Growth Rate and Gravitropic Curvature Studies in Roots of *Zea mays* L. Seedlings

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ABBREVIATIONS

ABA	abscisic acid
cm	centimeters
cv	cultivar
°C	degrees centigrade
°	degrees
e.g.	for example
E.R.	endoplasmic reticulum
G.A.	gibberellic acid
h	hours
I.R.	infra-red
IAA	indole-acetic acid
mm	millimeter
mmh^{-1}	millimeters per hour
min	minutes
mol dm^{-3}	moles per litre - molar
mol m^{-3}	moles per cubic meter
m	micrometer
nm	nanometer
%	percentage
s	second
S.D.	standard deviation
S.E.	standard error

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SUMMARY

Using infra-red video equipment it was possible, for the first time, to study the behaviour of roots grown and manipulated in total darkness, and to monitor continuously the growth and curvature of individual roots without the use of destructive sampling techniques.

The main objectives of this investigation were to rationalise the conflicting reports in the literature as to the growth rate changes, and amount of curvature, in roots, in order to obtain a clear indication of the behaviour of roots under defined environmental conditions.

The straight growth rate, gravitropic curvature, and the growth rate changes on the opposite sides of a gravireacting organ, were studied in individual roots, and the behaviour of the individual roots was compared to the mean response for each particular treatment to assess the validity of the use of such data which appear in published reports of experiments using destructive sampling techniques. Of particular interest were the growth rate changes on the upper and lower sides of a gravireacting organ, with regard to testing the validity of the Cholodny-Went hypothesis, as an explanation of the mechanism of gravicurvature in Zea roots.

The results of these investigations have revealed that:-

a) individual roots have a characteristic growth rate which is constant over time;

b) the growth rate of intact roots is reduced by as little as 10 minutes illumination, but the growth rate of decapped roots is unaffected by such treatment, thereby supporting reports of light

induced production of inhibitor in the rootcap;

c) white, red and blue light are capable of eliciting a reduction in growth rate;

d) decapping roots in darkness reduces the growth rate, indicating the possible presence of a promoting influence in darkness;

e) in both darkness and light gravitropic curvature develops after a lag phase of approximately 30 minutes; after this lag phase dark-grown, and some light-grown roots (type 1) bend to their maximum angle within 2-3 hours and then fluctuate about their final angle, which is slightly less than their maximum angle of curvature. Other roots in light (type 2) continue to bend throughout the whole of the observation period; the curvature pattern of individual roots was masked in the mean curvature and curvature was enhanced by illumination;

f) gravicurvature in Zea roots (cv. Fronica) developed as a result of a disproportionate increase in the growth rate on the upper side and a simultaneous, but statistically insignificant, decrease on the lower side; the increase on the upper side being twice as great as the reduction of the lower side. This disproportionality indicated that perhaps there was not merely a simple redistribution of a fixed amount of growth regulator from one side of the root to the other.

In addition to relating the growth rate changes to the observed direction and magnitude of curvature in roots under similar environmental conditions, they are discussed with reference to previous studies reported in the literature, the possible changes in growth regulator levels in the roots and the validity of the Cholodny-Went hypothesis.

CHAPTER ONE

INTRODUCTION

Plants, unlike most animals, tend to be sedentary organisms but they are capable of growth movements which are directionally related to external stimuli. These plant movements can be classified into 3 main types, tactic, nastic and tropic. Tactic movements are movements of the whole organism in response to external stimuli. Such movements are displayed by motile unicellular and multicellular algae, such as Chlamydomonas, Volvox and photosynthetic euglenoids, gametes such as those found in the Bryophytes and Pteridophytes and chloroplasts in higher plant cells. Nastic and tropic movements involve movement of parts of fixed plants. Nastic movements are those in which the plane of movement is determined by the anatomical structure of the organ and are thus independent of direction of stimulus. The rapid movements of sensitive plants such as Mimosa pudica and Dionaea fall into this category, as do the nyctinastic leaf movements of members of the Leguminosae. In tropic movements, however, the response is determined by the plane of symmetry established by the stimulus in the organ. In natural situations, this is usually related to the direction from which the stimulus originates. The most studied tropic movements are the phototropic and gravitropic responses of roots and shoots of dicotyledons and cereal species.

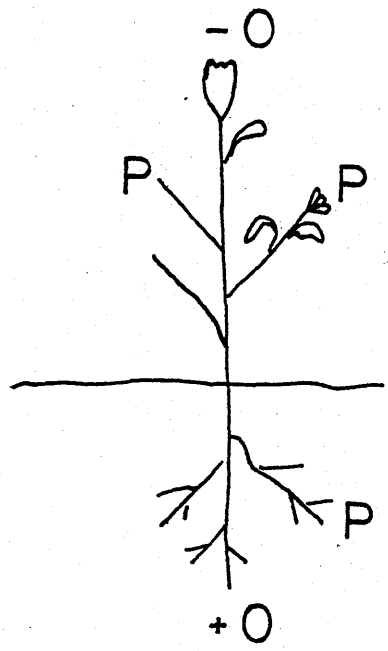
Tropic movements can be brought about by a number of environmental factors, such as light and gravity. In this thesis, attention is confined to the tropic response of roots to gravitational

stimulation. This response has, until recently, been termed geotropism (after Frank, 1868), but is now referred to as gravitropism. This change of nomenclature has taken place because the prefix 'geo' relates the response to the gravitational field of the Earth, whereas 'gravi' denotes the general dependence on mass acceleration. This difference will be especially relevant when gravity-related research is carried out in space.

A number of types of gravitropic response are known. The different types relate to the final stable angle adopted by the plant organ with respect to the gravity vector (Fig.1.1). Primary, or main stems and roots, grow parallel to the direction of gravity and are described as orthogeotropic (orthogravitropic). Lateral roots and branches assume various angles that are characteristic of their order, that is, whether they are first order or second order laterals, and the physiological condition of the plant. These organs are termed plagiogeotropic. Rhizomes and runners are special types of plagiogeotropic organs which grow horizontally, that is at 90°, to the direction of gravity. Such organs which grow horizontally are termed diageotropic, e.g. Aegopodium podograria, Agropyron repens.

