (dashed). Data represents dark control. Simulation models a single flow of inhibitor released at time $t = t_0$. Experiment 4.5.

FIGURE 4.9



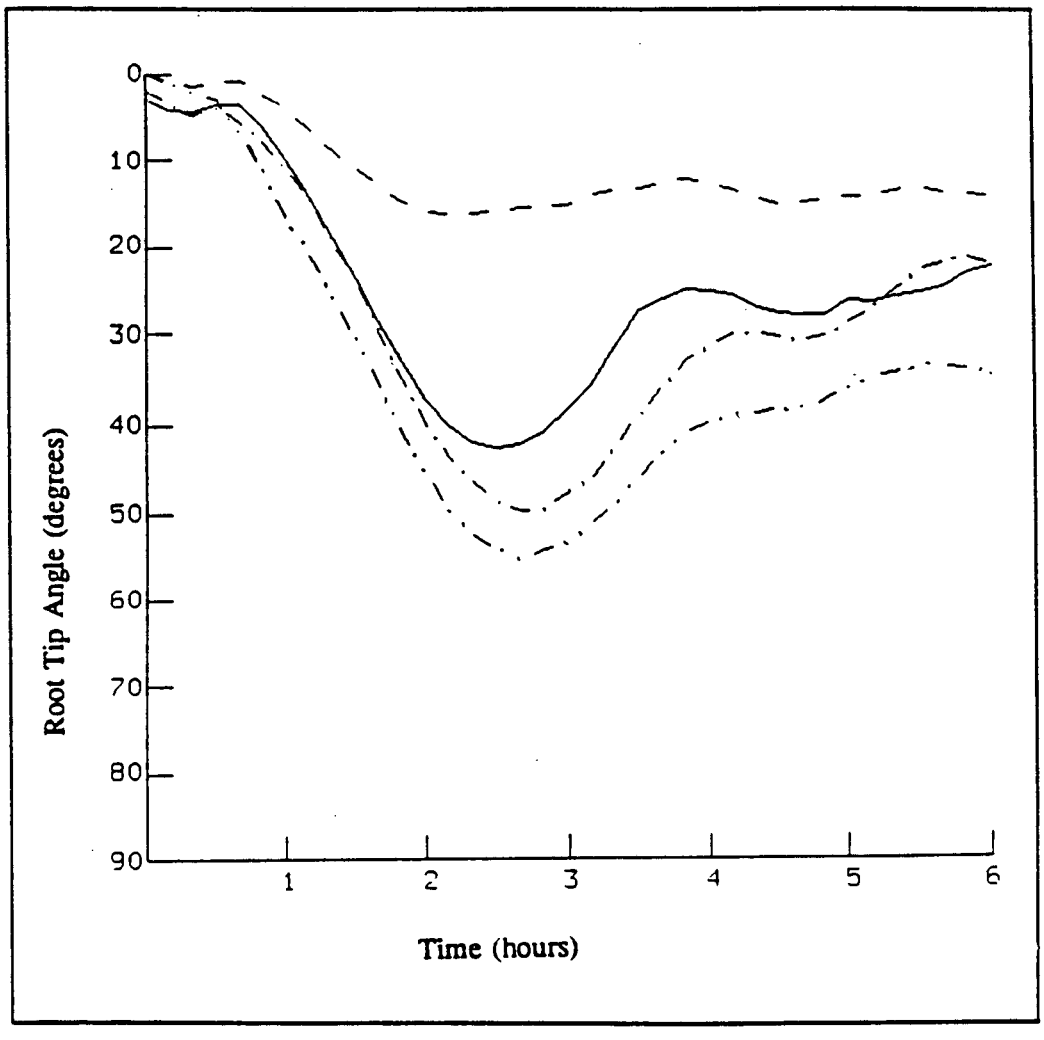
Early versions of the model: non-stop production of inhibitor without gravitational feedback (dashed-dot); non-stop production of inhibitor with gravitational feedback (dashed). Graph of averages from trial with incandescent light pulsed 6 sec./10 min. shown in solid curve. Experiment 4.8.

FIGURE 4.10A



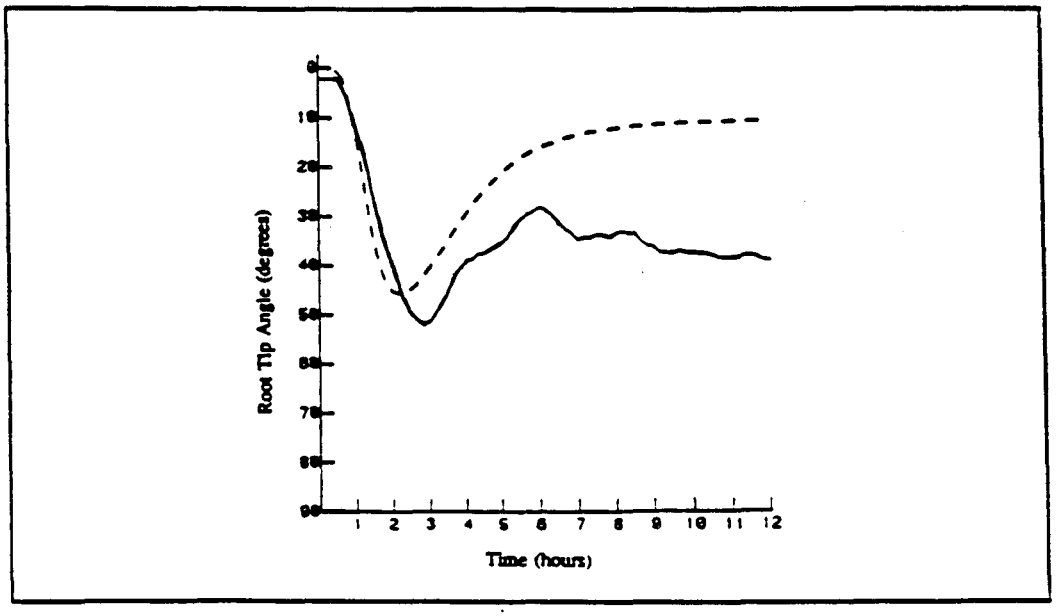
Mean georeaction curves for pulsed incandescent light trials frequency 6 sec./10 min., intensities 1.9W/m^2 (solid); 14.1W/m^2 (dashed-dot); 18.8W/m^2 (dashed-dot-dot). Dark control (dashed). Experiments 4.1, 4.18, 4.2, and 4.5, respectively.

FIGURE 4.10B



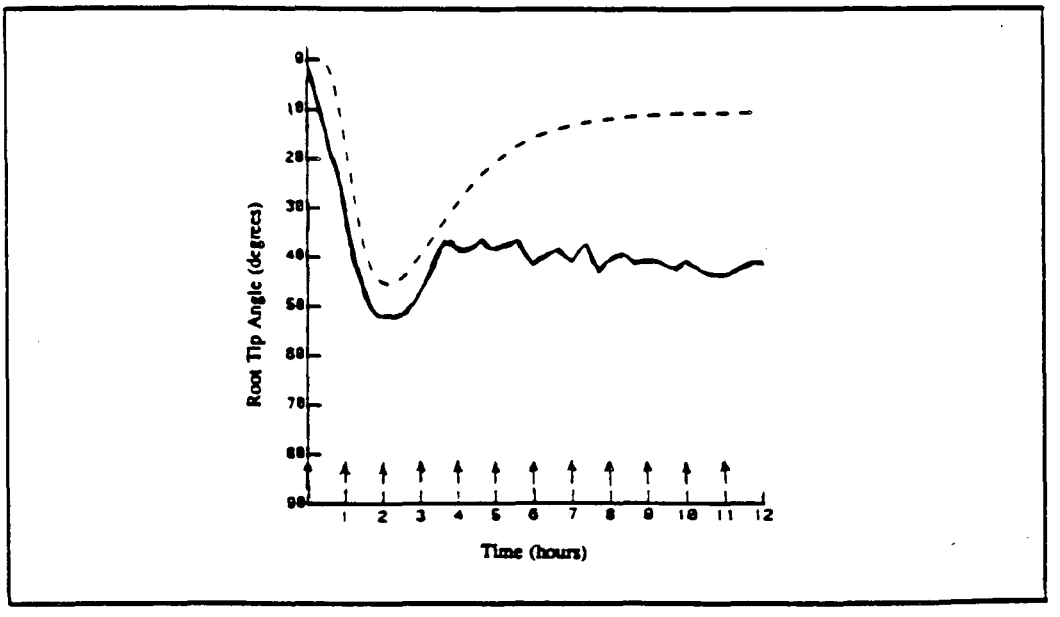
Mean georeaction curves for pulsed incandescent light trials frequency 6 sec./10 min., intensities 9.4W/m^2 (solid); 14.1W/m^2 (dashed-dot); 18.8W/m^2 (dashed-dot-dot). Dark control (dashed). Experiments 4.17, 4.18, 4.2, and 4.5, respectively.

FIGURE A-1A



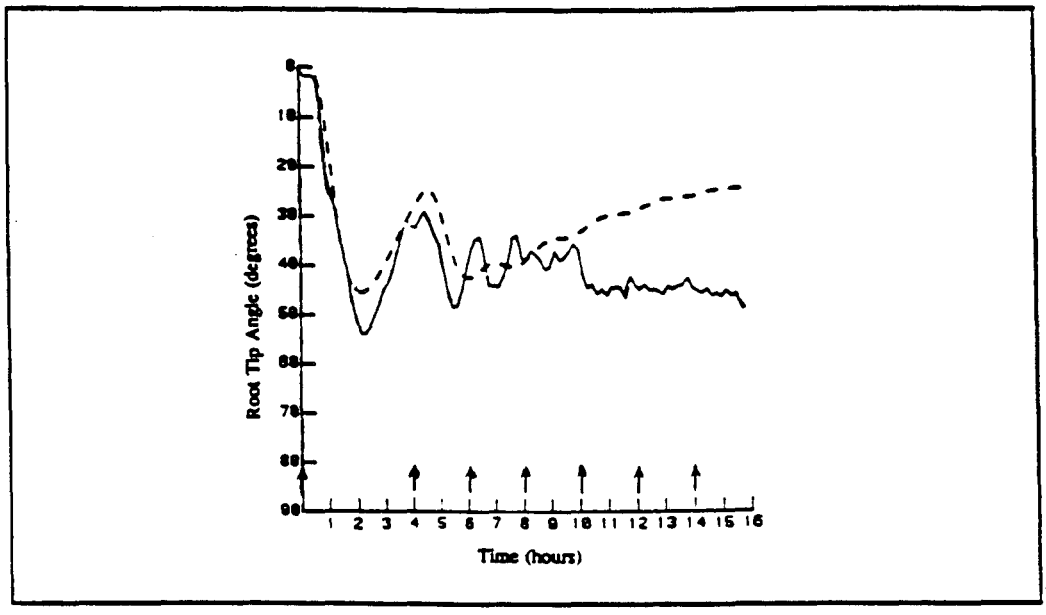
Mean georeaction curves of data (solid) and simulation (dashed). Data represents periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2. Simulation models inhibitor flow from tip along bottom and top of root released at time $t = t_0$. Experiment 4.8.

FIGURE A-1B



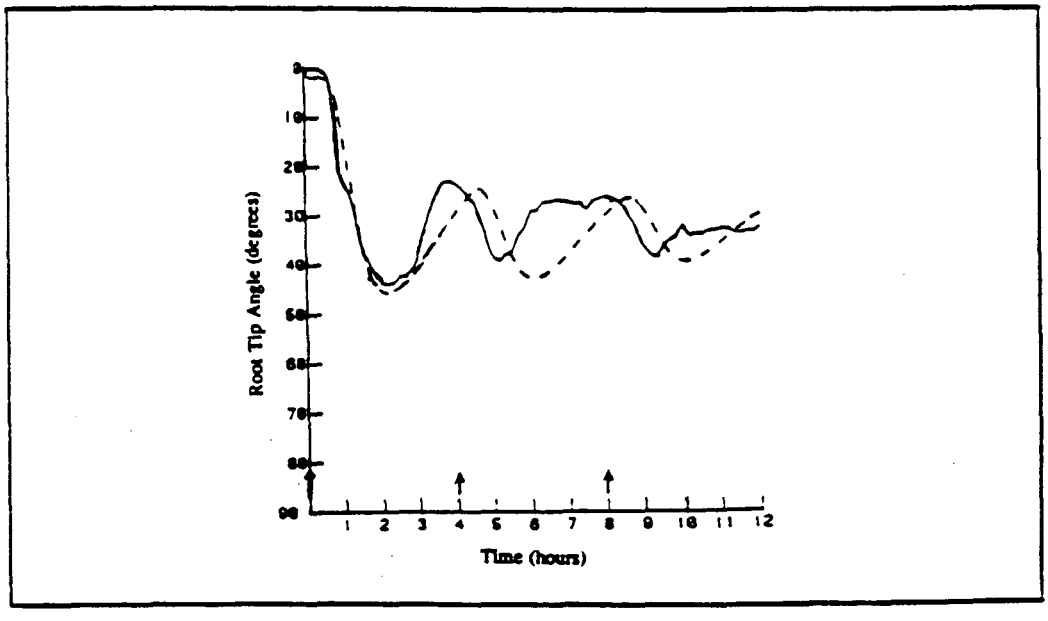
Mean georeaction curves of data (solid) and simulation (dashed). Data represents periodic incandescent illumination frequency 3 min./1 hr., intensity 18.8W/m^2. Simulation models inhibitor flow from tip along bottom and top of root released at time $t = t_0$. Arrows represent time of each new light pulse. Experiment 4.9.

FIGURE A-1C



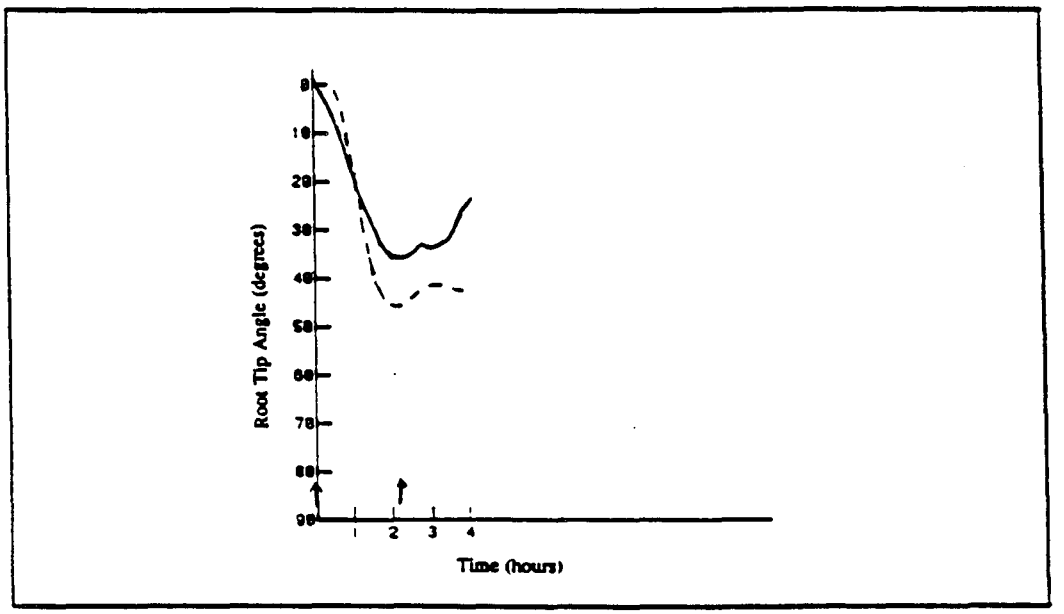
Mean georeaction curves of data (solid) and simulation (dashed). Data represents periodic incandescent illumination frequency 3 min./2 hr., except at +2 hours: intensity 18.8W/m^2. Simulation models new flow of inhibitor (bottom and top) of .5 times the previous flow following each light pulse, except for the pulse at +4 hours with is .7 time the original pulse. Arrows represent time of each new light pulse. Experiment 4.10.

FIGURE A-1D



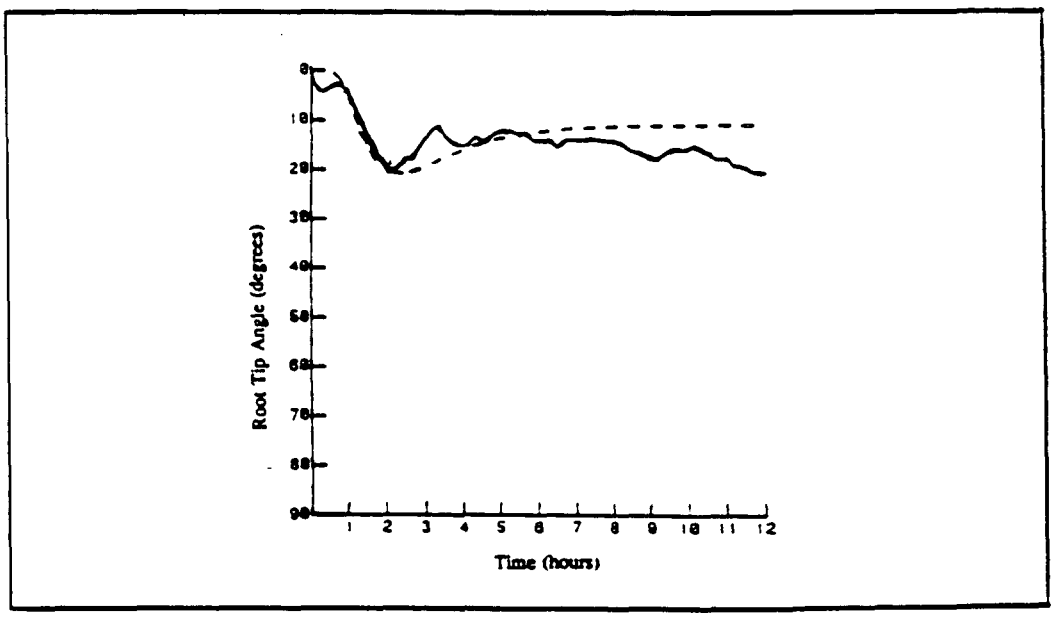
Mean georeaction curves of data (solid) and simulation (dashed). Data represents periodic incandescent illumination frequency 3 min./4 hr., intensity 18.8W/m^2. Simulation models new flow of inhibitor (bottom and top) of .7 times the previous flow following each light pulse. Arrows represent time of each new light pulse. Experiment 4.11.