Gravity has been thought to be a factor modulating the growth of plant organs for more than 300 years. It could not have escaped notice, even in the earliest times, that stems of trees grow vertically upwards and roots vertically downwards, regardless of the angle of the soil surface in the locality but, according to Audus (1969), Dodart in 1703 appears to be the first author to record this fact and give it attention.

Figure 1.1 Diagrammatic representation of the orthogravitropic (-O, +O) and plagiogravitropic (P) organs in a plant.



In 1709 Austruc had recognised that the upward curvature of a displaced stem was related to gravity. He suggested that the nutrient plant 'juices', because of their density, would move predominantly into the lower halves of horizontal organs. This would favour the growth of the lower side causing upward curvature.

The accounts by Dodart (1703) Austruc (1709) and their contemporaries are interesting as a record of scientific research at the

time, but were largely non-experimental studies. The first experimental work, which really established that plants were able to orientate themselves with respect to gravity, was carried out by Knight in 1806. He showed that a centrifugal acceleration caused both roots and shoots to execute growth curvatures. Knight attached seedlings on to the rim of a wheel that was rapidly rotated about a horizontal axis. The main axes of the seedlings assumed positions along the radii of the wheel; the main roots directed their tips outwards and the stems directed their apices inwards. Since the axis of the wheel was horizontal, a gravitational force could not act continuously on the seedlings in any particular direction. A centrifugal acceleration, generated by the rotation of the wheel, had overcome the gravitational acceleration. The fact that the roots grew in a centrifugal direction and the shoots in a centripetal direction, established the opposite nature of the response in these organs to mass acceleration and provided evidence that gravitational acceleration governs the orientation of plant organs.

Towards the end of the 19th century researchers, such as Ciesielski and Darwin, began to consider the question of whether or not the mechanism by which plant organs perceive mass acceleration stimuli was localised in the plant, in much the same way that specialised gravity sensitive organs occur in animals. The most obvious way to explore this possibility was to remove various tissues from the root and see whether the organ was still able to respond to gravitropic stimuli. Ciesielski (1872) removed the root-tips of a variety of seedlings and concluded that "when the roots of seedlings (Pisum, Ervum, Vicia) which had had their tips cut off, were laid

horizontally, they did not curve geotropically; when, however, the roots which had had their tips cut off were left for some days, they formed new growing points, and then they at once began to curve geotropically. From these facts Ciesielski (1872) inferred that the geotropic curvature of a root can only take place when the root possesses an uninjured 'growing point'" (cited from Vines, 1886 pp.467). Darwin (1880) carried out similar experiments, removing the root-tip from vertically orientated roots, before placing them horizontally and, like Ciesielski (1672), he found that no curvature occurred. If, however, the roots were placed horizontally for a short time prior to removing the root-tip, a curvature did develop. These experiments thus indicated that the site of perception was located in the root-tip and this finding established that the transmission of a 'message' from the root-tip to the elongation zone must be involved in the responses. Darwin (1880) described the tip as the site of geotropic 'irritability' and also established that even though decapitation abolished curvature it did not diminish the growth in length of the root, a fact which demonstrated that the loss of geotropic irritability was not due solely to a cessation of growth.

Although the experimental work of Darwin (1880) and Ciesielski (1872) appears to demonstrate quite conclusively that the site of perception of gravitropic stimuli is localised in the root-tips, it must not be forgotten that both experimenters used methods that involved surgically removing the root-tip, and it is possible that the observed loss of curvature was due to the effects of injury to the root. In 1898, Czapek reproduced Darwin's results, without surgical injury, by allowing the growing root apex to grow

into an 'L'-shaped glass-tube, so that the tip was kept at 90° to that of the regions behind it. If the apex was placed vertically, and the rest of the root horizontally, no curvature occurred; if, however, the apex was placed horizontally, within 24 hours the root had bent to reorientate the apex vertically. This finding again illustrated that the actively growing regions are incapable of perceiving gravitropic stimuli. At the end of the 19th century this experimental work appeared to demonstrate conclusively the localisation of the graviperception mechanism. However, today, with more knowledge of plant physiology, some caution is required in the interpretation of the results of this early work. Czapek's results (1898) may have been due to a number of factors other than the inability of the growing zones to perceive gravitropic stimuli. The root-tip, confined in it's glass-tube, may have been responding to it's restricting local environment. Under such conditions it is feasible that gaseous exchange is affected and bending could be induced by a build up of gases. For example, ethylene is produced under such conditions where the tissues become compressed or subjected to mechanical stress, and even at low concentrations, can induce curvature in a variety of organs such as pea roots (Chadwick and Burg, 1967; Burg and Burg, 1968). Other gases have also been shown to have an effect on plant growth; in pea roots, for example, CO_2 is found to suppress the gravitropic response. The same suppression is not, however, found in pea shoots but this has been taken as evidence in support of the involvement of ethylene in the response, since ethylene is not presumed to participate in shoot gravicurvature (Chadwick and Burg, 1967).

Furthermore, there could be a depletion of oxygen inside the glass-tube, and since the induction of the differential growth on the opposite sides of an organ has been shown to be dependent upon metabolic action during the perception stage (Brauner and Hager, 1958) it seems likely that a lack of oxygen could also lead to the absence of a gravitropic response. It is, therefore, necessary to be aware of the limitations imposed by a lack of knowledge at the time when these early researchers proposed their conclusions.

In addition to demonstrating the location of the site of graviperception, it was also necessary to establish where in the root the development of curvature took place. In 1887, von Sachs established that curvature took place only in growing roots and, in fact, only in the extension zone of such roots. In order to study the development of curvature it is necessary to divide the organ, under investigation, into recognisable regions. Sachs (1887) achieved this by marking the roots of Vicia faba with Indian ink dots at 2mm intervals. The marked roots were then placed horizontally in loose soil and allowed to grow for 7 or 23 h, after which time the positions of the ink marks were examined. At the same time it was possible to determine the increase in length of both the upper and lower surfaces, and compare it to that of a vertical root. It was established that no growth occurred in either the terminal 2mm, nor in the region behind the 8mm mark; growth was accelerated on the upper surface, and retarded on the lower surface, in comparison with that of a vertical root. Thus, Sachs (1887) showed that in roots curvature was brought about by unequal growth of the upper and lower halves of a horizontal root, and that this differential growth occurred in the region 2-8mm

behind the tip, that is, in the root elongation zone.

Thus, the early experimental work provided evidence showing that there was a distinct site of perception and a site of response in the root and, as a consequence, there must exist a mechanism for the transmission of information from the former to the latter.

Gravitropism can therefore be regarded as a classical sensory system with perception, transduction and response phases. The perception phase involves the interaction between the stimulus and a receptor mechanism in the organ, resulting in a change in the receptor. Transduction is the collective term for the sequence of processes leading from stimulus preception to the final response, involving the transmission of the 'message' to the response region. The final response phase is where the initiation and cessation of differential growth,and hence curvature,occurs in the plant.

Both the sensory and response mechanisms have been subjected to detailed investigation over the past 80-100 years. Two of the most important and far-reaching developments in the study of gravitropism during this time have been those concerned with graviperception, in 1900, and with the control of differential growth, in 1926. These theories were of tremendous importance when advanced and still form the basis of present day ideas on the nature of the sensory and the response mechanisms of gravitropism.

The first was the starch-statolith theory independently proposed by Haberlandt and Nemec in 1900. This resulted from the discovery of sedimentable starch granules in certain regions of plants. The hypothesis is based on the occurence of statolith-containing cells (statocytes) predominantly in

gravitropically sensitive zones of plants, such as root-cap cells. In the normal orientation of the plant organ the statoliths come to rest on the apical wall of the statocyst. Angular displacement of the organs causes the sedimentation of the statoliths to the walls and the establishment of an asymmetry in the organ, which initiates the processes that lead to gravitropic curvature. This hypothesis is described more fully later, together with an assessment of its validity.

The second theory is concerned with the response mechanism. This theory was proposed after the existence of growth-controlling hormones, especially the auxins, had been recognised in the 1920's. Cholodny (1926) and Went (1926), quite independently suggested the same hypothesis which stated that the lateral movement of auxin in horizontal organs would result in an asymmetric distribution, leading to differential growth and thus curvature. The Cholodny-Went hypothesis, as it is now known, has been subject to substantial criticism in recent years (e.g. Digby and Firn, 1976). The validity of this hypothesis will be discussed in more detail in this introduction since it is one of the objectives of this thesis to establish whether or not, the patterns of growth-rate changes in gravitropically responding roots and shoots, are compatible with the proposals of Cholodny and Went.

THE PERCEPTION OF GRAVITY

Noll's (1892) speculations upon the existence in plants, of structures, similar to the statocyst-like sense organs in animals, led to Nemec (1900) and Haberlandt (1900) studying gravity-sensitive

organs. They found that in all such organs, they examined, there were cells containing several starch-grains, which sedimented to the lowermost side, whatever the orientation of the organ. This finding led to their proposal of the starch-statolith hypothesis for graviperception, and subsequently many attempts have been made to correlate the occurrence of graviresponses in organs with the presence of sedimentable starch-grains. Even though it is over 80 years since the theory was proposed it is still not possible to establish its validity unequivocally. A number of different approaches have been used in testing this hypothesis, a number of which are outlined here.

Firstly, evidence consistent with the starch-statolith theory comes from the occurrence of gravitropically sensitive plants which only manufacture statolith starch and not storage starch; Crinium, Iris and Allium being three such plants (Audus, 1962). There are also examples of plant organs that contain statolith starch but are agravitropic and, conversely, gravitropically sensitive plants that contain no amyloplasts. The occurrence of these two types of plants seems, at first, to be inconsistent with the starch-statolith theory. The secondary roots of Myosotis palustris and Oxalis acetosella and the aerial roots of some epiphytic orchids, are examples of agravitropic organs containing movable starch. It is possible, in the roots of such plants, that although the perception mechanism is functioning normally, there is some breakdown in the sequence of events by which the 'message' is transmitted to the growing zones, and since the message is not received, no curvature develops. Audus (1962) proposes that these plant organs represent a step in evolutionary development that is leading to the loss of

gravitropic responsiveness. It is possible that a link between the sedimentation of amyloplasts and curvature has already been lost and the amyloplasts still remain, although they are useless. Especially in the case of the aerial roots of the epiphytic orchids a gravitropic response seems to be of little importance since the roots will hang downwards under their own weight without the need for precise orientation in response to gravity. In addition aerial roots are not performing an anchorage role for the plant where an inability of the roots to orientate themselves would be of greater importance.

Aerial roots of Laelia anceps Lindl., and the perianth of Clivia nobilis Lindl., are examples of gravitropically sensitive organs which apparently contain no movable starch-grains (Audus, 1962). In these organs it is feasible that other particles, such as calcium oxalate crystals, mitochondria, and ribosomes, could act as statoliths. Although these two organs represent a serious challenge to the validity of the starch-statolith hypothesis, the data and illustrations in the papers are of very poor quality and, as Audus (1962) points out, these findings need to be re-examined and reassessed.

A second approach to testing the hypothesis has been to correlate the 'presentation time' with the rate of sedimentation of starch-grains. The presentation time, which is specific for a particular organ, is the minimum time that an organ has to be displaced horizontally before a response is induced. If the hypothesis is correct there must be a close correlation between the rate of sedimentation of the statoliths and the presentation time. Hawker (1933) kept the stems of Lathyrus odoratus (sweetpea)

seedlings at different temperatures during horizontal exposure and determined the sedimentation velocity of the statoliths and the presentation times. If sedimentation of starch-grains is involved in the graviresponse it would be expected that a change in temperature would alter the viscosity of the cytoplasm and hence the rate of sedimentation, which should then be reflected in the changes in the presentation times. Hawker (1933) found a very close correlation between sedimentation velocity and presentation times over the temperature range 10-40 °C. Between 10-30°C there was an increased rate of fall of statoliths accompanied by a shortening of the presentation time. At 40°C, however, the rate of movement of statoliths decreased and there was an attendant increase in the presentation time.