FIGURE A-1E



Mean georeaction curves of data (solid) and simulation (dashed). Data represents light pulses at time $t = 0$ and $t = 130$ minutes, intensity 18.8W/m^2. Simulation models second flow of inhibitor (bottom and top) at .5 times the magnitude of the first flow. Arrows represent time of each light pulse. Experiment 4.12.

FIGURE A-1F



Mean georeaction curves of data (solid) and simulation (dashed). Data represents dark control. Simulation models a single flow of inhibitor (bottom and top) at time $t = t_0$. Experiment 4.13.

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CHAPTER 5

CIRCUMNUTATION OF MAIZE PRIMARY ROOTS

5.1 Literature Review

5.1.1: Circumnutation of Vertically-Oriented Roots

M. Spurny (1966, 1967, 1973) has energetically devoted himself to the examination of the detailed patterns traced by roots elongating in three dimensions. He determined that roots of *Pisum* and *Zea* sometimes, but not always, elongate in a helical pattern when they are oriented vertically downward. Spurny photographed the growing roots from two orthogonal directions in order to obtain a three dimensional view of the patterns described during elongation. He characterized the parameters of oscillation of *Pisum* and *Zea* with the following mean values: pea roots---period of oscillation 8 hours, amplitude 0.26 cm; maize roots---period of oscillation 1 hour 32 minutes, amplitude 0.027 cm (Spurny, 1973).

Ney and Pilet (1981) studied the three dimensional growth pattern of cv. LG 11 maize primary roots oriented vertically and exposed continuously to white fluorescent light (1.85W/m^2 at 21°C). A 0.4-second pulse of incandescent light (198W/m^2) was delivered each minute for filming purposes during a 4-hour observation period. These authors conclude that most roots examined show oscillational activity with a period of about 1 hour and with an amplitude of from 10° to 20°. However, they note that some roots show no spiral oscillations whatever. Data is presented for three presumably typical nutating roots.

5.1.2: Circumnutation of Horizontally-Oriented Roots

Ney and Pilet (1981) argue in favor of the gravitropic feedback model with data presented from primary maize roots (cv. LG 11) started horizontally and allowed to grow and georeact for

periods up to 10 hours in fluorescent light. Several such roots were filmed at one-minute intervals in order to characterize precisely the kinetics of root geocurvature. These authors concluded that roots, oriented horizontally in humid air, show three distinct phases of gravicurvature. First, a 20-minute lag time is experienced following reorientation of the roots from the vertical to the horizontal and before the start of geocurvature. Next, a steep decline phase lasting about 3 hours occurs in which the root tip approaches an angle of approximately 70°. Finally, a feedback phase begins in which the root tip executes a series of oscillations with an amplitude of from 5° to 20° and with a period of about 1 hour.[1]

These authors believe their data is consonant with the gravitropic feedback model since the steep decline phase is not interrupted by any upward movements which might otherwise indicate the presence of an endogenously-controlled cyclic pattern of movement. They also present data from a single "typical" root, started vertically, showing the time course of oscillations as the elongating root performs cyclic movement around the preferred direction of growth. The period of these oscillations is also in the range of 1 hour. Because of similarities in period length, Ney and Pilet assume that the cyclic motion following gravicurvature of horizontal roots arises from the same source as the nutational movement which they observed in vertically-growing roots. However, they do not apply rigorous statistical measures in analyzing their data. Therefore, it is difficult to properly evaluate their conclusions.

Heathcote (1982) uses conventional regression methods to re-examine the single georeaction curve which Ney and Pilet use for illustration purposes (Figure 5.1). He concludes that there is, in fact, a shoulder present 130 minutes after the start of geostimulation. He argues that this shoulder represents an oscillational event whose presence would not be expected unless

[1]This is, of course, the same phenomena explored in detail and modeled in Chapter 4 above.

an autonomous oscillator were driving circumnutation.

In truth, there are difficulties with both approaches. Ney and Pilet cannot fairly use the presence of a steep, uninterrupted positive phase of gravicurvature as proof of the feedback model because an internally driven nutational signal might still exist, but be completely overpowered by the strength of the gravitropic signal. Secondly, the fact that these authors produce data describing the geocurvature of only one root, rather than on a larger sample, makes it impossible from the outset to assess the accuracy of their conclusions. On the other hand, Heathcote does not disprove Ney and Pilet's argument merely by presenting a regression line instead of the original authors' graph of averages. There is at least one test for linearity (§4.2.3) which measures the statistical likelihood that the regression plot for an entire population reflects the true linearity of the data. Such a test could be employed in this case to unscramble the data. The discussion regarding the nature of cyclic gravicurvature in connection with roots is resumed below in §5.2.2.

5.2: Results and Discussion

5.2.1 Circumnutation of Vertically-Oriented Roots

I investigated the three dimensional growth parameters of maize primary roots oriented vertically downward in order to establish if helical growth around a vertical axis is consistently observed; and if there is a preferred orientation, i.e., clockwise or counterclockwise, of the pitch of the helix. I was also curious to discover whether roots oscillated in a toroidal (screw-like) pattern as they descended. There are some reports in the literature of nutating organs which elongate in a toroidal manner, e.g., the sporangiophore of Phycomyces blakesleeanus (Russo and Gallant, 1980). There are, however, no earlier reports in the literature in which toroidal root elongation has been observed.

Five cv. Merit maize primary roots were oriented vertically downward, marked with India

ink 0.15, 0.3, 0.45 and 0.6 cm from their caps and allowed to elongate for 17 hours 30 minutes in continuous incandescent and fluorescent light ($6.8 W/m^2$). Photographs were taken at half-hour intervals from two orthogonal angles with the roots viewed in profile from each camera position (Figure 2.3). The ink spots were visible only from one camera position and were at right angles to the other at the start of the experimental period.

With one exception, the ink spots continued to face in the same direction throughout the 17 hour 30 minute trial. Between 2 and 3.5 hours one of the roots rotated 90° clockwise around its longitudinal axis and it remained visible from the second camera throughout the rest of the experimental period. This experiment was repeated twice in trials lasting 12 hours; but with photographs taken at 10-minute intervals. The result was that no other roots showed evidence of toroidal movement.

I next explored the question of whether the same roots adopted a helical growth pattern as they descended. The root tip position was projected onto an x-y grid, taking a top down view of the elongating root. The two dimensional trace of the tip position was then plotted against time. The result was that the root tips showed some directional movement as the roots grew downwards. However, this motion could not really be considered helical.[2] The paths taken by several vertically-oriented roots are depicted in Figures 5.2 and 5.3.

The sample size was not large enough to establish general space-time parameters for the motion of cv. Merit primary roots oriented vertically downwards in continuous incandescent and fluorescent light. However, as noted, the results do suggest the absence of both toroidal motion and generalized helical growth.

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[2]I carried out Fourier transforms of the data described which indicated the absence of significant cyclic activity. Entire data sets were included in computing the power spectra.

5.2.2 Circumnutation of Horizontally-Oriented Roots

Our goal here is to discover the nature of the forces which lead to cyclic root motion for roots started horizontally. We begin by attempting to reason the issue according to the classical lines of debate from the literature on circumnutation: if gravitropic and nutational curvatures are distinct processes which affect the direction of growth of a given organ; then, in the simplest case, the effect of each on the overall curvature of the organ will be additive, in a vectorial sense. In this event, the nutational process would modulate the magnitude and/or the direction of gravitropically-derived curvature but nutation would still operate as an autonomously-controlled process. When the primary root is horizontal, the strength of the gravitropic signal may completely outweigh the nutational component even in the event that these processes exist independently of one another. This could, for example, lead to the transient obliteration of the oscillational motion until the strength of the gravitropic stimulus decreases to a level where the nutational signal can produce noticeable oscillatory motion. In such a case the kinetic profile of organ curvature would be indistinguishable from the motion described by the root if nutation were solely dependent on the response of the organ to gravitation.

I conducted two additional trials as a means of further exploring the nature of the forces governing the nutation of gravistimulated roots. I reasoned that altering the angle of the root tip, at critical points in a gravitropic trial with predictable space-time parameters, would help distinguish between the gravitational feedback model and the endogenous oscillator model---especially in the event of certain results. In the first of these trials (Figure 5.4A), roots were initially positioned horizontally and allowed to georeact for 2 hours 30 minutes in the presence of periodic, incandescent illumination (frequency: 6 sec./10 min.; intensity: 18.8W/m^2). After 2 hours 30 minutes, the roots were reoriented upwards approximately 50°. I argued that repositioning the roots upward towards the horizontal, just prior to the onset of the rising phase,

would impose a new gravitational stimulus which would constitute a challenge to the root's intended upward movement. I would have considered it strong evidence in support of the autonomous oscillator model if the roots had bent upwards after approximately 2 hours 40 minutes as occurs in Experiment 4.8 (Figure 4.1B). The reason is that if the negative phase of nutation were strong enough to overcome what is the known contrary force of gravitation, then the motion upwards could plausibly be considered of independent origin. A contrary result, i.e., positive gravicurvature following reorientation upwards, would not necessarily invalidate the internal oscillator model. The reason is that such a result might merely demonstrate the relatively greater strength of the positive gravitropic effect by comparison with the tendency of roots to curve upwards.

The result obtained shows the presence of a small but discernible descent phase following upward reorientation. The average root tip angle declined 15.3 degrees from 13.6° to 28.9° between 2 hours 40 minutes and 3 hours 50 minutes. We first consider the result from the point of view of the internal oscillator model. The simplest explanation is that the rising phase of gravicurvature does not occur after reorientation because the upward force of nutation is not as strong as the downward gravitational force. Now consider the result with the assumption that circumnutational motion is gravity-dependent. From this standpoint the overshoot of the liminal angle during the descent phase is compensated for by lifting the root. By this logic, the negative curvature phase is unnecessary and as a result it does not occur.

In a second trial cv. Merit roots were reoriented upwards approximately 28° at 2 hours 30 minutes. The result is depicted in Figure 5.4B. The graph of averages shows a nearly flat curve after reorientation. This result seems to imply a balancing of oppositely-directed forces whose relationship, however, remains obscure. One explanation is that after root reorientation the upward force of circumnutation and the downward force of gravitropism just balance to produce

a static phase in the curve.

The above discussion remains doggedly faithful to the terms of the debate over the forces governing circumnutation as presented in §1.5 and §1.6. (Recall that Heathcote and Johnsson strictly distinguish between the gravitational feedback model and the endogenous oscillator model.) I believe now, however, that the same arguments used to distinguish between the two competing models for vertically-positioned roots cannot be applied effectively to the discussion of the motive force behind nutational movements seen in gravistimulated roots. The following discussion is pertinent to this point.

It is difficult to imagine a process driven solely by a gravitational feedback mechanism which would lead to the patterns of curvature described in §5.1.2. It seems logical that if a gravitational feedback mechanism were in operation alone then the gravistimulated root would bend downward towards and beyond the plumb line. When the tip passed the vertical on its downward swing the statocytes would register this fact and signal the cells in the growth zone to reverse the polarity of asymmetrical elongation. The result would be movement of the tip in the opposite direction. A self-sustained pattern of oscillations around the vertical might be maintained in such a manner.

I believe that it is correct to conclude that the mechanism which actually underlies the curvatures described is not simply based on a gravitational feedback principal for the following reasons:

1: It is a well-established fact that horizontally-oriented maize primary roots tend to attain liminal angles in the range of 45° to 90° below the horizontal without passing the plumb line on their downward arc. In the case of my own data, the greatest mean maximum angle achieved did not exceed 67° in any of the trials described. The report of Ney and Pilet (1981) indicates that the initial descent phase is terminated when the root tip reaches an angle of about 70° while according

to the data of Hillman and Wilkins (1982) the original decline phase ends when the gravistimulated root reaches about +90°. In fact, a search of the literature does not reveal any reports in which horizontally-positioned roots develop a rhythmic pattern of motion around the plumb line.

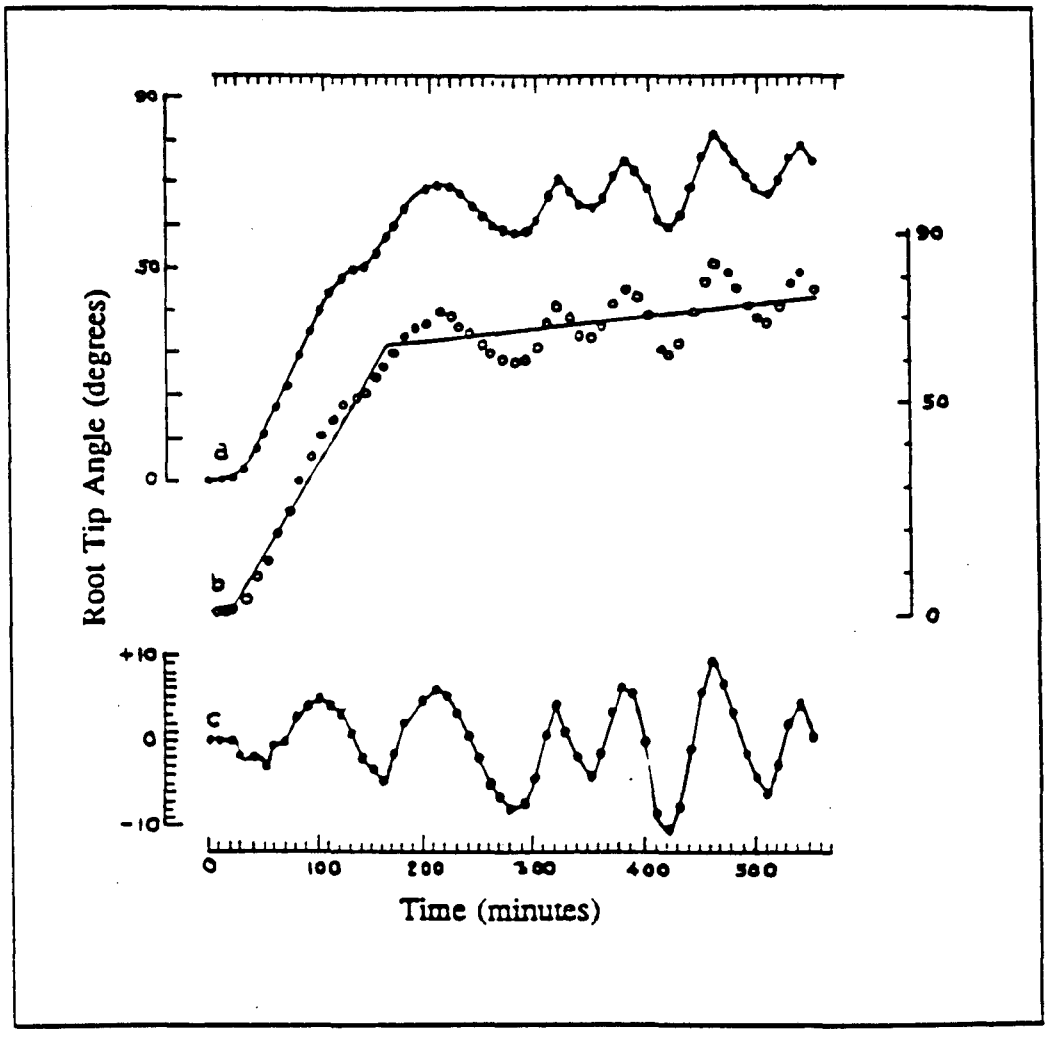
2: What then of the possibility that, as the root bends downward, either a gravisensitive or graviresponsive cell layer, canted at an angle to the root axis, might become aligned with the gravity vector leaving the root to oscillate around an angle between 0° and 90°? A morphological peculiarity of this sort could account for a self-sustained pattern of oscillatory root tip motion while still managing to fit the parameters of my data as well as that of Ney and Pilet and Hillman and Wilkins. This is highly unlikely, however, since the maize root is generally organized in a radially-symmetric fashion and in any event no such non-symmetrical, off-axis gravitropic structures have ever been identified.

Such considerations lead us to the view that nutational root movements following gravistimulation in the presence of light are not simply based on a gravitational feedback process. The model presented in §4.2.7 does contain a gravity feedback term (see Equation 5 in §4.2.7). However, this feedback loop is not strictly needed to produce a rising phase in the curve of root gravicurvature as a function of time. The simulation shown in Figure 4.9 (dashed-dot line) supports this assertion. The curve shows the presence of a rising phase although the model used to produce it does not contain a gravity feedback term. The primary model from §4.2.7 contains a gravity feedback term, but it is principally the circumferential transport of inhibitor in the growth zone that is responsible for the reversal of the direction of the root tip. In the model, we propose that there is significant circumferential movement of inhibitor only when the root is gravistimulated in the light. Therefore, light-aided gravistimulation, rather than a gravity feedback process, per se, precipitates inhibitor movement in the growth zone. Finally, it is precisely

because circumferential flow of inhibitor is stimulated by light and gravity that it can not be considered an endogenously-derived process. This conclusion differs from either of the two main competing models for circumnutation.

In conclusion, the endogenous oscillator model and the gravitropic feedback model are twisted together by the physical phenomenon of gravitation. Since gravitation plays an important role in each, the two are almost impossible to untangle definitively in normal terrestrial conditions. In the autonomous oscillator model, gravitation can be viewed as a confounding factor. Gravitation affects the direction of organ movements in common with the nutational process, but the latter process is considered to exist irrespective of the presence of gravitation. By contrast, the feedback model posits gravitation as the sole motive force for circumnutation. A single 0 g satellite experiment has been performed which attempted to distinguish between the competing models (see §1.5, final paragraph). However, other such investigations are needed to tell the broad truth about nutational movements in various plant organs and species.