A third way of testing the starch-statolith theory is to demonstrate that removal of the starch-grains from the organs leads to an associated loss of responsiveness. In practice statolith starch is very persistent, and even when plants are starved, although they rapidly use reserves from other parts of the plant, they will not utilise the starch in the amyloplasts. Zollikofer (1918) starved germinating plants of Tagetes, Dimorphotheca and Helianthus by giving the plants a 2-4 day light treatment before growing them in darkness, since this accelerates the starch breakdown compared with plants totally grown in darkness, which, even after 4 days, contain some starch. In the starch depleted plants no gravitropic reactions were seen. Protic (1928) used a similar starvation treatment, and Hawker (1933) cold treatments, to reduce the amount of statolith starch, and in these two cases also, there was an attendant loss of gravitropic

responsiveness. In all 3 cases, when the plants were returned to normal conditions, the starch-grains in the statoliths reformed, and the organs regained gravitropic responsiveness. It has already been stated that statolith-starch is very persistent, and even if it were possible to prove that these treatments led to a total loss of starch, there is still the remaining problem that starved organs may be unable to respond to gravity for reasons other than the lack of statoliths. For example, the growth rate may be extremely low, or interference with normal hormonal metablism may have taken place. Only one of the cited investigations (Zollikofer, 1918) established that the starved organs were still growing, and moreover, still able to respond phototropically.

Another method of removing starch-grains was used by Pickard and Thimann (1965,1966). This method involved the incubation of coleoptiles of Triticum vulgare L. in a solution of 6-furfuryl-amino purine (kinetin) and gibberellic acid (GA_3) at 30°C, for 34 h, in darkness. Pickard and Thimann (1966) detected no loss of gravitropic responsiveness with the disappearance of starch, a finding which appeared to refute the view that starch-grains formed a critical part of the graviperception mechanisms. Compared with the controls, the treated coleoptiles developed a smaller curvature, and this did not begin until about 5 h after the onset of gravistimulation. In addition, the growth rate of destarched coleoptiles was retarded, although the ratio of curvature to growth rate was the same for treated, and control coleoptiles. The slower response might indicate that there could be the sedimentation of other smaller particles, such

as mitochondria, in the root apex and, thus, the root is still able to respond albeit more slowly.

Iversen (1969) applied the same destarching treatment as Pickard and Thimann (1966) to roots of Lepidium sativum L.; however, Iversen used slightly higher temperature of 35°C, for 29, rather than 34 hours. After incubation the roots were totally free of sedimentable starch and there was a total loss of gravireponsiveness. Iversen (1969) also demonstrated that the growth rate of the starch-depleted roots was only slightly less than that of control roots, incubated in water and, thus, a cessation of growth was not the cause of the lack of curvature. These results led Iversen (1969) to the opposite conclusion to Pickard and Thimann (1966), that is, without starch-grains the roots are unable to detect their orientation in a gravitational field. When the destarched roots were placed in water and illuminated, after 20-24 h, the starch-grains reformed and at the same time, the gravitropic responsiveness was regained.

A number of years after providing evidence in support of the starch-statolith theory in roots, Iverson (1974) repeated the destarched coleoptile experiments of Pickard and Thimann (1966). After incubation in the kinetin-GA solution, Iversen (1974) used light- and electron-microscopy to examine the shoot tissues and both techniques revealed the presence of small amounts of starch. This residual starch could, therefore, have been the cause of the 18.4% curvature that Iversen (1974) himself observed, and also that reported earlier by Pickard and Thimann (1966). Iversen (1974) tested this possibility by incubating the coleoptiles at 34°C for 36 h, and this treatment resulted in a total loss of amyloplast-starch. Furthermore,

no curvature was observed even after 24 h horizontal displacement, despite the fact that the shoots were still able to elongate. It, therefore, appears that in both roots and shoots there is a correlation between the hormonally induced disappearance of starch-grains, and a loss of curvature. In roots, there is also the additional evidence of the simultaneous reappearance of starch-grains and gravitropic sensitivity after the cessation of the hormonal treatment (Iversen, 1969, 1974).

In the light of more recent knowledge with regards to the involvement of growth regulators in the gravitropic response (Gibbons and Wilkins, 1970; Shaw and Wilkins, 1973; Pilet, 1971a, 1973b) it is necessary to reconsider Iversen's (1969, 1974) conclusions, since the incubation in kinetin and gibberellic acid may have caused the cessation of production, or the inactivation of the critical growth inhibiting regulator, on which the response is dependent, as well as leading to the removal of starch-grains, and the loss of response. A critical test of whether the loss of graviresponsiveness is caused by the treatment affecting growth-regulator transport, or simply by removing the starch-grains, is suggested by Wilkins (1976b). He proposes that in view of the research by Gibbons and Wilkins (1970), the response elicited by half-decapping, destarched, roots would resolve the problem. If the production and basipetal transport of the inhibitor continued, then curvature towards the remaining half-cap would occur. On the other hand, if no curvature developed, it could be argued that Iversen's results (1969, 1974) possibly reflect a disruption of the hormonal control mechanism of the root, as well as removing the starch-grains. No report of such an experiment has

appeared in the published literature.

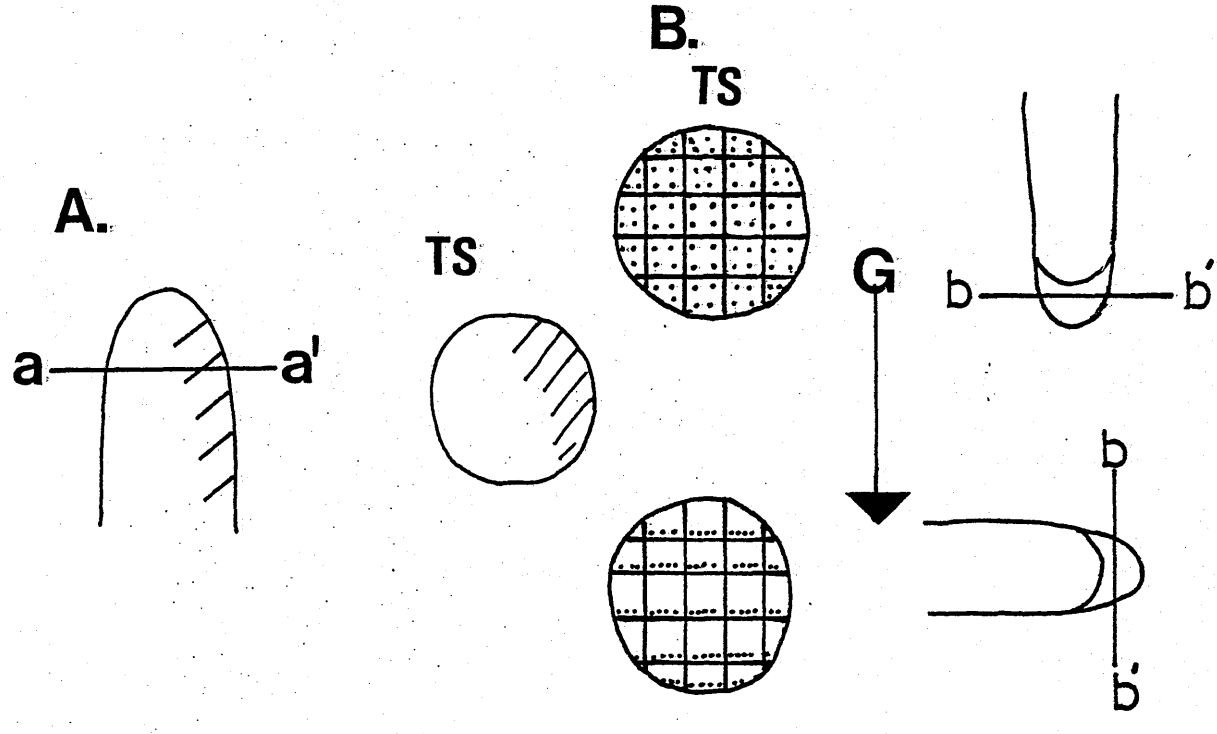
Removal of the root cap, the site of the statolith containing cells, from the apex of Zea mays roots resulted in a loss of gravitropic responsiveness (Juniper et al., 1966) and thus appeared to provide evidence in favour of the starch-statolith theory. However, difficulty in accepting the theory arose when light- and electron-microscopic studies of the roots of Triticum vulgare and Z. mays showed that graviresponsiveness was regained 14 hours after decapping, which is before a new cap regenerates at about 3 days (Pilet, 1973a; Barlow, 1974a, 1974b). However, it was discovered subsequently that amyloplast starch formed in the proplastids in the cells of the quiescent centre, the immature xylem and the cortical tissues of the root apex, immediately after decapping, and were very prominent after 24 h (Barlow and Grundwag, 1974). On regeneration of a new cap, 72 h after decapping, it was found that starch was no longer formed in the cells of the root apex. Thus, the decapped roots are in possession of starch-grains although their involvement in the perception of the stimulus was not established.

More recently, some indication of the role of these newly formed starch-grains has been found by Hillman and Wilkins (1982). They have shown that in decapped roots of Z. mays the graviresponse returns quite suddenly between 12 and 24 h after removal of the root cap. By examining individual roots, sedimentation of the newly formed starch-grains in the root apex was observed in at least some of the cells in roots which had regained their gravitropic responsiveness. However, no such sedimentation was observed in roots which had not regained their capacity to respond gravitropically. As there was no

substantial size difference between amyloplasts in the root apex 12 and 24 h after decapping, a change in weight could not account for the onset of sedimentation. Hillman and Wilkins (1982) suggested that the occurrence of sedimentation was due to changes in the physical characteristics of the cytoplasm. This change in viscosity would allow movement of the amyloplasts, and hence, the return of graviresponsiveness. Thus, there is now some evidence for a close correlation between the return of gravitropic responsiveness, and the ability of the newly formed amyloplasts in the root apex to sediment to the lowermost side of the statocytes. These findings indicate that the root apex can take over the role of graviperception in the root, when the root cap is absent, and this situation allows a graviresponse to occur before a new cap has regenerated.

The nature of gravistimulation is somewhat different from that of the stimuli of light, chemical, and physical contact, which elicit phototropic, chemotropic and thigmotropic responses respectively. This difference arises because gravity acts equally on all cells in the organ, whereas light, for example, gives a larger stimulus to the cells on the side facing the source, than those on the shaded side. In order to elicit a tropic response an asymmetry must be established in the organ; in the case of light this asymmetry is self evident, in that the stimulus acts at the level of the organ (Fig. 1.2A).

Figure 1.2 Diagram to illustrate A) the asymmetry set up in an organ in response to a light stimulus. B) the asymmetry set up in the root cap by the sediment - Transverse section (b-b^1) of (i) a vertical root. (ii) a horizontal root showing the arrangement of the amyloplasts (black dots). Gravity acts in the direction of the arrow G.



In the case of gravity, which is also a unilateral stimulus, the establishment of an asymmetry is more complex and appears to involve the movement of particles, and hence the establishment of an asymmetry in the organ at the cellular level, which in turn leads to an asymmetry in the organ as a whole. The result of this asymmetry is to set up a lateral polarity in the cells from the bottom to the top of the horizontal root (Fig. 1.2B). Exactly how the statoliths act in

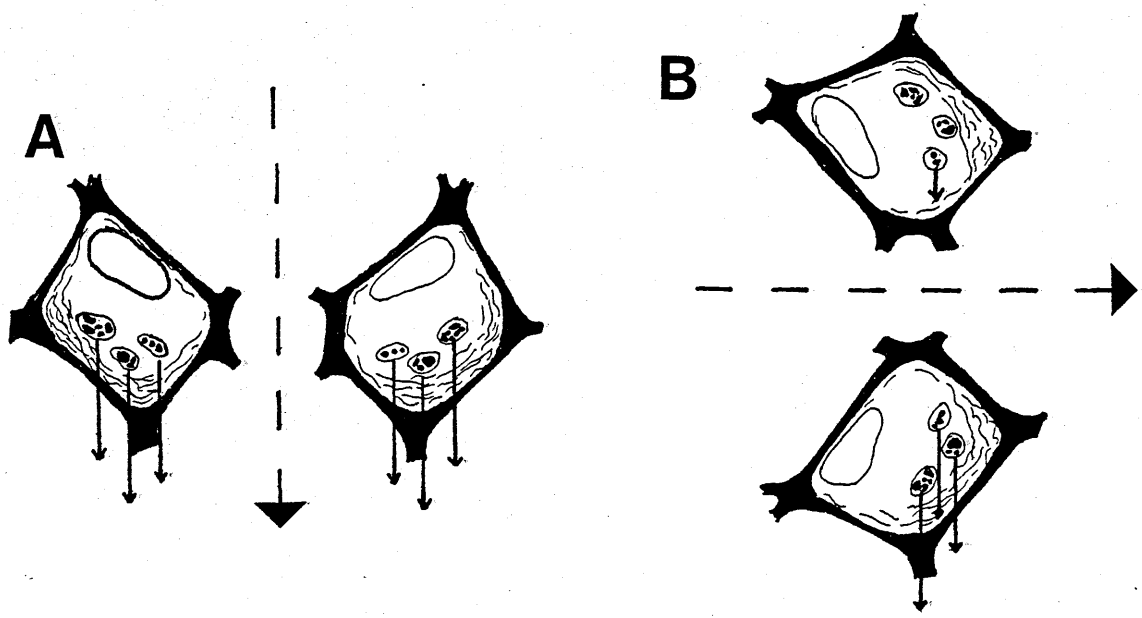
the perception mechanism is unknown, but in some way the physical signal is changed into a physiological one.