FIGURE 5.1

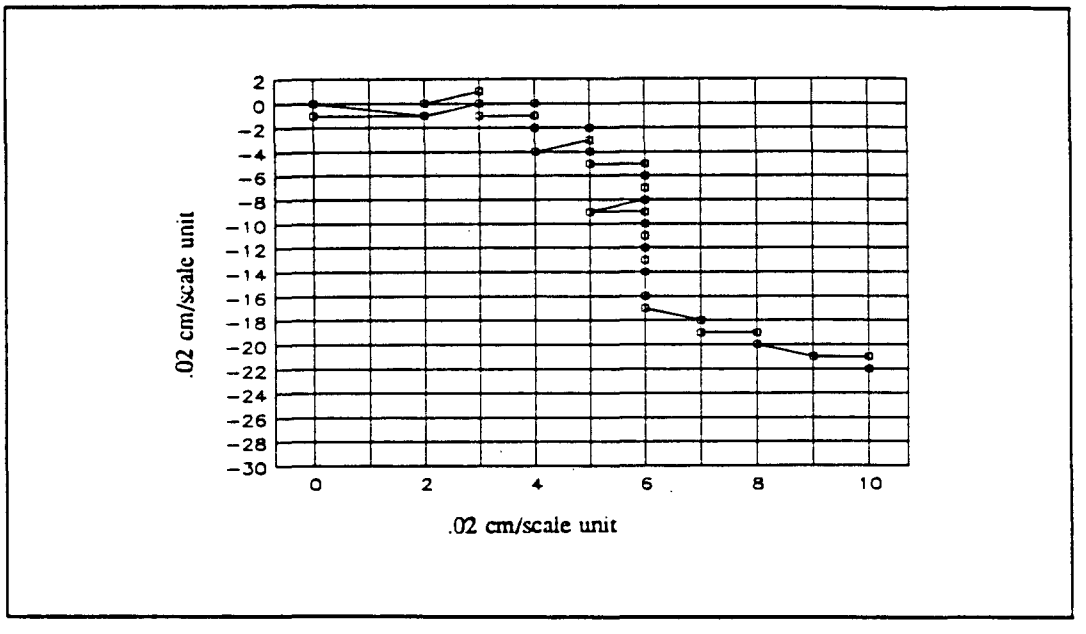


A: Graviresponse curve of primary root of *Zea mays* (cv. LG 11) showing positive gravicurvature and nutation phases (Ney and Pilet, 1981).
B: The same data with fitted trend lines indicated. The regression equation for the graviresponse phase is: $f(t) = -7.61+0.44t$: the equation for the plateau phase is: $f(t) = 56.92+0.03t$.
C: The computed difference between the time series data and the fitted trend lines. The different time course is a measure of the nutational oscillation which continues after the graviresponse curve.
Redrawn from Heathcoate (1982).

FIGURE 5.2

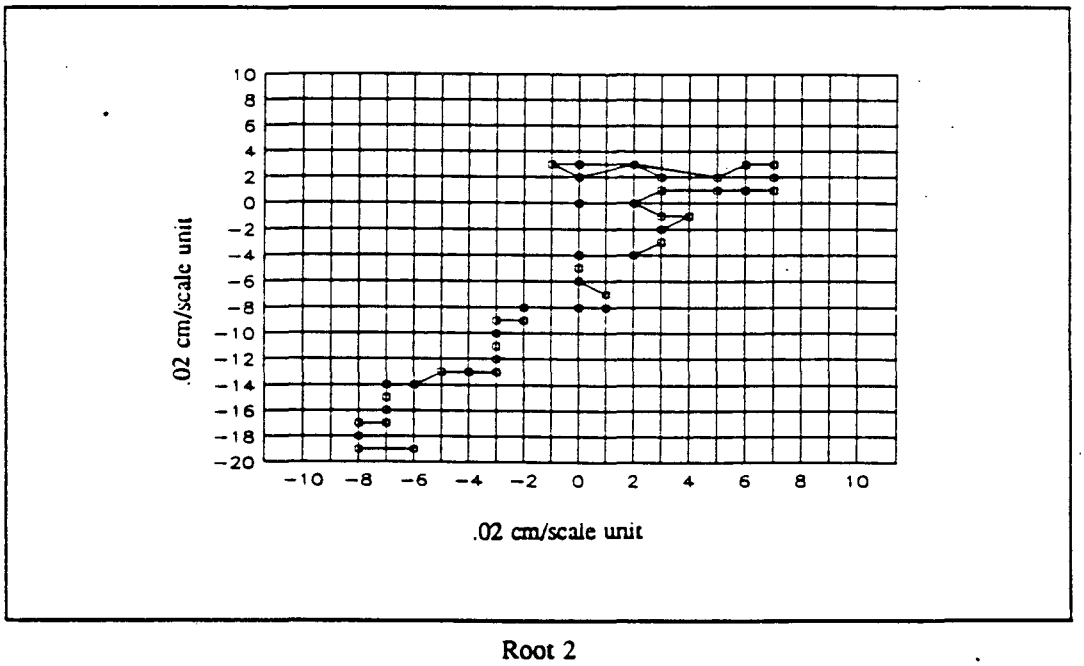
Cv. Merit maize primary roots oriented vertically downward at time $t = 0$, and subjected to continuous incandescent and fluorescent illumination, intensity $6.8 W/m^2$. Projection of individual root tip position onto xy-plane recorded at 30-minute intervals for duration of 17 hour 40 minute experiment.

FIGURE 5.2A



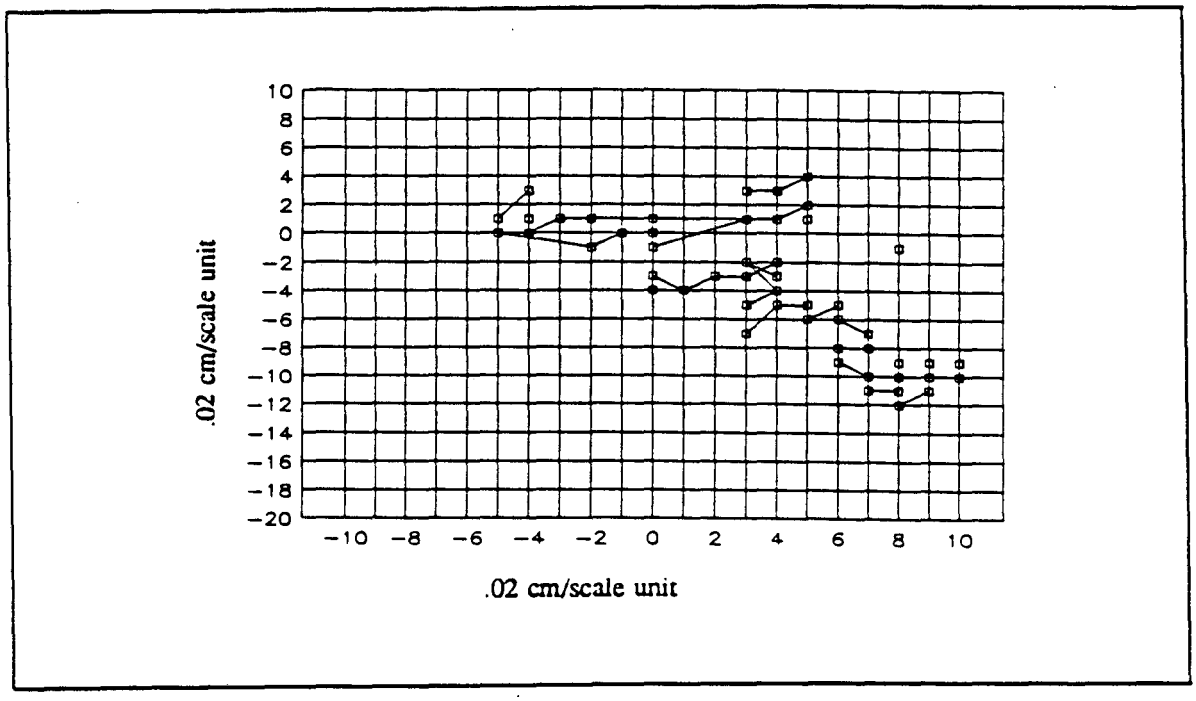
Root 1

FIGURE 5.2B



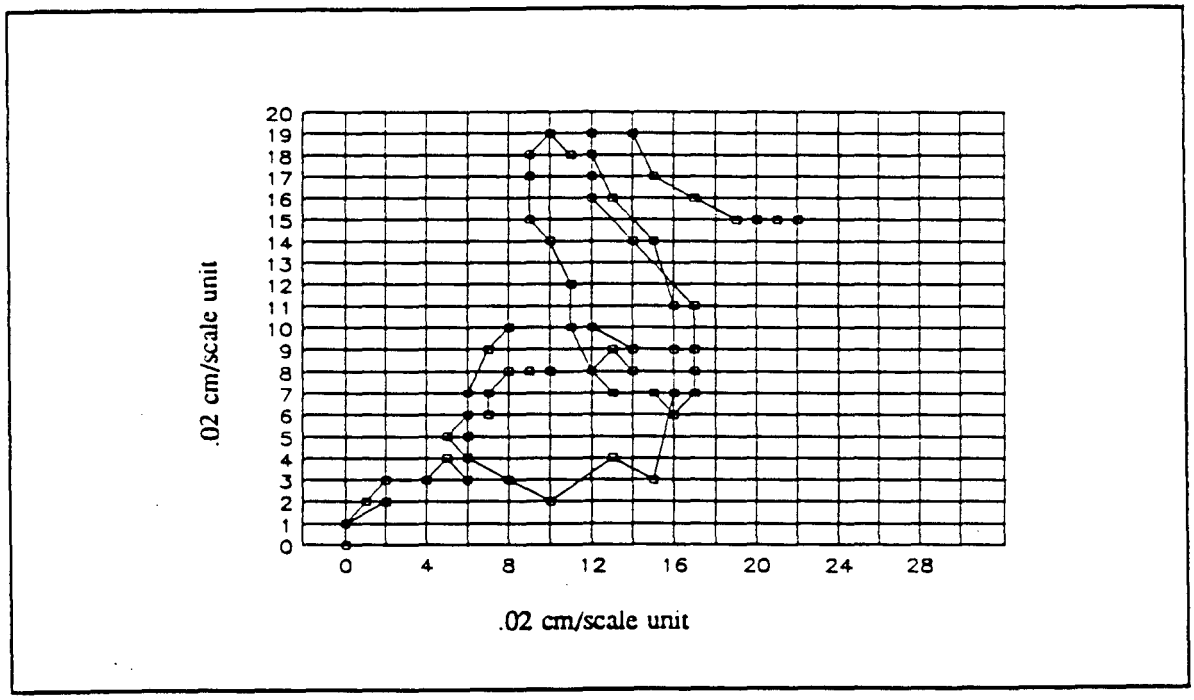
Root 2

FIGURE 5.2C



Root 3

FIGURE 5.2D

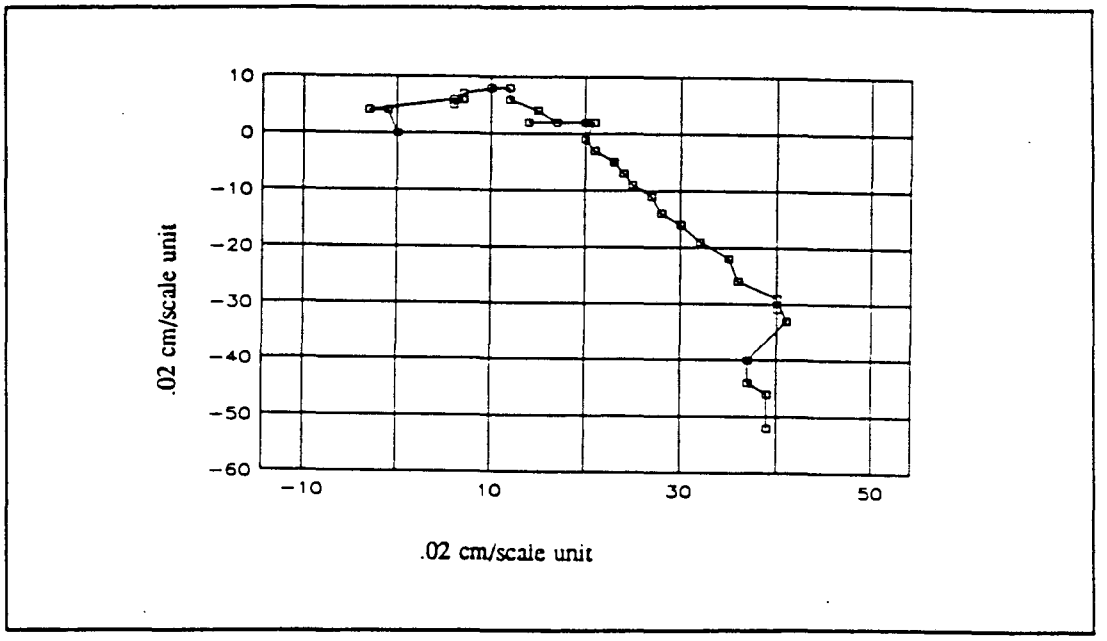


Root 4

FIGURE 5.3

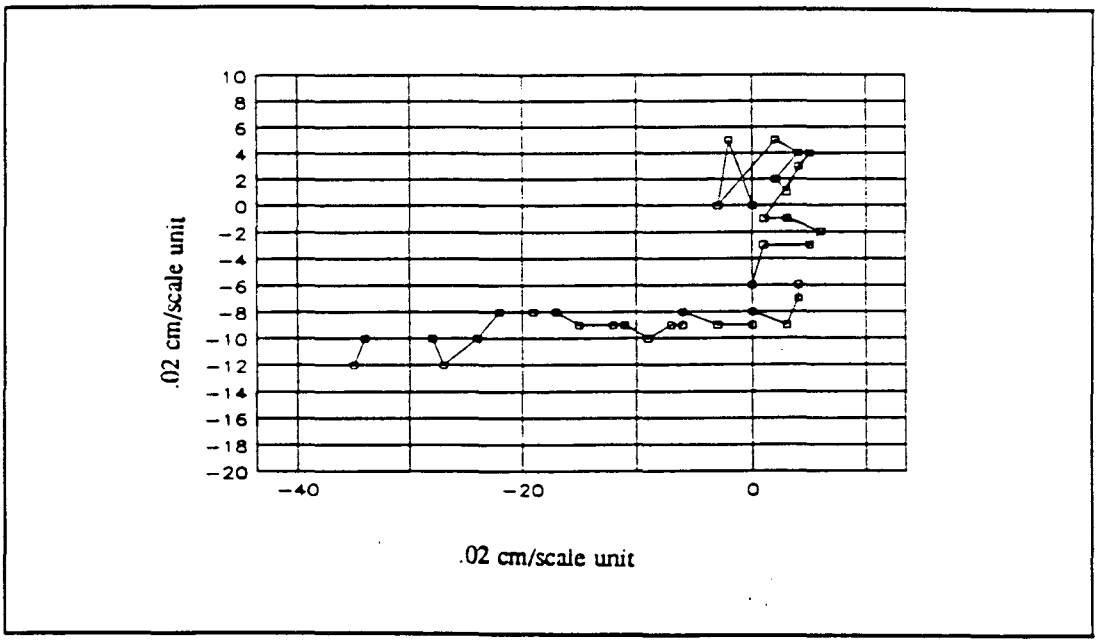
Cv. Merit maize primary roots oriented vertically downward at time $t = 0$, and subjected to continuous incandescent and fluorescent illumination, intensity 6.8W/m^2. Projection of individual root tip position onto xy-plane recorded at 10-minute intervals for duration of 12 hour experiment.