There are several ways in which this transduction of the signal could occur; the most obvious way is by the exertion of a physical pressure. During sedimentation the amyloplasts could fall onto some sensitive part of the lateral, lowermost, side of the cells and thus trigger the sequence of events that leads to transduction, and finally, the response. It is also possible that the statoliths have their own specific metabolism, and when the organ is displaced, this metabolism becomes concentrated on the lowermost side of the cell. It could be that the amyloplast membrane carries an electrical charge, which could cause a polarity between the upper and lower surfaces of the statocytes following their sedimentation. Alternatively, their mass could displace other metabolically active cell constituents away from the sensitive regions of the plasmalemma, in the lowermost part of the cell, to the uppermost part. This could result in the upper part of the cell having a higher metabolic activity, and would cause a gradient between the upper and lower surfaces of adjacent cells, in a vertical series. This gradient, would be in favour of the upper half of the lowermost cell, and could form the basis for the induction of a polar movement of specific substances from the upper to the lower cell via a specific carrier mechanism.

Audus (1962) has presented evidence that the amyloplasts cannot exert a pressure of more than 2-4 dyne cm^{-2}, and he questions whether such a pressure is of sufficient magnitude to induce the gravitropic response.

It has, however, been proposed that the pressure caused by the precipitation of amyloplasts onto the endoplasmic reticulum (E.R.) complex, forms the basis of graviperception. Sievers and Volkmann, (1972, 1977; Volkmann and Sievers, 1979) have offered an explanation of graviperception involving the sedimentation of amyloplasts onto the statocyte E.R. complex which is asymmetrically distributed in certain root cells of Lepidium sativum. When the root is orientated vertically (Fig. 1.3A) the pressure exerted by the amyloplasts on the E.R. will be equal in the two cells and thus the root grows normally. Any deviation from the vertical will change the pressure exerted. If the root is placed horizontally (Fig. 1.3B) the amyloplasts will exert a pressure on the E.R. only in the lowermost cell, and this inequality in pressure will cause asymmetric growth.

Figure 1.3 Diagram to illustrate A) the equal pressure exerted by the amyloplasts on the endoplasmic reticulum in statocytes on either side of the root axis. B) the unequal pressure exerted by the amyloplasts in a horizontal root. The solid arrows represent the direction and magnitude of the pressure of the amyloplasts on the E.R. and the dashed arrows the direction of the root-tip (after Sievers and Volkmann, 1972).



Sievers and Volkmann believe that the pressure exerted is the important factor in graviperception, and only a small amount of spatial movement of the amyloplasts would be possible in the short presentation times in Lepidium roots (12 s) (Wilkins, 1984).

Although this hypothesis seems feasible for Lepidium roots it must be stressed that it involves the precise shape of the statocytes and asymmetric distribution of the E.R. within the apical part of the cells. In many other species the shape of the statocytes, and distribution of the E.R. is not the same as in L. sativum. In Lens culinaris, Daucus carota, and Allium cepa, this particular pattern of E.R. arrangement is found (Volkmann, 1974; Wilkins, 1984), but not in the statocytes of Z. mays (Juniper, 1976), Vicia faba (Griffiths and Audus, 1964) nor the statocytes of stems, such as those of grass-nodes (Osborne and Wright, 1977; Wright and Osborne, 1977).

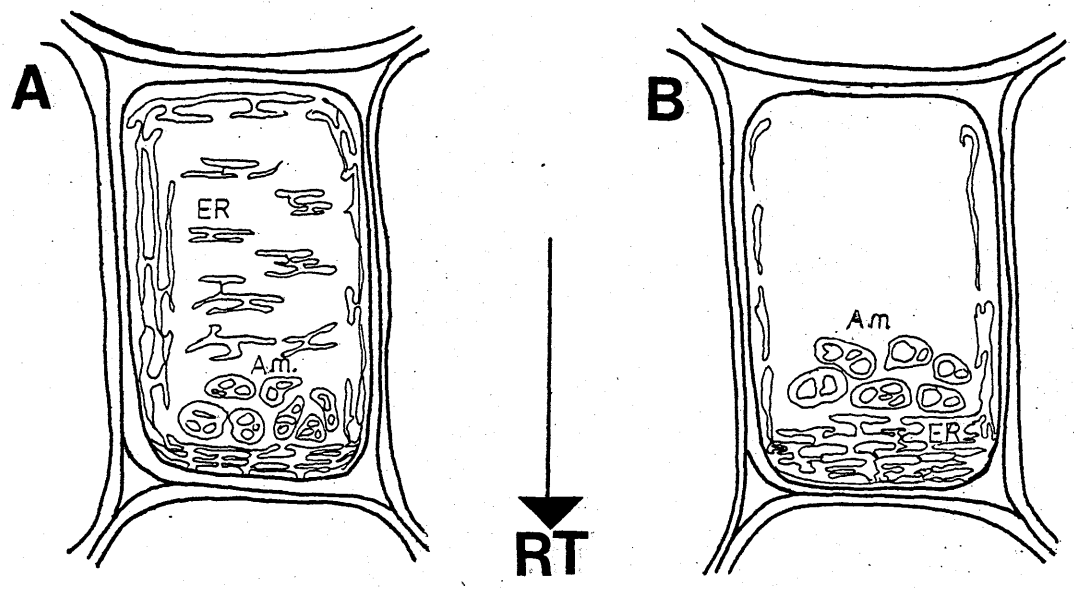
Sievers and Heyder-Caspers (1983) centrifuged seedlings of L. sativum for 20 min at 50g, and thereby disrupted the structural polarity of the statocytes; the E.R. complex being displaced by the other, heavier, cell organelles. After several minutes the original cell polarity was re-established, and after 7.5 minutes, the E.R. was located in the distal cell pole, and the amyloplasts were found sedimented on the E.R. complex. This, especially rapid reorganisation of the distal cell pole of the statocytes, demonstrates the stability of the cell polarity, and Sievers and Heyder-Caspers (1983) suggest that this must be of prime importance for the principle functions of the statocytes in graviperception. A supportive piece of evidence comes from the fact that the time taken for most statocytes to rebuild

their distal cell poles equals the increase in the latent period of the graviresponse.