FIGURE 5.3A



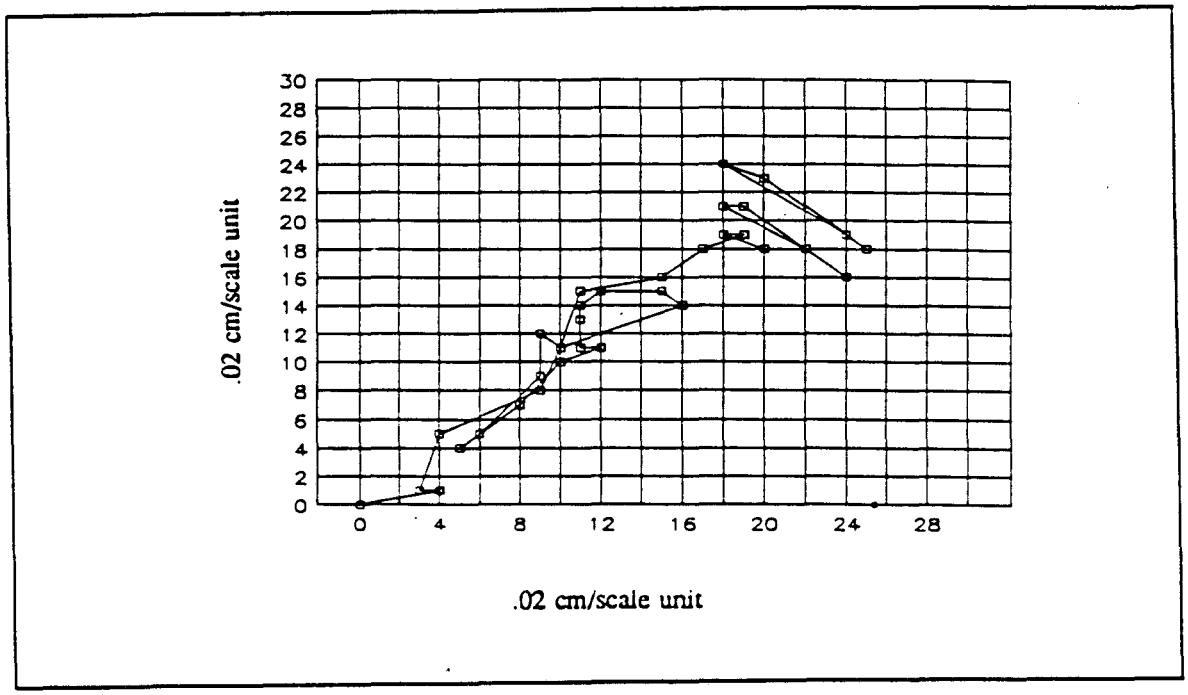
Root 1

FIGURE 5.3B



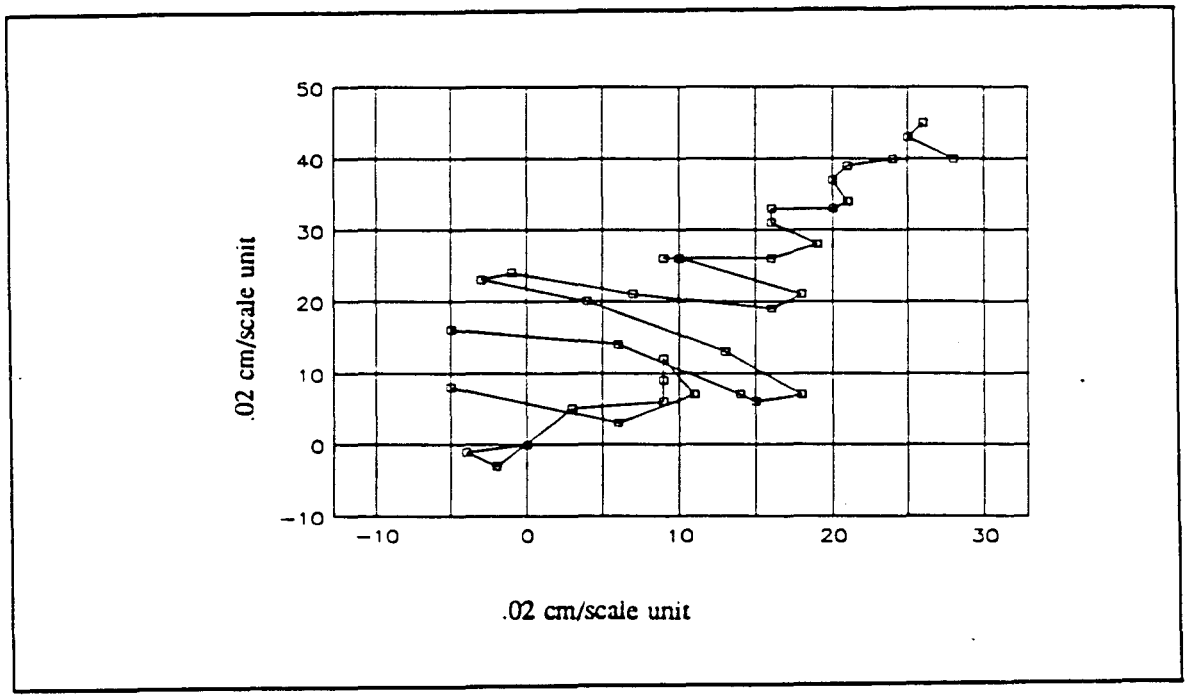
Root 2

FIGURE 5.3C



Root 3

FIGURE 5.3D

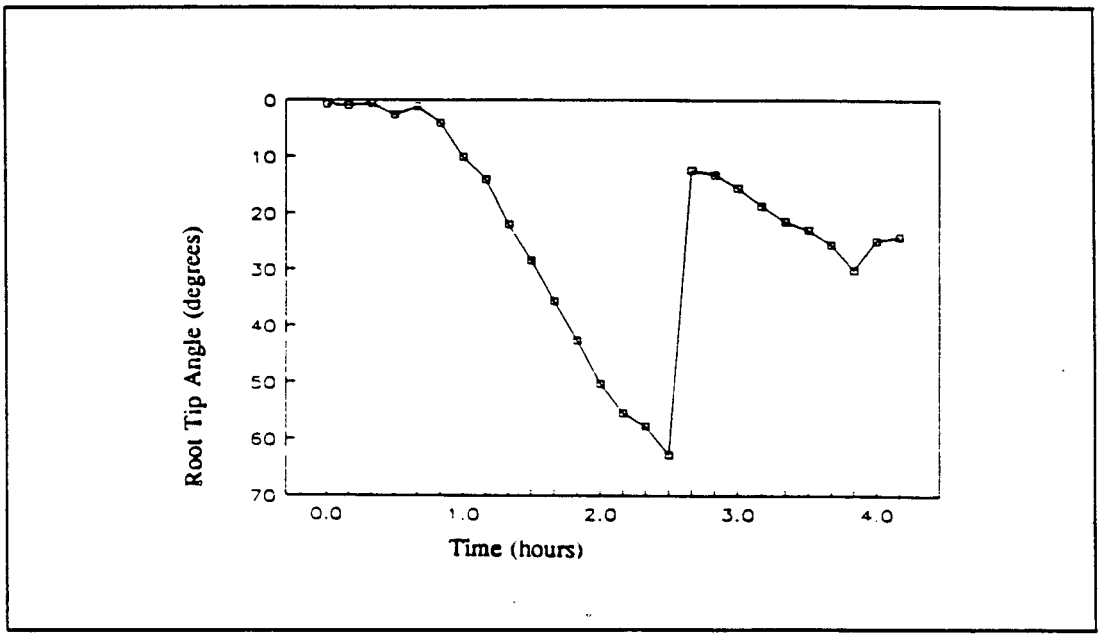


Root 4

FIGURE 5.4

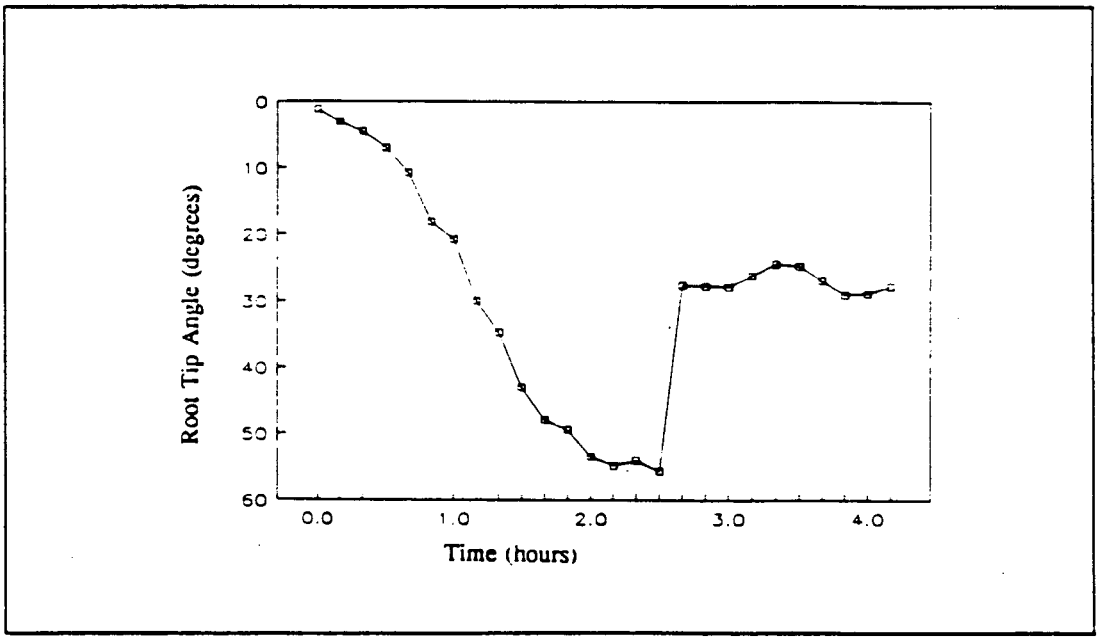
Graphs of averages for cv. Merit maize primary roots started horizontally at time $t = 0$, and subjected to periodic incandescent illumination frequency 6 sec./10 min., intensity 18.8W/m^2.

FIGURE 5.4A



Roots re-oriented upwards 50.3°(±6.9°) at +2 hours 30 minutes. Duration of experiment 4 hours 10 minutes.

FIGURE 5.4B



Roots re-oriented upwards 28.1°(±6.2°) at +2 hours 30 minutes. Duration of experiment 4 hours 10 minutes.

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CHAPTER 6

IN SEARCH OF A GENETIC COMPONENT FOR MAIZE ROOT GRAVITROPISM AND CIRCUMNUTATION

6.1: Gravitropism

6.1.1: Introduction

Why is it that some maize primary roots subjected to gravitropic stimulation exhibit rapid downward curvature while others in the same population show much less gravitropic activity in the same time period? Two basic explanations of this phenomenon present themselves. Either the genetic background of the organism in some way dictates the extent and rapidity of root gravitropic movement; or the environmental stimuli, which the seedling has experienced, determine the progress of positive root curvature under the influence of gravity. These factors may also exist in combination.

As a result of work presented here, I am now satisfied that there is no simple, i.e., one- or two-variable gene expression system, governing this response in the two varieties of midwestern maize which I investigated (cv. Merit and cv. Pioneer).

6.1.2: Results and Discussion

As noted earlier (§4.2.3) Merit is an example of a maize cultivar whose roots require light to develop a strong positive gravitropic response. In the summer of 1986, I measured gravicurvature as a function of time for parental (P1) and filial (F1) Merit primary roots subjected to periodic incandescent illumination (frequency: 6 sec./10 min.; intensity: 18.8W/m^2). The seedlings were then grown to maturity and self-pollinated. The F1 progeny were tested under the same conditions. Some similarities in the graviresponse profile existed between individual P1 and

F1 roots which provoked further exploration of this problem (Figures 6.1 and 6.2).

In May of 1987, I discovered that when untested Pioneer P1 plants were sibbed and selfed, the F1 population exhibited a strong gravitropic response in the absence of all light. In fact, the response was as great as that shown by light-exposed Merit roots. At the same time I found that Merit x Pioneer F1 seeds were also strongly gravitropic in the dark.[1] (By contrast, as noted in Chapters 3 and 4, dark-exposed Merit roots were fairly unresponsive to gravitropic stimulation.) The working hypothesis became, therefore, that gravitropic responsiveness in the dark is a dominant, single-gene trait (Figure 6.3A).

In order to test this theory I back-crossed Pioneer/Merit F1 tested plants to Merit P1 tested plants in the summer of 1987. If the trait were heritable, I might have expected a bimodal distribution among the offspring. As is made evident in Figures 6.4 and 6.5, however, no such two-peaked arrangement could be demonstrated. The smeared distribution implies one of two things. Either there are several genes involved in the gravitropic response pathway, and due to the large variability in responsiveness among roots in a given population, the various genetic contributions become masked in the absence of very large sample sizes; or the response is simply not under genetic control. The latter conclusion appears very likely, because even with a complicated multi-gene control system, the ends of the distribution should breed true. In other words, the self-pollinated progeny of extremely gravitropic roots should produce extremely gravitropic offspring, while unresponsive roots which are selfed should yield unresponsive progeny.

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[1]Average angle computed for the entire population over the 6-hour period of gravistimulation: dark-exposed Pioneer sibs and selfs, 32.2°; light-exposed Merit (frequency 6 sec./10 min.; intensity 18.8W/m^2), 34.2°; dark-exposed Pioneer x Merit, 34.5°.

6.2: Circumnutation--Results and Discussion

I investigated a similar question in connection with oscillatory root movements. Do seeds with primary roots exhibiting a strong tendency to oscillate during gravitropic stimulation produce offspring with a similar phenotype when they are self-pollinated or is the variability of this behavior also under non-genetic control? As discussed extensively in §4.2.5, a small percentage of gravistimulated roots exhibit a rhythmic pattern of curvature in the presence of periodic incandescent illumination; frequency: 6 sec./10 min.; intensity: 18.8W/m^2 (Experiment 4.2 in Table 4.3). A gravistimulated root is considered to be cyclic, for the purpose of this discussion, only if it contains a significant power spectrum spike according to the Shimshoni-modified Fisher test. Ten seeds, representing both cyclic and non-cyclic phenotypes, were tested, grown to maturity and self-pollinated in the summer of 1986. Their offspring were subsequently tested and no phenotypic correlations could be established between generations that would have warranted further genetic testing. Only one of the ten P1 roots displayed a pattern of cyclic gravicurvature. Its offspring did not show a higher incidence of cyclic activity as is depicted in Figure 6.6.

It is extremely difficult to pinpoint the cause or causes of an observed phenomenon like this one, not obviously under genetic control. However, the vagaries of the particular environmental conditions to which the developing seed are subjected can account for significant variability in either form or behavior. For example, the summer growing season of 1987 was particularly damp and cool and many ears had to be harvested before the seeds had hardened sufficiently on the cob. Seed hardening usually occurs 50 to 60 days after pollination but in the summer of 1987 it did not take place at all; in many cases, even after 85 days. Therefore many ears were harvested with unhardened seeds, and seed desiccation was finally induced in the warmth and dryness of the greenhouse, rather than outside on the plant. Presumably a trait under genetic control would continue to express itself through such anomalies in the weather from

season to season but it still remains unclear which particular environmental factor or factors led to such variations in the kinetics of root curvature described above.

The study of maize genetics requires extreme patience. Only two generations of maize can be propagated each year and thus the lag time between the dawning of an idea for an experiment and the availability of hard results in connection with the experiment can be months or even years. Mistakes are also not readily tolerated because they can inevitably lead to delays lasting up to 6 months, or the time required to complete another generation. Thus when one embarks on a "fishing expedition", in this case involving the search for new genes, it is sensible to have clear goals and, if necessary, to know when to suspend the investigation.

There may indeed be genetic control over some aspects of light-induced gravitropic sensitivity, or over nutational movement in maize roots (in fact with over 300 races of maize known, it would be surprising if there were not). However, given the results taken from two typical midwestern corn-belt maize cultivars no such genetic findings could be unearthed and the decision was made to suspend the project.

6.3: Possible Genetic Control of Other Aspects of Primary Root Gravitropism

There are several other issues which warrant further discussion in connection with possible genetic involvement in the phenomenon of gravitropism in maize roots. To begin, my own observations of the general condition of primary roots subjected to gravitropic stimulation in the presence of light reveal a possible connection between the development of root hair and the tendency for the root to express gravitropic curvature. Specifically, I have noticed that roots which tend to show sparse root hair development, as noted at the conclusion of a 6-hour test period, sometimes show a pattern of straight plagiogeotropic growth during the same period. As a rough estimate, no more than 5 or 10 percent of roots show both minimal gravitropic curvature

and sparse root hair development. It would, of course, be a straightforward, if somewhat time consuming matter, to establish a firm genetic linkage between these seemingly coupled phenomena. On the other hand, as with the observation of oscillatory root movements described above, a finding which established no inter-generational connection between root hair development and gravicurvature would provide quick and convincing evidence that the two phenomena are not linked through inheritance.

A second issue of interest emerges from an examination of the graphs of averages describing gravicurvature against time in the presence of incandescent light for Merit and Pioneer primary roots. As described in §4.2.4, gravistimulated Merit roots exposed to light bend upward towards the horizontal after the initial descent phase. In contrast, Pioneer roots show a considerably reduced or a non-existent rising phase depending on the type of seed used (Figure 6.7). Although the numbers of Pioneer roots tested is small compared to the sample sizes for Merit, the differences are notable enough to be pursued in terms of a possible genetic component.

I have carried out several Pioneer x Merit crosses for which the gravitropic response profile in the presence of periodic incandescent light has been examined for each parent. Some crosses were performed between parental Pioneer primary roots showing the complete absence of a negative curvature phase and parental Merit primary roots exhibiting a typically strong negative curvature phase. The offspring of these crosses will be evaluated in the future to determine, for example, if a clear pattern of genetic dominance exists. The pursuit of a gene expressing the presence or absence of a negative curvature phase would be continued if a pattern of dominance expressed itself in the offspring of some carefully selected crosses.

Finally, there are examples of cultivars of non-light requiring gravitropic primary roots cited in the literature, such as cv. Anjou 210. Pilet (1978) found that apical segments of cv. Anjou 210 primary roots exhibit a strong graviresponse after six-hours gravistimulation in the

dark, and only a slightly greater response in the presence of light. I find it surprising that no one has discovered if this particular cultivar of maize is strongly graviresponsive in the dark as a direct consequence of an allelic variation. It would be a valuable contribution to establish whether such a genetic component exists in cv. Anjou 210. The problem could be analyzed in a straightforward manner using the methods outlined in this chapter.

FIGURE 6.1

Graviresponse curves comparing cv. Merit P1 primary root with six different roots from the F1 generation. The P1 plant was self-pollinated in the summer of 1986 to produce the F1 population. Each plot contains two curves representing graviresponse profile of the same P1 root with a different F1 root. Roots oriented horizontally at time $t = 0$ subjected to periodic incandescent illumination 6 sec./10 min., intensity 18.8W/m^2 throughout 6-hour experimental period.

FIGURE 6.1A

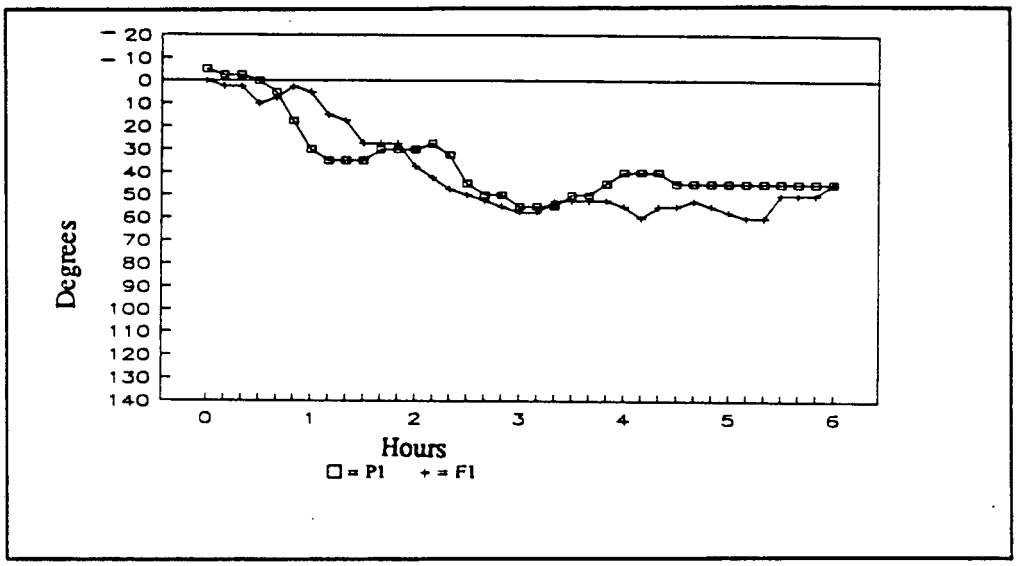


FIGURE 6.1B

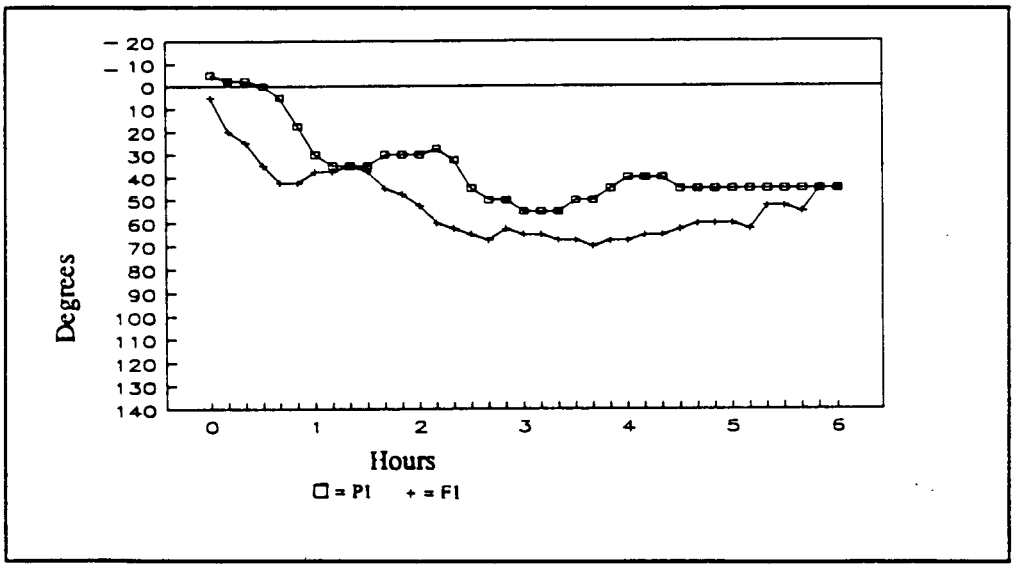


FIGURE 6.1C

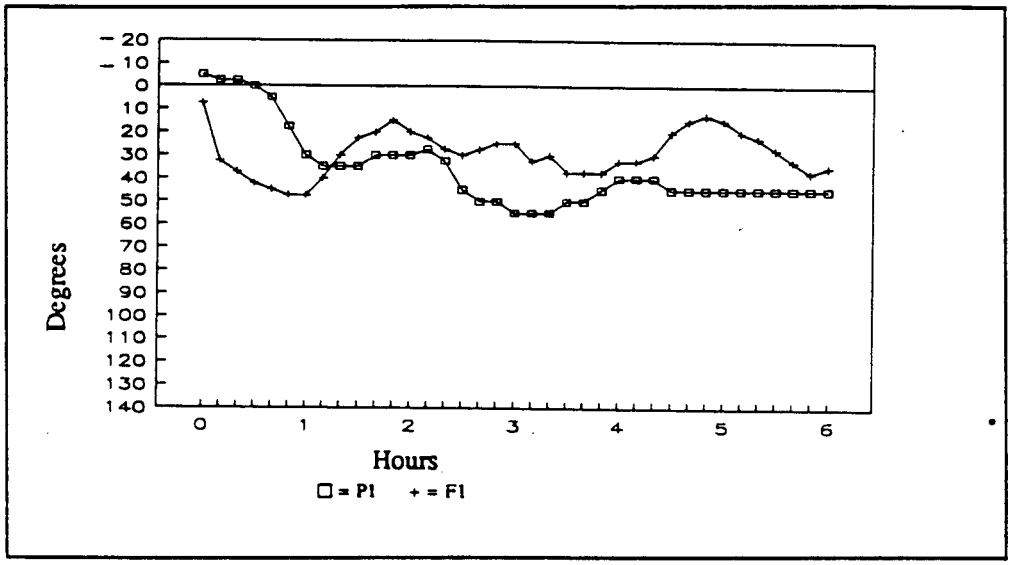


FIGURE 6.1D

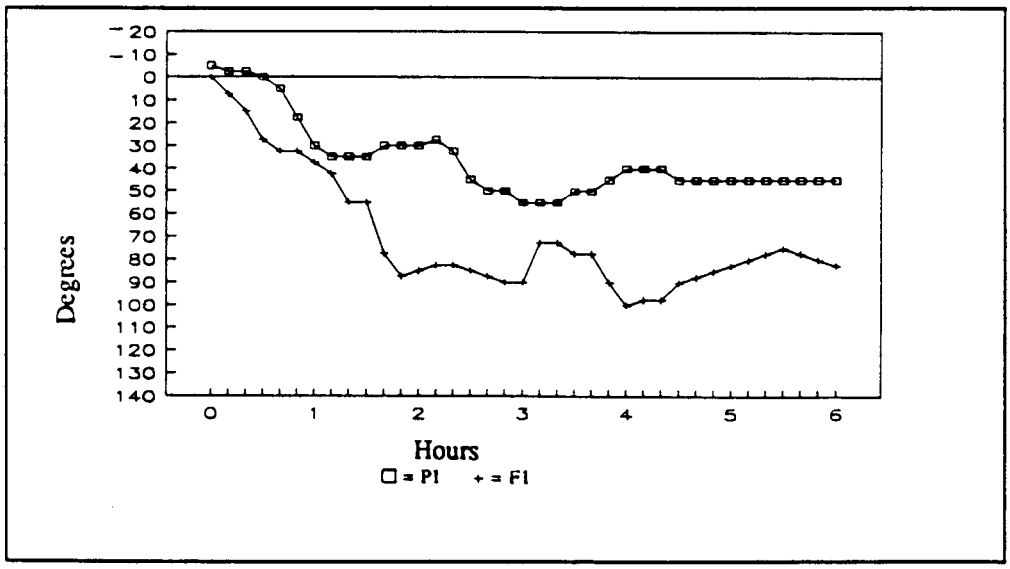


FIGURE 6.1E

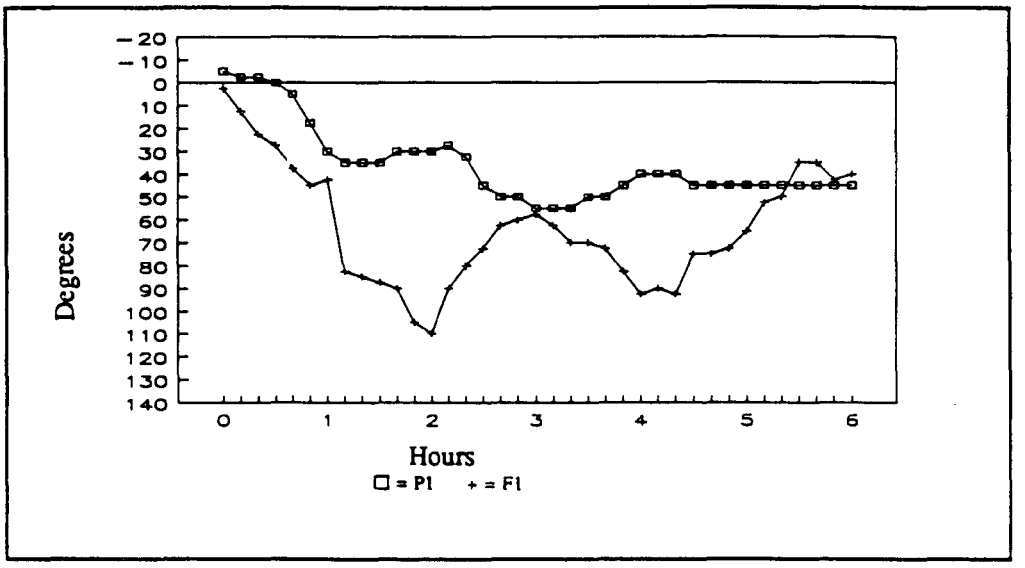


FIGURE 6.1F

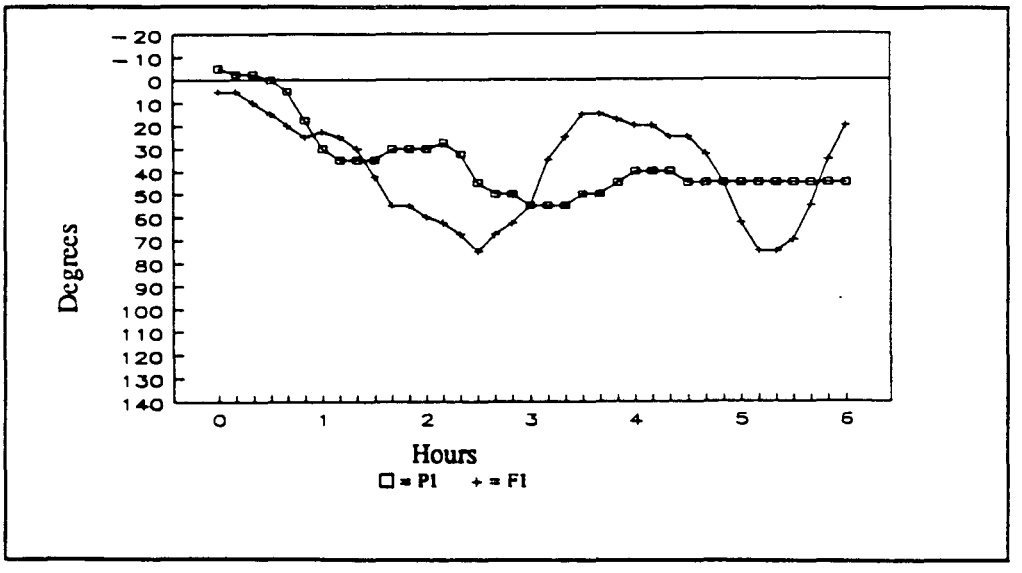


FIGURE 6.2

Graviresponse curves comparing cv. Merit P1 primary root with six different roots from the F1 generation. The P1 plant was self-pollinated in the summer of 1986 to produce the F1 population. Each plot contains two curves representing graviresponse profile of the same P1 root with a different F1 root. Roots oriented horizontally at time $t = 0$ and subjected to periodic incandescent illumination 6 sec./10 min., intensity 18.8W/m^2 throughout 6-hour experimental period.