Electron-micrograph studies have made it possible to make detailed examination of the E.R. complex in the root cap cells. Such studies have revealed that amyloplasts sedimenting onto the E.R. complex cause localised compression of the cisternae, which results in the distance between successive elements in the granal stack being different (Sievers and Volkmann, 1972, 1977). Such evidence, for the deformation of the E.R., answers Audus's query (1962) as to whether the amyloplast is of sufficient mass to induce a pressure that causes a change in the E.R.

Further support for the E.R. complex being the sensitive structure in the cell, comes from studies by Olsen and Iversen (1980) using an agravitropic mutant of pea, Pisum sativum var. ageotropum. They found that the only major anatomical difference between the root cap cells of the mutant and a normal pea was that the E.R. was differently distributed in the 2 types. The E.R. in the normal pea statocyte was found to be concentrated in the distal part of the cell, whilst, in the mutant, it was evenly distributed throughout the cell (Fig. 1.4). This difference in distribution between the 2 types, would result in a difference in the way that the amyloplasts and the E.R. interacted. This report supports the idea that the interaction between the E.R. and the amyloplasts might bring about the biophysical and biochemical changes which are of basic importance for the initial phase of the perception of gravity.

Figure 1.4 A semi-schematic representation of statocyte cells in an agravitropic (A) and a normal (B) pea root. The distribution of the E.R. and amyloplasts with starch grains (Am) in columella cells kept in the normal vertical position. RT and arrow indicate the direction of the root-tip (after Olsen and Iversen).



Unfortunately, it was not possible to extend this morphological difference to the mutant and wild form of Arabidopsis thaliana (Olsen et al., 1984). Studies of this species did not show any difference in the E.R. distribution in the statocytes. In both the wild-type, and the 2 mutant species examined (aux-1 and aux-2) the E.R. distribution was similar to that in normal pea and cress, with

the amyloplasts resting on the dish of distal E.R., which extends upwards close to the longitudinal wall when the roots are in the vertical position. It, therefore, seems that ultra-structural differences cannot be used to explain agravitropic behaviour due to the fact that differences in E.R. distribution, in normal and agravitropic roots, appears to be species related, rather than a general phenomenon.

As previously mentioned, physical pressure exerted by the amyloplasts need not be the only way that a polarity is established in the cells. Wilkins (1978) suggested that if amyloplasts were electrically charged their sedimentation could create a cell polarity that might affect the permeability, and transport properties, of the nearby plasmalemma. Recently, Sack et al. (1983) have demonstrated a surface charge on isolated maize coleoptile amyloplasts. They confirmed the existence of the net negative surface charge by ultrastructurally binding cationised ferritin to amyloplasts. This demonstration of a charge on the amyloplasts supports Wilkins's hypothesis (1978) but further investigation is necessary to establish whether the amyloplast charge has a role in the graviperception mechanism.

In summary, there seems to be little doubt that sedimentable amyloplasts are a prerequisite for gravity perception. The only 2 cases cited here which seem to oppose this conclusion, are the aerial roots of Laelia anceps and the perianth of Clivia nobilis, which were quoted earlier as examples of organs where gravity perception is apparently achieved in the absence of amyloplast-starch. However, even if the starch-statolith theory can be supported by the increasing

volume of correlative evidence in its favour, a more definite indication as to how exactly sedimentation of amyloplasts initiates the graviresponse is still wanting.

THE RESPONSE MECHANISM

At the present time the most favoured explanation for the development of gravitropic curvature in plant organs is the Cholodny-Went hypothesis which was advanced to account for the curvature of both roots and shoots. It states that auxin (an endogenous plant growth regulator) is produced at the tip of the organ and moves basipetally, in such a way, that it is symmetrically distributed in vertical organs. In horizontal organs a downward, lateral transport of auxin occurs, giving rise to an asymmetric distribution in favour of the lower half of the organ. This asymmetry leads to differential growth and, thus, curvature. It has been demonstrated several times, firstly by bioassay techniques (Dolk, 1929,1936; Gillespie and Thimann, 1961), and later with radioactive IAA (IAA-^{14} C) (Gillespie and Thimann, 1963; Goldsmith and Wilkins, 1964), that when IAA is applied to the apical end of decapitated, horizontal, coleoptiles and shoots, it becomes asymmetrically distributed, with more accumulating on the lower side of the growing zone than the upper side; Shaw *et al.* (1973) were able to show that this asymmetry was not peculiar to decapitated tissues, but was also established in whole coleoptiles. The increase in the levels of IAA leads to greater growth on the lower side of the organ and, thus, an upward curvature. A similar mechanism has also been proposed for roots, but there are doubts about its validity. The opposite curvature

responses in roots and shoots have been explained by the belief that the auxin concentration in roots is supraoptimal, and, therefore, further accumulation on the lower side results in a decreased growth rate; conversely, a decrease in concentration on the upper side, leads to an increase in the growth rate. These changes initiate the differential growth and give rise to downward curvature. Exactly what is meant by "concentration" in this context, and its significance, is discussed later.

Much research has been carried out since 1926, when the hypothesis of Cholodny and Went was proposed, but there is still no evidence to prove unequivocally the existence of this response mechanism in plant organs. The validity of this hypothesis, depends upon the establishment of two postulates; firstly, the growth regulator in the apex of the root or shoot must be chemically identified, and secondly, this compound must be shown to undergo downward, lateral, transport, and accumulate in the lower half of the horizontal organ. An assessment of the evidence for and against the hypothesis is presented below; shoots and roots are considered separately.