FIGURE 6.2A

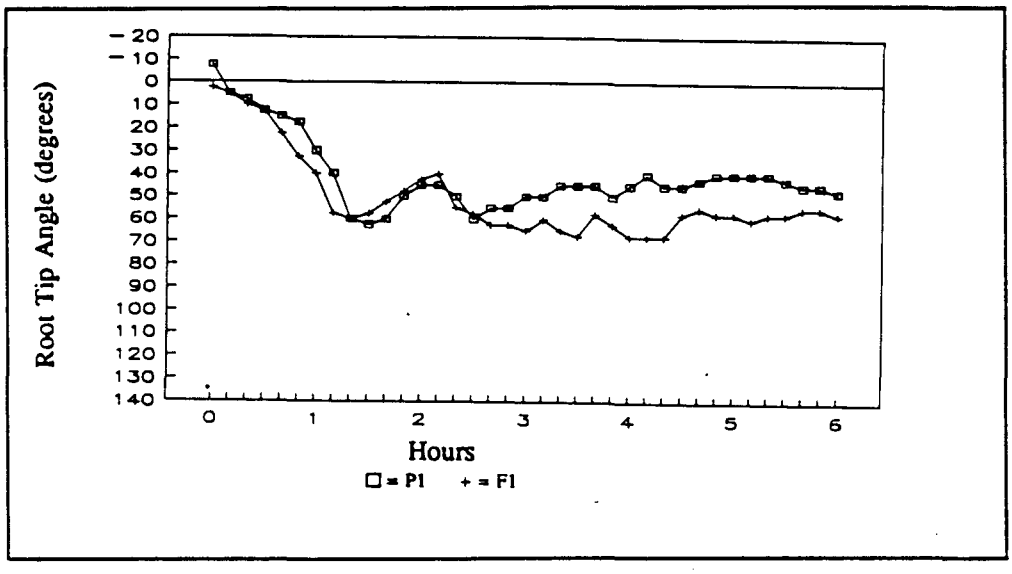


FIGURE 6.2B

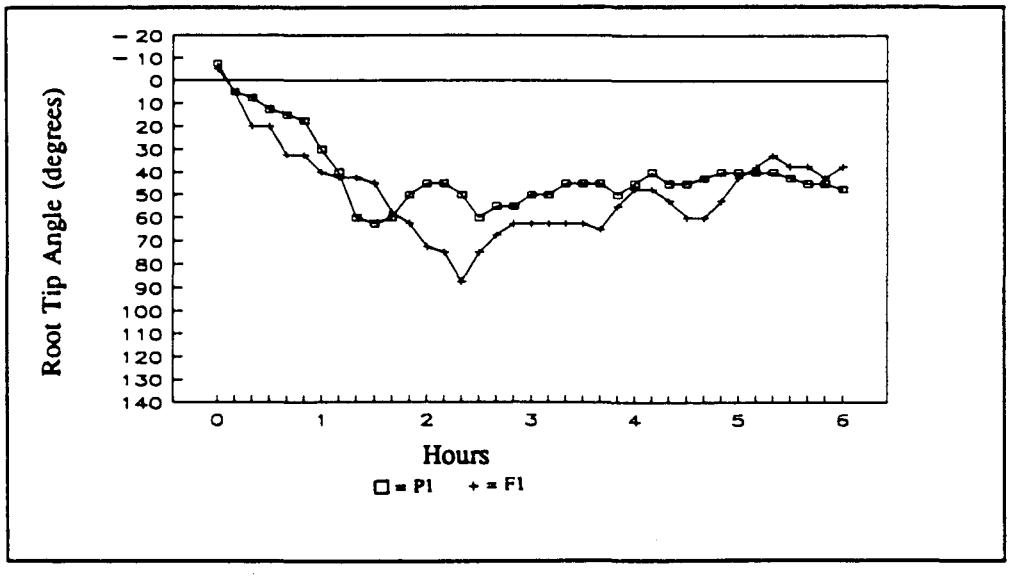


FIGURE 6.2C

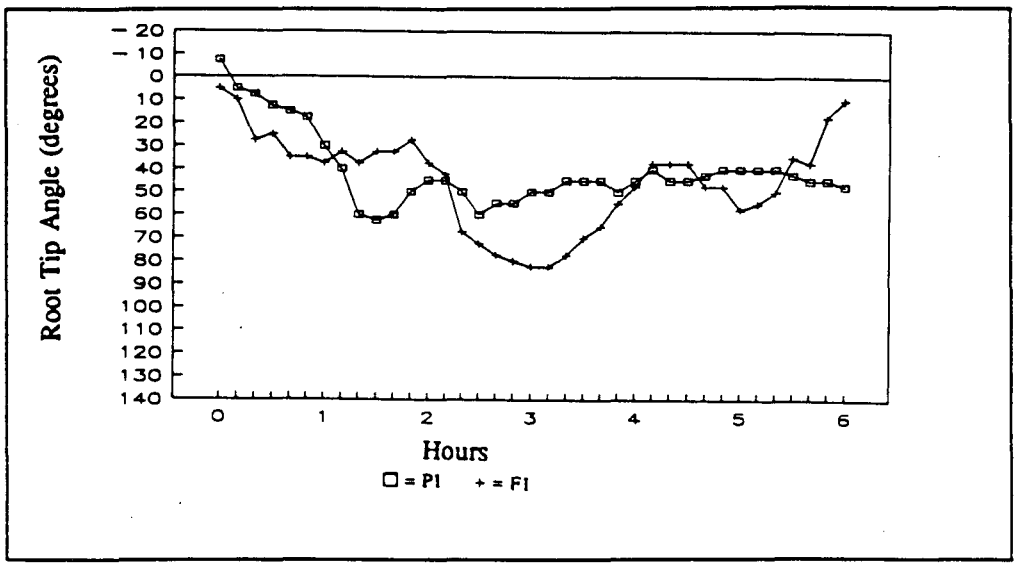


FIGURE 6.2D

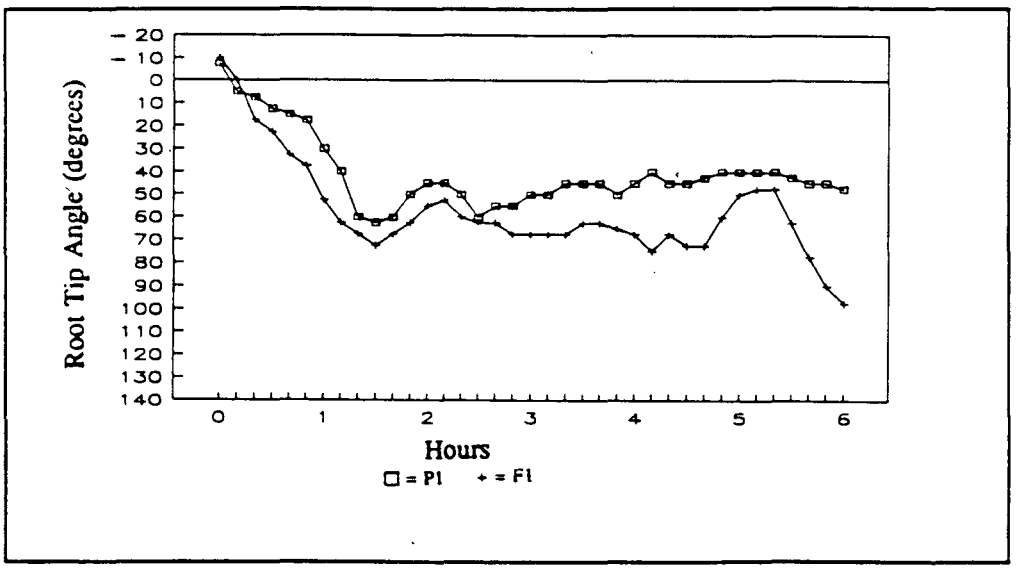


FIGURE 6.2E

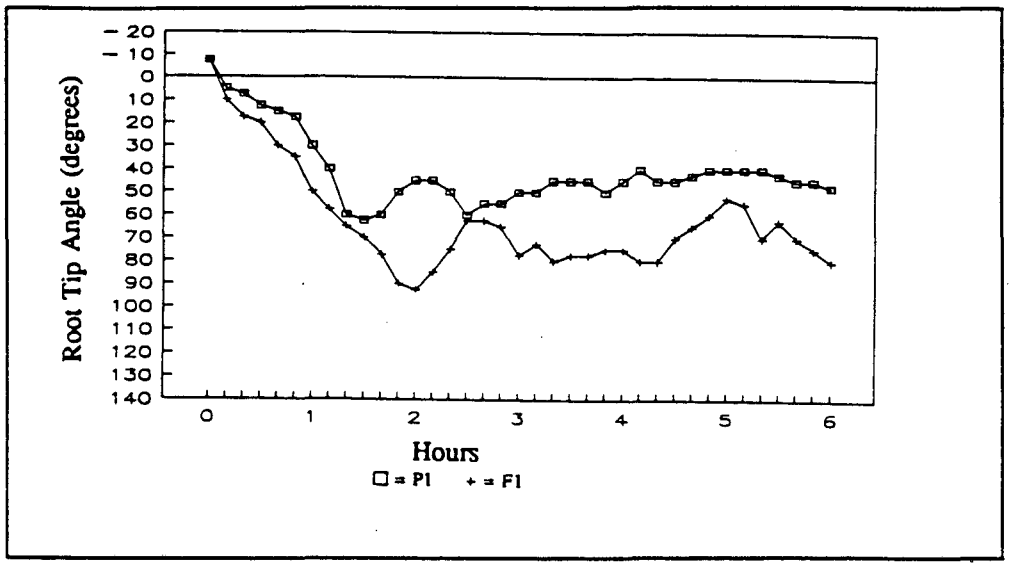


FIGURE 6.2F

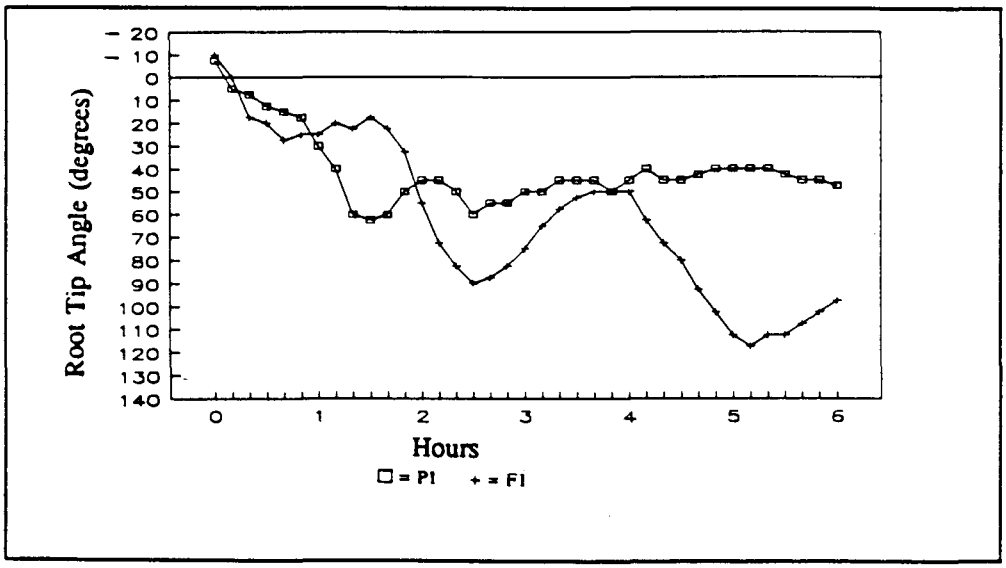
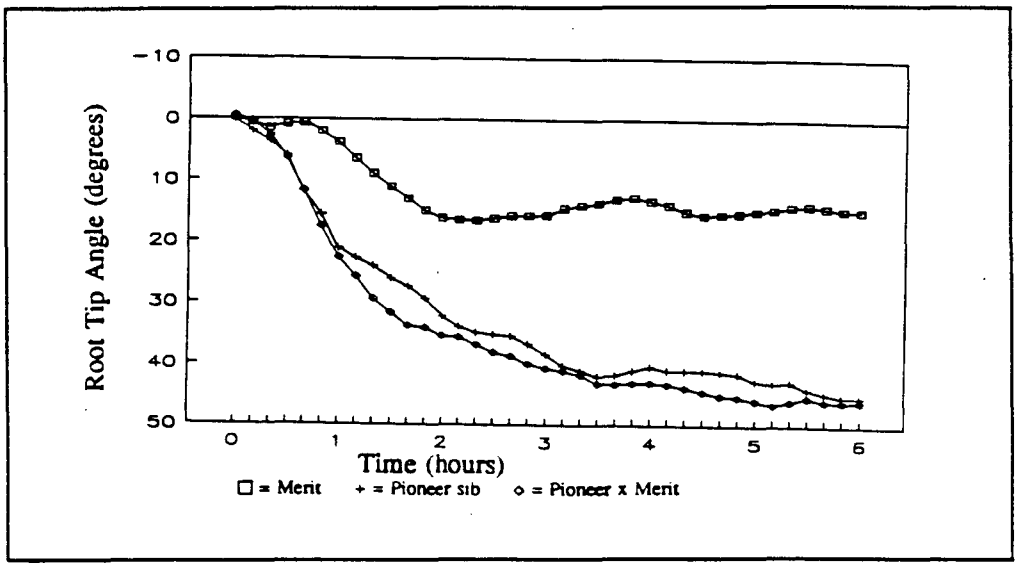


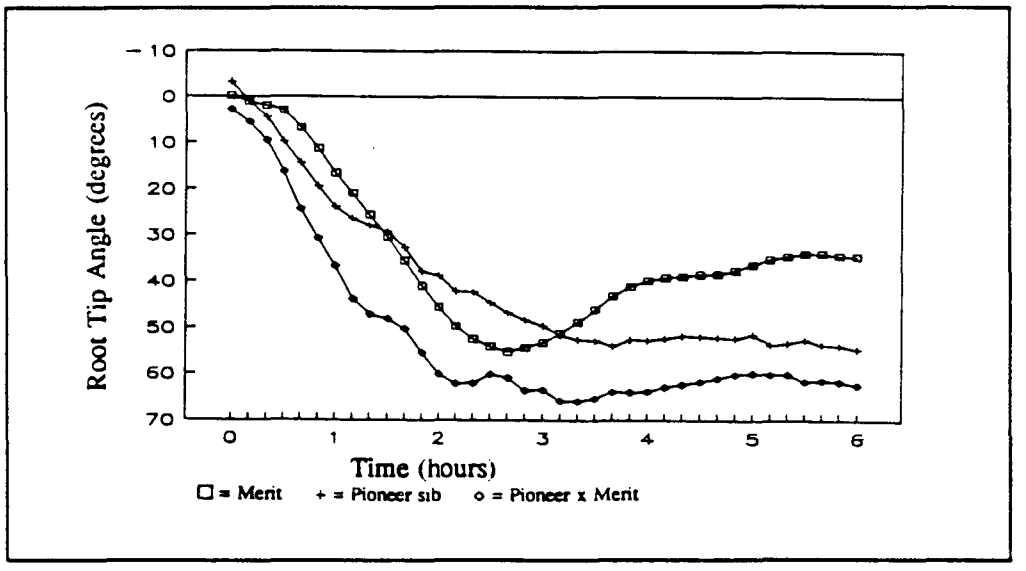
FIGURE 6.3

FIGURE 6.3A



Graphs of averages for three groups of dark-exposed maize primary roots started horizontally at time $t = 0$ and allowed to develop gravitropic curvature throughout 6-hour experimental period.

FIGURE 6.3B

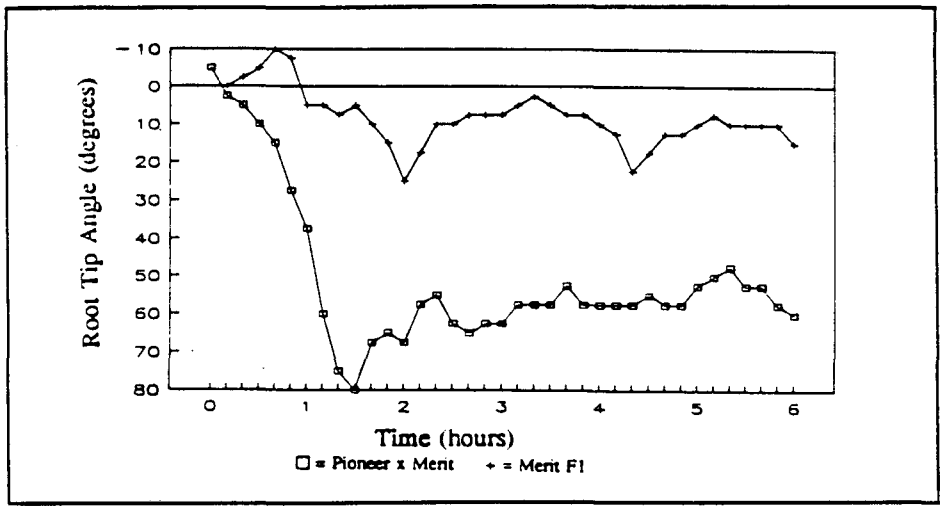


Graphs of averages for three groups of maize primary roots started horizontally at time $t = 0$ and subjected to periodic incandescent illumination 6 sec./10 min., intensity 18.8W/m^2 throughout 6-hour experimental period.

FIGURE 6.4

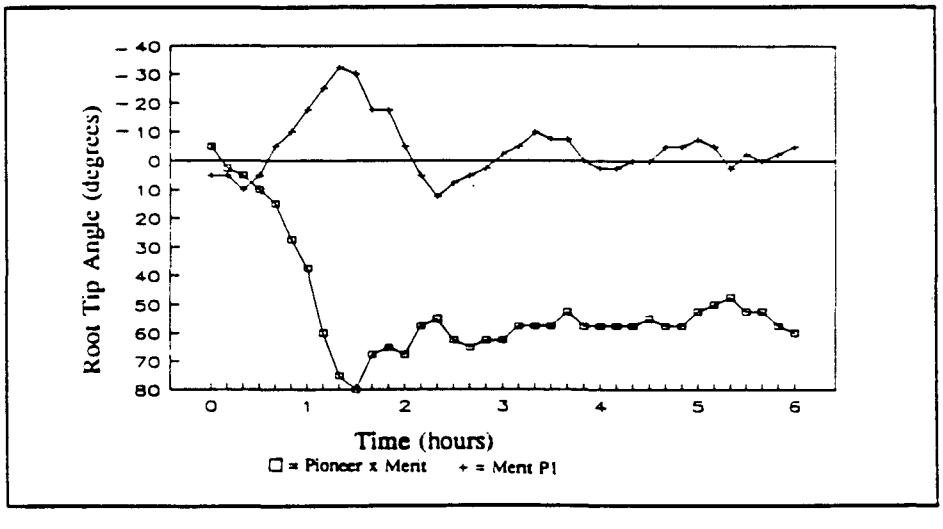
Graviresponse curves for dark-exposed primary roots representing in each case an extremely gravitropic female parent with an extremely ungravitropic male parent. The A and B ears from the same female parent were used in Figures 6.4A and 6.4B, respectively. The roots were oriented horizontally at time $t = 0$ and were allowed to develop gravitropic curvature during the 6 hour test period.
Figure 6.4 illustrates the primary root graviresponse profile of the plants which were crossed to produce the population depicted in the frequency distribution displayed in Figure 6.5.

FIGURE 6.4A



Pioneer (P1)/Merit(P1) × Merit(F1)

FIGURE 6.4B

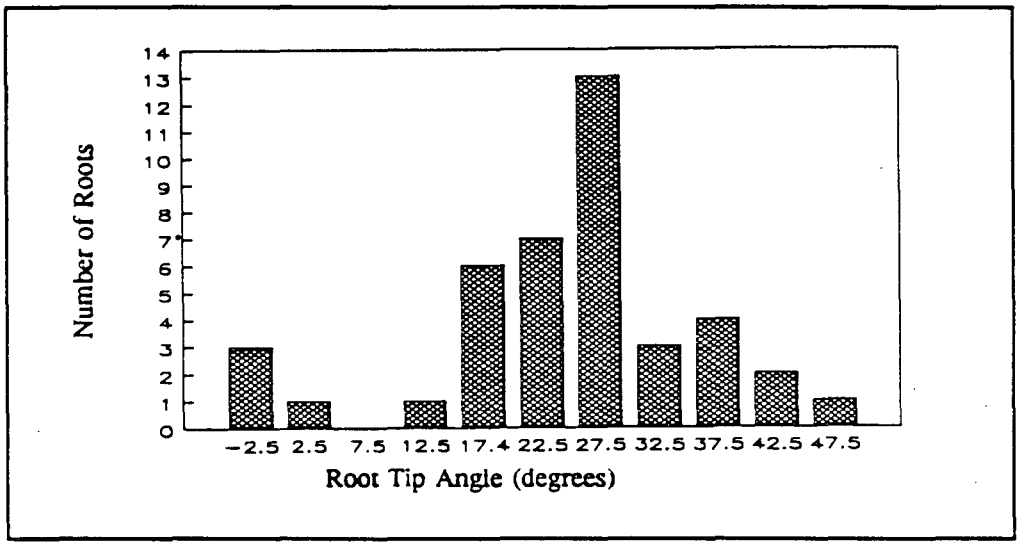


Pioneer (P1)/Merit (P1) × Merit(P1)

FIGURE 6.5

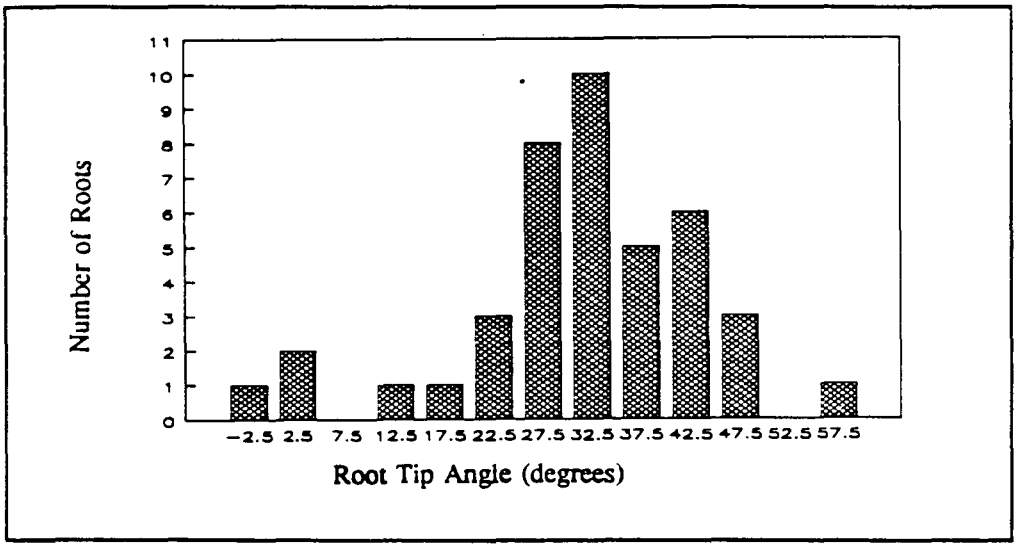
Frequency distributions representing average curvature between 0 and 6 hours for individual roots (Figure 6.5A) and curvature at hourly intervals between 2 and 6 hours (Figure 6.5B-6.5F) for the progeny of the crosses described in Figure 6.4. Each bar represents a 5° increment centered on the indicated value and including the upper bound of that interval. The values represent the combined progeny of crosses described in Figures 6.4A and 6.4B. $N = 41$.

FIGURE 6.5A



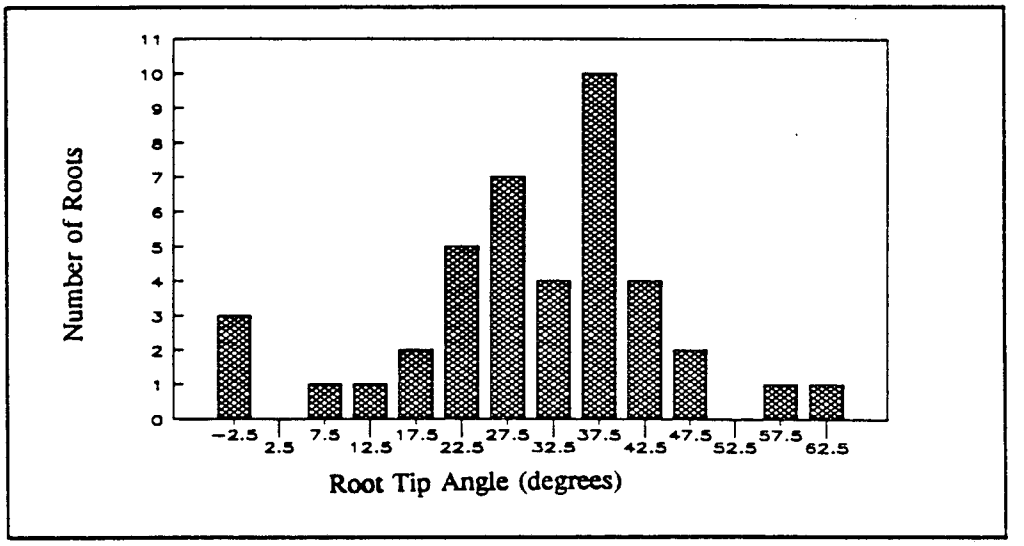
Average root tip curvature from 0-6 hours (frequency distribution)

FIGURE 6.5B



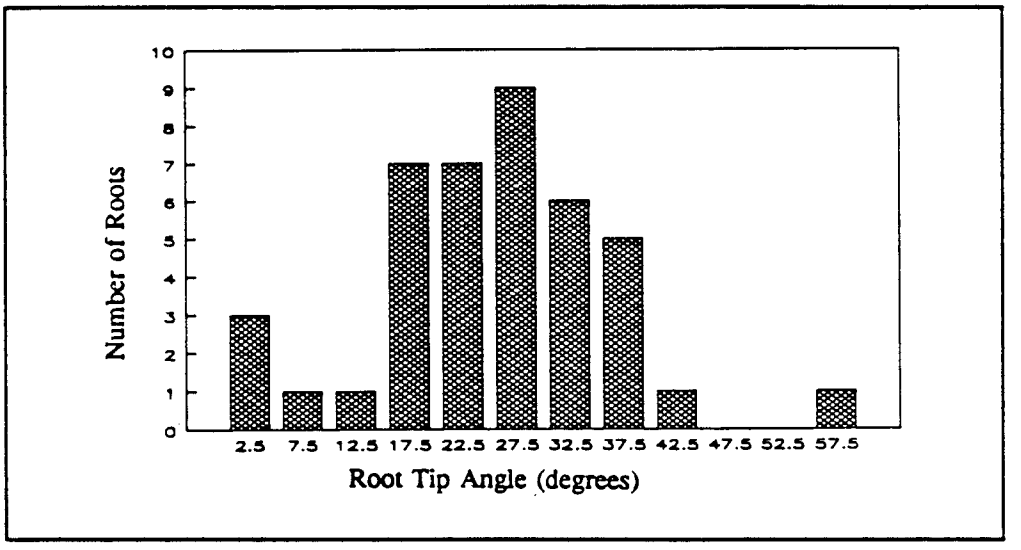
Root tip curvature at 2 hours (frequency distribution)

FIGURE 6.5C



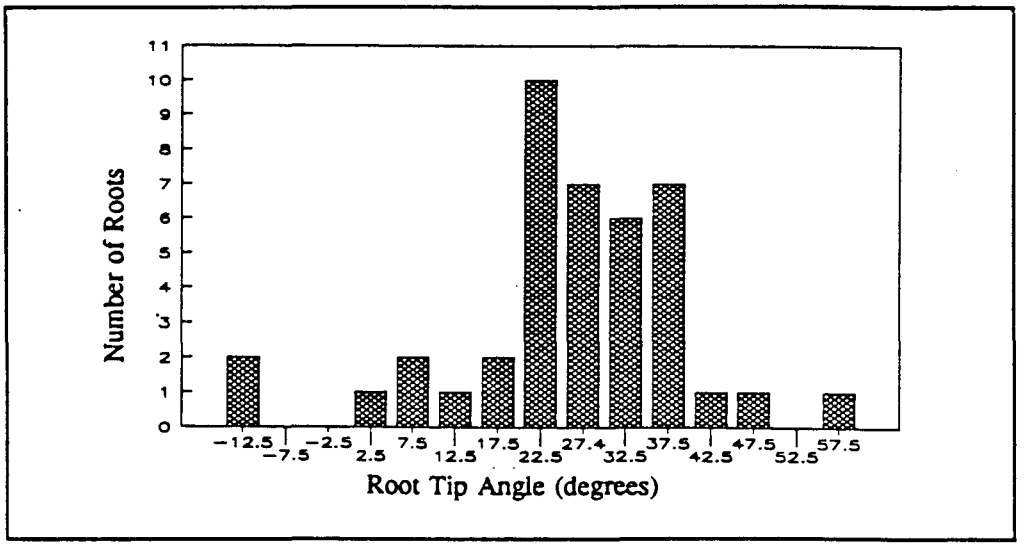
Root tip curvature at 3 hours (frequency distribution)

FIGURE 6.5D



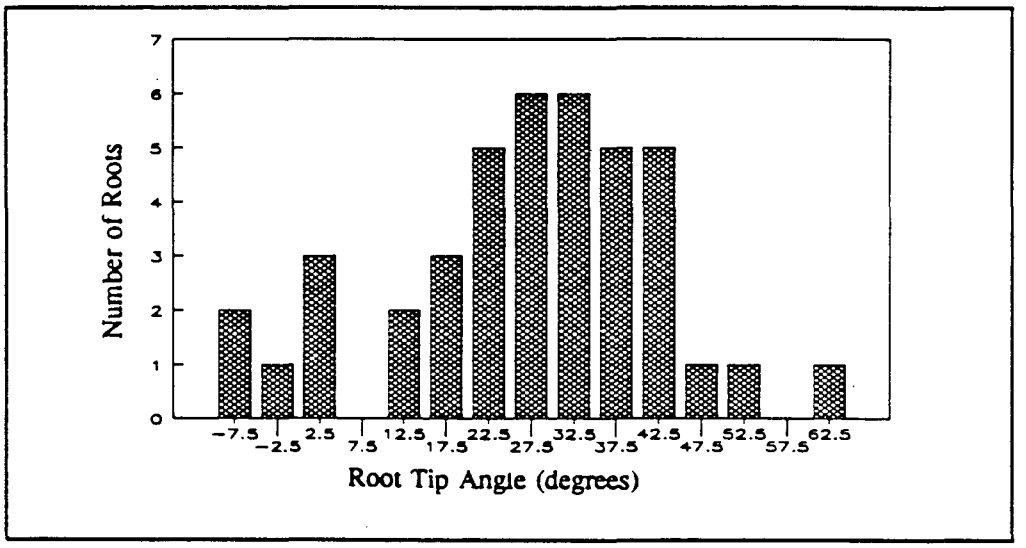
Root tip curvature at 4 hours (frequency distribution)

FIGURE 6.5E



Average root tip curvature at 5 hours (frequency distribution)

FIGURE 6.5F



Root tip curvature at 6 hours (frequency distribution)

FIGURE 6.6

Graviresponse curves comparing cv. Merit P1 primary root with nine different roots from the F1 generation. The P1 plant was self-pollinated in the summer of 1987 to produce the F1 population. Each plot contains two curves representing graviresponse profile of the same P1 root with a different F1 root. Roots oriented horizontally at time $t = 0$ and subjected to periodic incandescent illumination 6 sec./10 min., intensity 18.8W/m^2 throughout 6-hour experimental period. The F1 curve in Figure 6.6F resembles the P1 curve in its cyclic profile. Otherwise, the F1 curves do not resemble the extremely oscillatory P1 curve.

FIGURE 6.6A

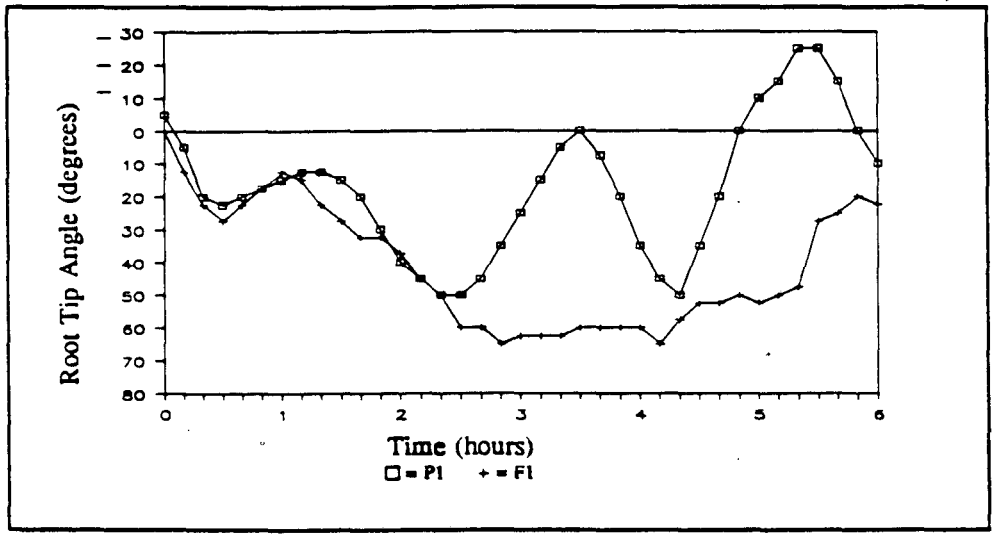


FIGURE 6.6B

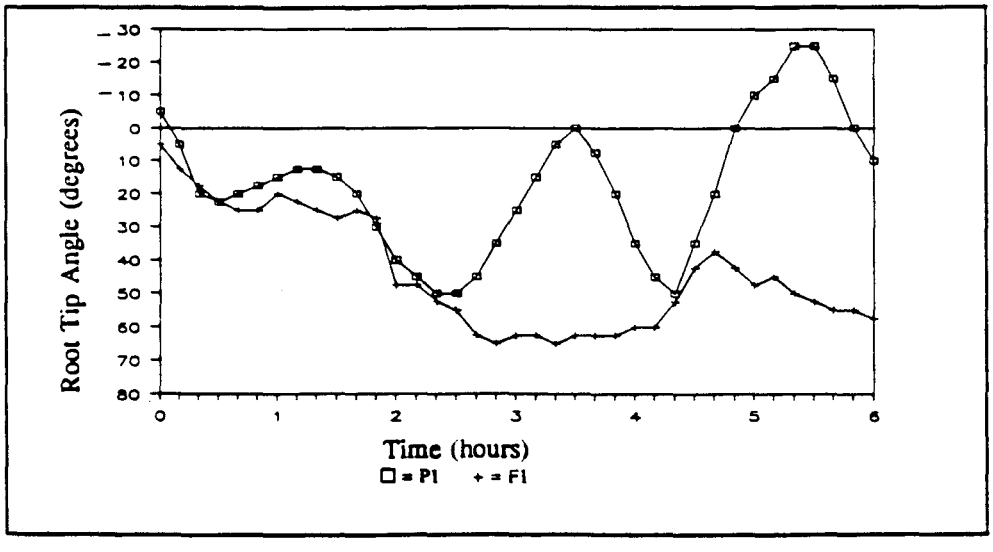


FIGURE 6.6C

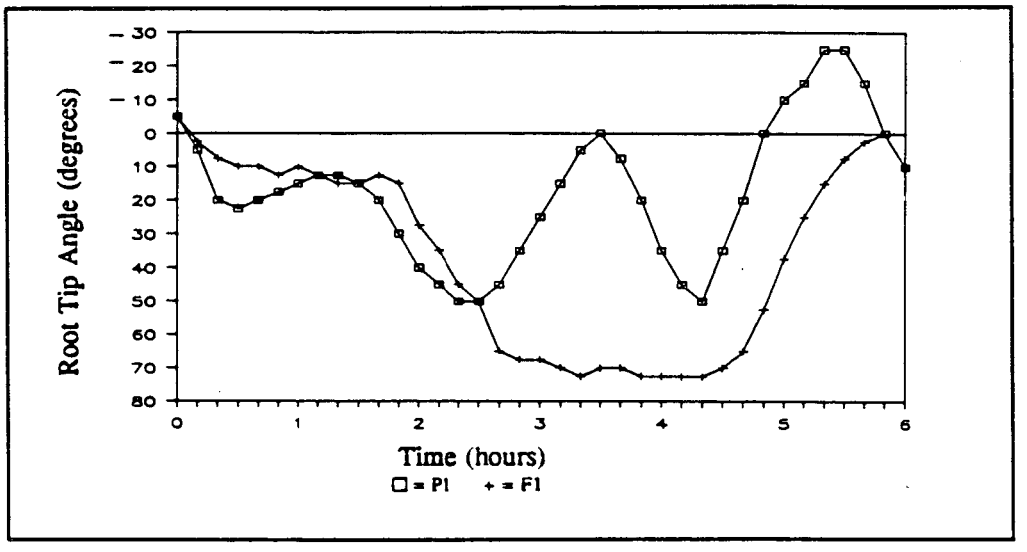


FIGURE 6.6D

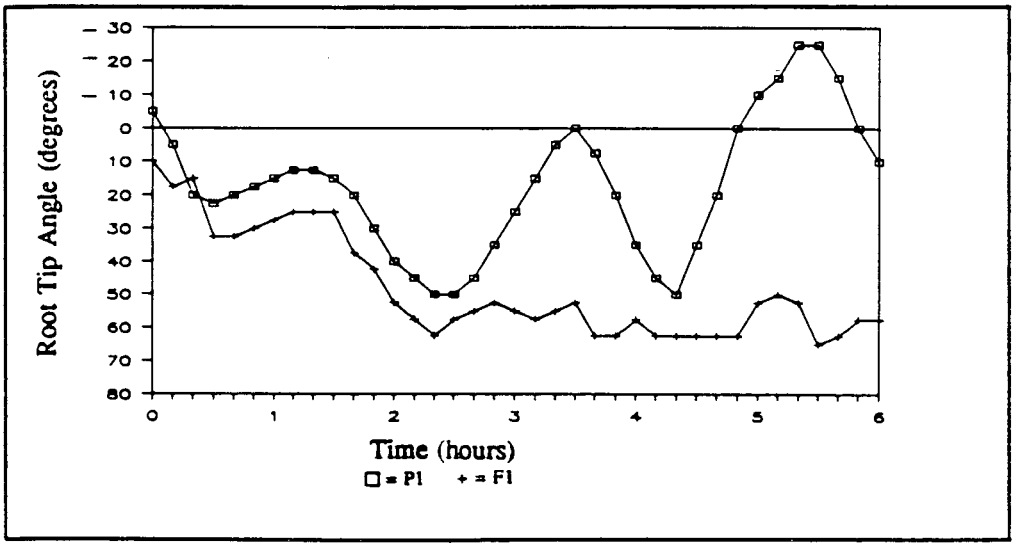


FIGURE 6.6E

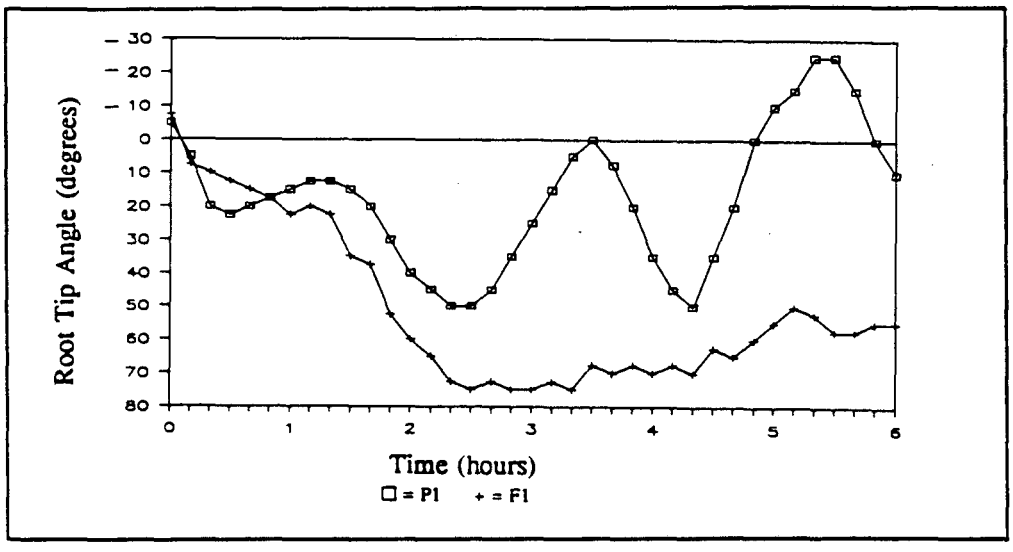


FIGURE 6.6F

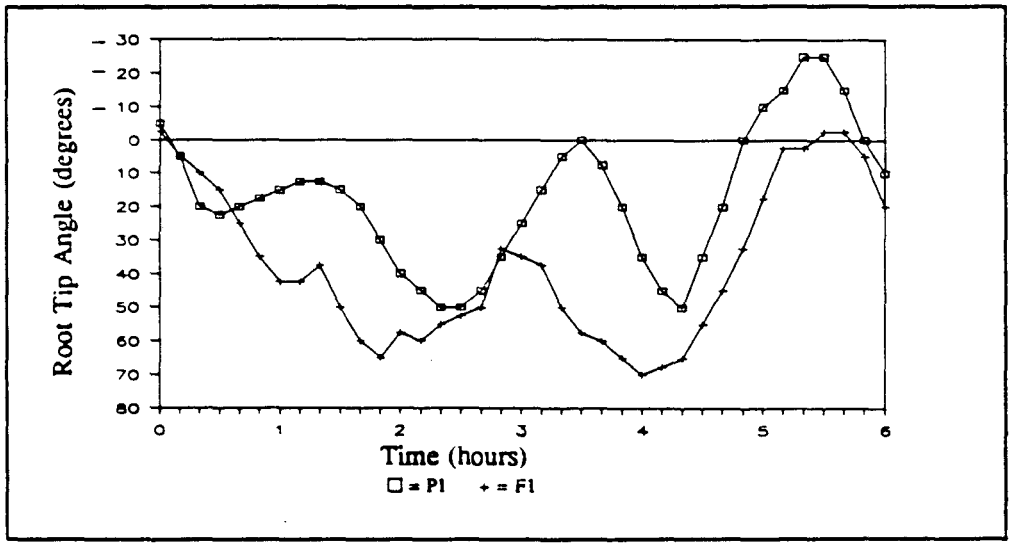


FIGURE 6.6G

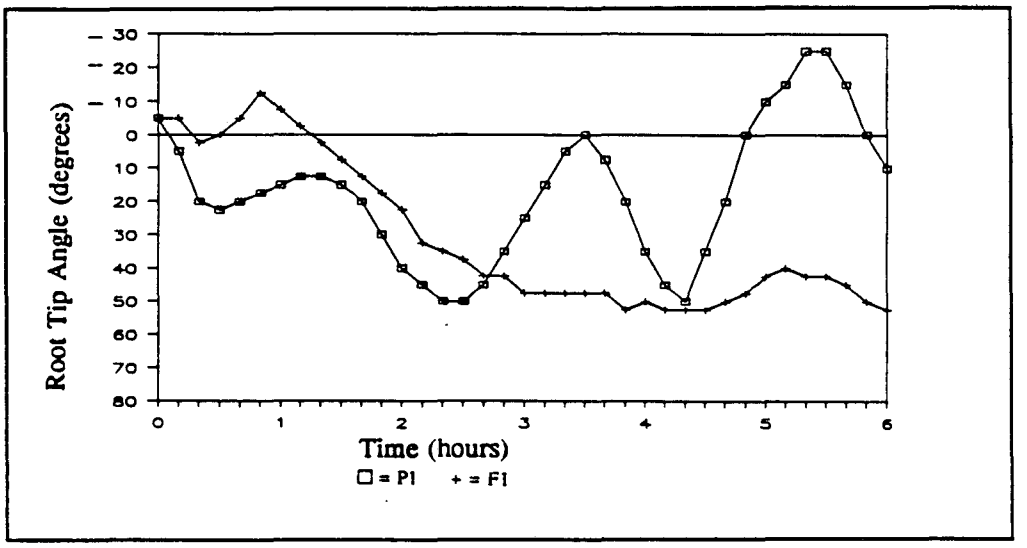


FIGURE 6.6H

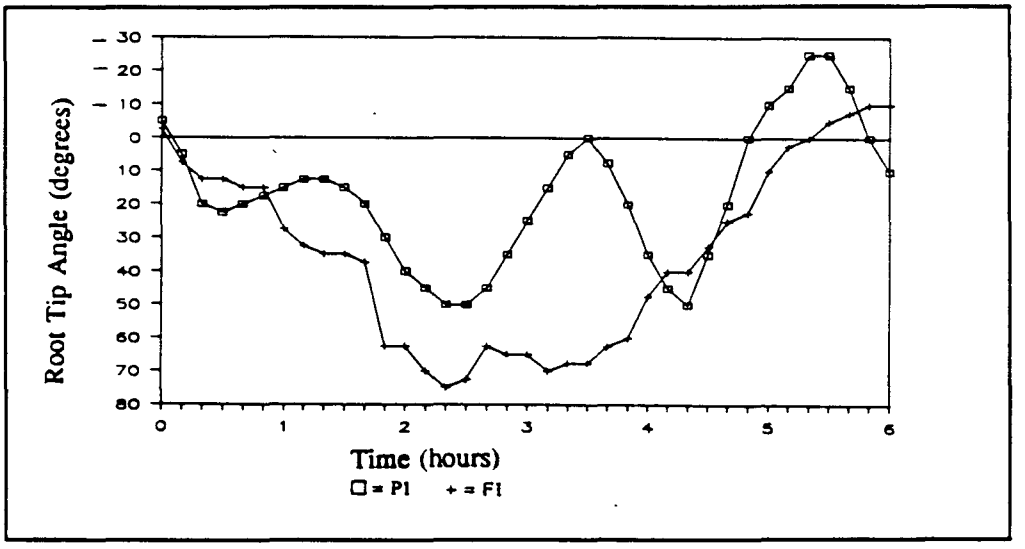


FIGURE 6.6I

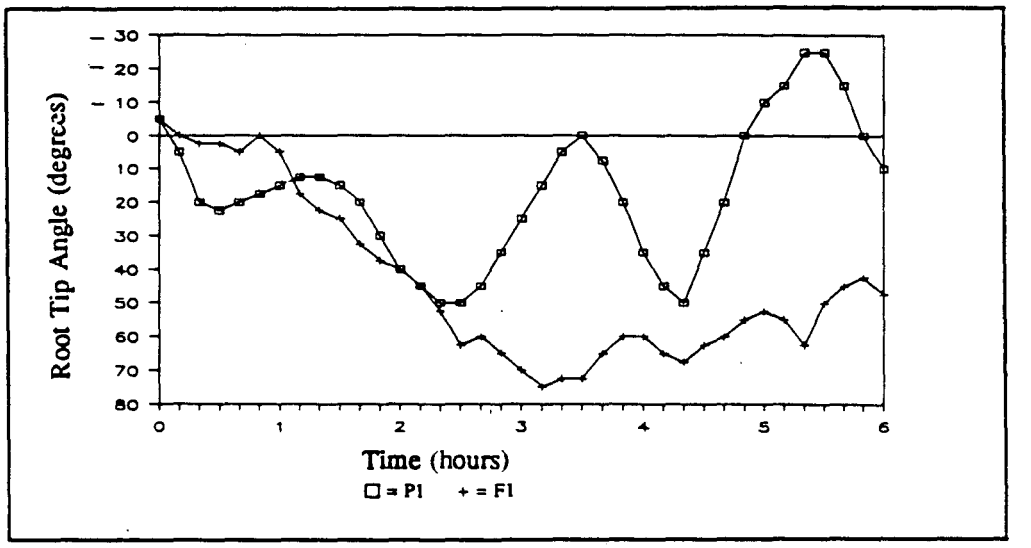
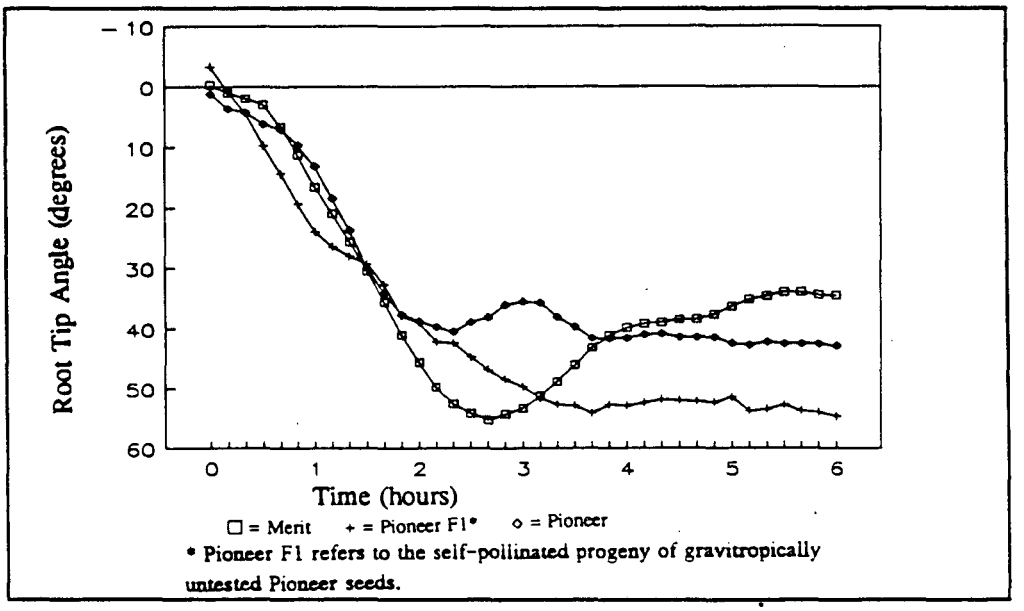

Root Tip Angle (degrees)
30
20
10
0
10
20
30
40
50
60
70
80
0
1
2
3
4
5
6
Time (hours)
□ = P1 + = F1

FIGURE 6.7



Graphs of averages for three groups of maize primary roots started horizontally at time $t = 0$ and subjected to periodic incandescent illumination 6 sec./10 min., intensity 18.8W/m^2 throughout 6-hour experimental period.

CHAPTER 7

SOME PHYSIOLOGICAL RESPONSES PECULIAR TO THE LAZY MUTANT IN MAIZE

7.1: Literature Review

The maize mutant designated Lazy derives its name from the prostrate growth habit of the adult plant. The Lazy mutant was first discovered in 1923 by Emerson, Beadle and Fraser (1935), who classified Lazy as a recessive mutation on chromosome four of the maize genome.

Overbeek studied some of the physiological aspects of Lazy in a series of publications written in the 1930s. Overbeek (1936) characterized lazyness in the adult plant as an ageotropic mutation meaning that the stalk of the Lazy plant is gravitationally indifferent.[1]

Overbeek also demonstrated that the stems of Lazy and normal plants grown from seed in the dark quickly resume a negative gravitropic growth pattern (upward growth) after the containers in which they were planted were turned 90° after 5 or 6 days. This result demonstrated that the Lazy mutation does not express itself very early in the development of the seedling.

In a second experiment Overbeek grew homozygous Lazy plants in the greenhouse, presumably in the presence of light. The seedlings adopted a normal, vertical growth pattern in the first ten days after germination. The plants were then reoriented 45°, 90° and 180° from the original stem-up configuration and were allowed to grow for several weeks. The stems of the lazy plants re-directed 45° and 90° from their starting position, continued to elongate in the direction in which they were pointed, except that as the plants aged the stalks grew heavier and began to sag downwards. "This reaction is to be distinguished from geotropism, as a purely mechanical

[1]Ageotropism contrasts with both diageotropism and positive geotropism. A diageotropic plant tends to resume growth in the horizontal direction after being inclined at an angle to the horizontal. A positively geotropic plant stem resumes growth downwards along the plumb line after being displaced from the preferred downward direction.

effect of gravity, distinct from the physiological growth reaction of geotropism" (Overbeek, 1936). The Lazy plants which were turned 180° (inverted) maintained a growth pattern downwards along the plumb line for several weeks.

Overbeek (1938) demonstrated that auxin is present in a higher concentration in the upper halves of the stem apices of prostrate Lazy plants. This contrasts with the pattern of auxin distribution which he found to exist in horizontally-oriented normal plants in which the auxin levels were higher in the lower halves of stem apices.[2] Overbeek measured auxin levels using an ether extraction technique in the mature plant at a point immediately prior to shedding of the pollen.

7.2: Results and Discussion

I carried out a series of experiments to determine more about Lazy's physiological characteristics. In one such experiment, 25 Lazy plants were grown from seed in peat pots in the greenhouse and spaced such that no seedling shaded any other seedling during the 28-day growth period. The result was that 21 plants became oriented toward the south, while 4 became oriented northward. The Lazy phenotype begins to express itself within 10 days to 2 weeks of shoot emergence. It is inevitable that the shoot will begin to bend downward as the plant matures. This finding however, points to the fact that there is a preferred direction of downward growth; that being via the south, or in the direction of maximal light exposure. The positive geotropic curvature displayed by Lazy plants (in the direction of maximal light) is however distinct from normal phototropic bending. In the course of this experiment, I also discovered that Lazy's phototropic mechanism is intact, as was born out by the observation that its leaves are able to

[2]The mature, normal plants, grown in pots in the greenhouse, were turned horizontally and maintained in this position for one day or more by placing empty flowerpots on the stems.

orient themselves toward the source of light.

In another experiment I attempted to determine the extent of Lazy's requirement to grow upward from seed. A flat was arranged with a wire mesh bottom containing openings large enough to accommodate the shoot of a maize seedling. The flat was filled with moistened dirt and suspended in the south-facing window of the greenhouse. Ten homozygous Lazy seeds were planted near the bottom of the flat in the dark. The flat was then covered with a light-tight lid so that the developing seeds could only be illuminated from below through the wire mesh screen. After 12 days the lid was removed, revealing that 7 of the 10 seedlings had sprouted upward. These all had the characteristic appearance of etiolated plants with whitish-yellow leaves, long internodes and spindly stems. The 3 remaining seeds failed to germinate. Ten out of 10 heterozygous controls sprouted upward from seed in similar conditions. It is interesting that the Lazy trait did not express itself this early in the development of the seedling even when the light was unilaterally directed from below.

In reality, the outcome of the experiment was predictable since it is difficult to imagine a set of circumstances in which a mutant would be capable of surviving to produce seed when the initial growth direction of its stem was anything but upwards (even a Lazy mutant would have to emerge from the soil first before it could develop into a viable plant).

I confirmed in a follow-up test that in Lazy mutants as in non-Lazy plants, it is not the presence of light that triggers the shoot to grow upward from seed. Of 8 Lazy seeds grown for 3 weeks in complete darkness, 6 emerged upward, while 2 did not sprout at all. In similar conditions, all 8 heterozygous controls sprouted upward. In this test it was difficult to characterize differences between dark-grown Lazy and non-Lazy seedlings since the elongation pattern among etiolated plants is somewhat haphazard in its general upward trend. Moreover, the stems of dark-grown seedlings are so light and spindly that they appear to be unable to support the full weight

of the growing plant, sometimes taking sideways jogs before righting themselves. In any event, it was not clear whether the Lazy trait could be distinguished in the dark.

The following tests were carried out in order to investigate Lazy's proclivity to establish a downward or positively gravitropic orientation as opposed to a diageotropic or ageotropic growth pattern. A single homozygous Lazy seedling, grown in a peat pot, was inverted at the point when the shoot emerged from the soil. The seedling was suspended in a hanging basket in the greenhouse, in such a way that the newly-emerged stem pointed straight downward. The result was that the shoot continued to elongate downward for 5 weeks at which point it fell from its anchorage, since the roots which developed could not establish a secure enough purchase to support the maturing plant. It is remarkable that the plant appeared to be developing normally, having reached the six-leaf stage, as it continued to grow directly downward. One of two conclusions can be drawn from this result: either the Lazy trait is characterized by a genuinely positively-gravitropic growth tendency, or the native tendency is diageotropic and the process of inversion is too strong of a stimulus for the plant to overcome. As noted, Overbeek (1936) proposed a third explanation: that the mature Lazy plant was, in fact, gravitationally indifferent.

Two additional observations are worth making in this connection. In the field, the average Lazy plant bends downward slowly and in such a way that the bottom one third of the plant is almost always arched above the ground. Above this point however the plant continues to grow pressed close against the soil. If the Lazy plant encounters a depression, such as a plough furrow, at right angles to its path, it contours along the ground growing into the furrow and back out again while continuing to press against the ground. Secondly, when Lazy plants are grown in pots placed on high shelves in the greenhouse the stalks bend slowly downward until all plants eventually assume a totally inverted position. It is not the weight of the stalk which compels the Lazy mutant to direct itself downward. In fact, the measured force generated by a Lazy plant is

several times as great as the force produced by a plant whose weight alone results in downward motion (Neuffer, 1968). This fact, combined with Overbeek's finding that a higher concentration of auxin is present in the upper apices of prostrate Lazy plants, leads to the conclusion that the positive gravitropic curvature exhibited by the Lazy plant is an active rather than a passive process. Furthermore, these results point to the conclusion that Lazy's preferred direction of growth is in line with the gravity vector rather than perpendicular to it.

Another question which arises in connection with Lazy is whether the behavior of the mutant is confined to the cells of the shoot apex; or instead, is present in other parts of the plant. For example, is root orientation normal with respect to gravity in homozygous Lazy plants? I observed that the alignment and general elongation pattern of roots in mature Lazy plants is indistinguishable from the root structure and orientation of heterozygous and wild type plants. This conclusion was reached following the examination of the root structures of several plants which had been carefully excavated.

Primary roots of homozygous Lazy seeds were also tested for gravitropic sensitivity under the influence of incandescent light (frequency: 6 sec./10 min.; intensity: $18.8 W/m^2$). The result was that the roots underwent substantial positive gravitropic curvature (Figure 7.1). Thus, the normal gravitropic effect is shown to be present in the roots of Lazy mutants just as it is widespread in non-Lazy plants with diverse genetic backgrounds. While I have demonstrated that the roots of Lazy plants do not differ in terms of gravitropic orientation from non-Lazy plants it remains an open question as to whether the Lazy trait is exclusively confined to a particular organ or cell layer; for example, the cells of the shoot apex.

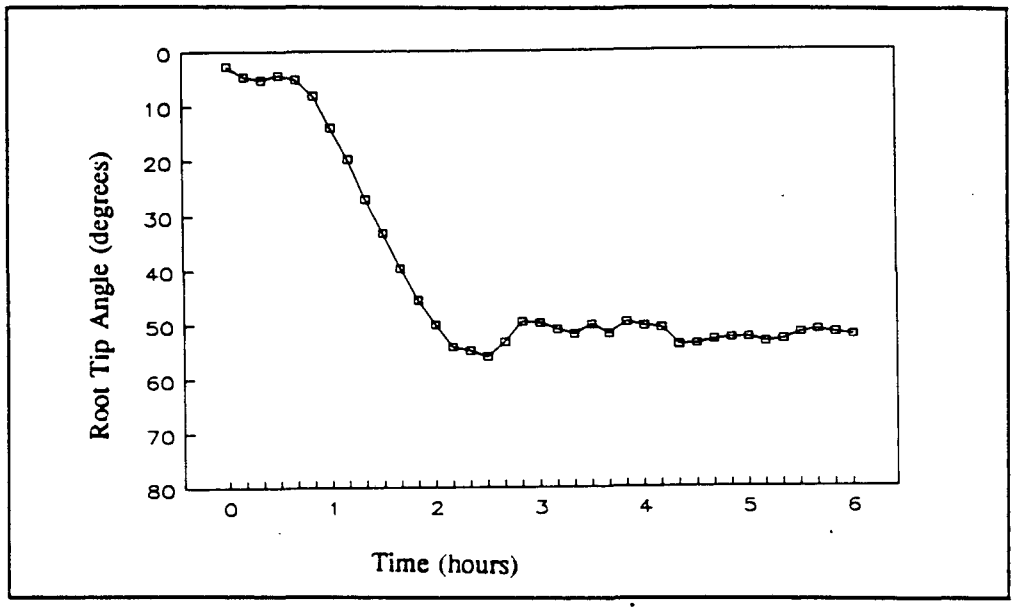
7.3: Where To Go From Here With Lazy Maize

The next logical step is to isolate the product of a Lazy gene. In maize, this would

require crossing a line in which transposons are actively integrating into chromosomes as female with *la/la* testers. Transposons Ac, Ds2, Spm, Mu1, Mu3 and MuR have all proven to be successful as insertional mutagens and, subsequently, as tags with which to identify cloned genomic fragments that contain the gene of interest (Freeling, 1988).

Although an amino acid sequence of the *La* product would not necessarily imply a function, it would certainly point the way.

FIGURE 7.1



Graphs of averages for homozygous Lazy primary roots started horizontally at time $t = 0$ and allowed to develop gravitropic curvature throughout 6-hour experimental period.

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M. G. Neuffer, L. Jones and M. S. Zuber, The Mutants of Maize, Crop Science Society of America, Madison, Wisconsin, p. 25, (1968).

J. van Overbeek, *J. Hered.*, vol. 27, pp. 93-96, (1936).

J. van Overbeek, *J. Hered.*, vol. 29, pp. 339-41, (1938).

CHAPTER 8

SUMMARY AND CONCLUSIONS

We arrived at the following conclusions with regard to the motion of maize primary roots under the combined influences of light and gravity (unless otherwise noted, cv. Merit is the particular variety of maize used throughout the course of research):

1. The horizontally-positioned primary root bends downward to a greater extent in light than in darkness. The magnitude of descent varies between approximately 35° and approximately 55° depending on the duration of the light exposure, the light intensity and the light source. In the dark, the horizontally-positioned root bends downward about 15°. The trough of the descent phase is reached from 2 to 3 hours after the beginning of gravistimulation.

The magnitude of downward curvature is shown to increase as a function of light intensity, within a certain range. Thus, the response is not considered to be controlled by a gaited (all-or-nothing) type of mechanism.

2. The root cap is the site of the photo-active trigger which stimulates positive curvature beyond the dark control level. This result was established by probing the root at various sites along its length with focused light from a helium neon laser.

3. Gravistimulated roots in the presence of light show a negative curvature phase after the initial descent. The magnitude of the rising phase is approximately one-third that of the descent. The rising phase occurs irrespective of the light intensity or the light regimen, i.e., continuous or pulsed light treatment. In addition, the rising phase occurs under both helium neon laser and

incandescent light sources.

4. It is possible to stimulate secondary and tertiary cycles of root curvature by pulsing incandescent light every 2 to 4 hours. When the schedule of light pulses is more frequent than 2 hours, however, secondary and tertiary cycles of curvature are suppressed.

5. We developed a fluid-mechanical model to explain the above results in terms of the physical forces and molecular flows presumed to be involved in light-stimulated gravitropic curvature. The mathematical expression of the model incorporates the following physiological features:

a. Geosensors in the columella cells of the root cap sediment under the influence of gravity.

b. An inhibitor of cell extension is formed or activated under the joint influences of the sinking geosensor and a photo-convertible pigment molecule.

c. The inhibitor diffuses along the bottom margin of the root from root cap to extension zone where growth retardation occurs leading to downward curvature.

d. The inhibitor is transported by convection, circumferentially around the extension zone where it retards growth at all points in its path at a rate equivalent to its concentration. (The concentration of inhibitor diminishes at an exponential rate from the time of its formation.)

e. When the concentration of inhibitor at the top of the extension zone exceeds that at the bottom, the root begins to bend back upwards.

6. Model-generated curves are presented with experimental data as one measure of the efficacy of the theory presented.

7. In view of the model advanced, arguments are put forward that light-aided gravitropism, per

se, is responsible for the pattern of root movements which follow the horizontal positioning of the primary root. This is in contrast to the explanation of nutational curvature offered by either the gravitropic feedback model or the endogenous oscillator model.

8. The growth direction of maize primary roots positioned vertically downward was explored. Toroidal (screw-like) motion was not observed. Further, there was no evidence of a helical growth pattern although the roots showed considerable movement. Evidence is presented that the root tip sometimes became displaced up to .5 cm in a lateral direction away from the original root axis.

9. Differences in the magnitude of positive gravitropic curvature in cv. Merit and cv. Pioneer primary roots was explored in terms of possible genetic control. Merit roots require light to develop a strong gravitropic response while Pioneer roots are graviresponsive even in the dark. The working hypothesis was that graviresponsiveness in the dark was governed by a dominant-single-gene expression system. Members of the two populations were first interbred and then back-crossed to Merit plants. When the progeny were tested for graviresponsiveness in the dark the result was a smeared rather than a bimodal distribution. This indicated that the trait was not under allelic control.

10. We established that the pattern of nutational curvature seen in some gravistimulated maize roots was not found to be under genetic control. This was established by carrying out inter-generational studies of nutational curvature with cv. Merit roots.

11. Physiological and genetic aspects of the Lazy mutant in maize were examined. Experiments

supported the idea that the stem of the adult homozygous Lazy plant is positively gravitropic rather than agravitropic in its growth habit. Finally, a method is introduced for isolating the Lazy gene using a transposon-tagging method.

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