Shoots.

In 1972, using high-resolution mass spectroscopy, Greenwood et al. were able to identify the auxin present in coleoptile tips of Z. mays; from the fragmentation pattern of the molecule, and the high resolution molecular mass of the sample, they found that the auxin was indole-3yl-acetic acid (IAA).

Dolk (1929, 1936) carried out early studies of the

distribution of growth regulators in Avena coleoptiles. Excised coleoptile tips were placed in a horizontal position with their cut end in contact with 2 agar blocks. After leaving them for a number of hours, the agar blocks were removed and the net growth-promoting activity present assessed by the Went Avena curvature test. Dolk (1929) found an asymmetrical distribution of regulator in favour of the agar block that had been in contact with the lower side of the horizontal coleoptile tips. Although the experiments of Dolk (1929) provided evidence of an asymmetry of net growth promoting activity it was not possible to ascertain how this asymmetry was established. The availability of radioactive IAA, made possible the examination of how the asymmetric distribution of radioactivity arose in plant organs. Gillespie and Thimann (1961, 1963) demonstrated that there was a greater amount of radioactivity (IAA-^{14}C) retrieved from the receiver blocks of agar in contact with the lower halves of Avena (1961) and Zea (1963) coleoptiles, and that there was an asymmetric distribution of radioactivity in the upper and lower tissues of Zea (1963). Whilst substantiating the findings of Dolk (1929), and providing evidence that IAA may be the growth- regulating compound found in coleoptiles, these experiments still did not give any indication as to whether or not the asymmetry had arisen due to a lateral transport of IAA. Goldsmith and Wilkins (1964) were able to demonstrate unequivocally, that downward, lateral, transport was responsible for this asymmetry in horizontal shoots.

They placed donor agar blocks, containing radioactive IAA, asymmetrically onto the apical end of Zea coleoptiles, which they then orientated horizontally, or vertically. This procedure resulted in

different proportions of the total amount of radioactivity in the organ occurring in the non-donated part of the segment. Since the only source of radioactivity was the agar donor block, the different amounts, found in the non-donated half of the coleoptile, can only have arisen as a result of a change in lateral transport.

These studies were, however, carried out using coleoptile segments, and it could be argued that the lateral transport reported is just a feature of the isolated tissue; for example, the magnitude of the response might be reduced in a segment. A strong, polarised, downward, lateral, transport, was however, demonstrated in gravitropically stimulated, intact, coleoptiles by Shaw et al. in 1973 using a micro-application technique. This technique involved the use of glass micro-pipettes to apply (5-^{3}H)-IAA, at predetermined points on the coleoptiles with the minimum amount of damage to the tissues (Shaw and Wilkins, 1973).

From the above evidence, it appears that the gravitropic response of Z. mays and A. sativa coleoptiles is explicable by the downward, lateral, transport of IAA. However, this evidence in favour of the redistribution of auxin causing gravitropic curvature, has been questioned by Hall et al. (1980). They believe that the auxin concentration gradients that have been found in horizontal coleoptiles are not consistent with the observed growth changes. By fitting the changes in growth rate of the upper and lower surfaces, onto a typical dose-response curve for auxin action on cell elongation, it is possible to predict changes in concentration of auxin. Hall et al. (1980) carried out the above process and found that these changes in concentration were an order of magnitude too small to account for the

observed growth rate changes. It is, however, possible to accommodate such growth rate changes if it is assumed that prior to gravistimulation the amount of IAA in the shoot is such that the shoot is growing at its maximum rate; that is, at the point where the dose-response curve reaches a plateau. At this point a large depletion in the amount of IAA on the upper surface would result in the growth rate falling to zero, but an equally large addition of IAA on the lower surface would have no effect since the IAA is already at its optimal level. These changes in IAA concentration are very large and although such changes seem improbable, until the actual changes in endogenous inhibitor levels in the shoot are known, this possibility cannot be ignored.

In addition to this criticism, it is also known that downward, lateral, transport is not the only change that occurs in the shoot upon gravistimulation. On gravistimulation the basipetal transport of IAA in the tissue increases, with a greater movement along the bottom half of a horizontal coleoptile; this phenomenon was demonstrated by Naqvi and Gordon (1966) using ^{14}C-methylene labelled IAA, and by Cane and Wilkins (1969) using opened out segments of coleoptiles. Other compounds such as gibberellins and cytokinins may also be involved in the induction of differential growth and one or more of these compounds could play a role in the development of gravitropic curvature.

The gibberellins are one group of compounds that has been studied in recent years in connection with a possible role in the gravitropic response of shoots and roots. Gibberellin-like activity was shown to be asymmetrically distributed between agar-blocks in

contact with the upper and lower halves of the basal end of Helianthus annuus shoots and Z. mays coleoptiles (Phillips, 1972; Railton and Phillips, 1973). Ten times more gibberellin activity was found to be present in the lower half of the shoot than the upper half.

Wilkins and Nash (1974) investigated the movement of radioactivity supplied as (^3H)-GA_3 in sub-apical segments of Z. mays coleoptiles. They could find no evidence of a downward, lateral, transport of radioactivity in the tissue, following application of asymmetric donor blocks. Webster and Wilkins (1974) carried out a more detailed study of the movement of ^{14}C-gibberellic acid in gravitropically stimulated coleoptiles, and primary roots of intact seedlings of Z. mays, and they reported an upward, lateral, movement of radioactivity in both roots and coleoptiles. This upward movement of ^{14}C from gibberellic acid, is not consistent with the finding of a greater concentration of gibberellic acid on the lower side of a horizontal coleoptile (Railton and Phillips, 1973). However, naturally occurring gibberellic acids may have been displaced downwards, and may have emerged in the receiver blocks. Alternatively, synthesis or release of other gibberellins may mask, or reverse, the upward transport of GA_3, since, despite the fact that ^3H GA_3 is used as radioactive-tracer, the naturally occurring gibberellins in Zea coleoptiles have not yet been identified and GA_3 may not be among them (Webster and Wilkins, 1974; Crozier, 1984 - personal communication).

In addition to the asymmetric distribution of growth-regulating molecules in gravistimulated shoots, there have been studies which have shown that there is an asymmetry in the

concentrations of inorganic ions, such as Ca^{2+}, K^{+} and ^{32}P (Goswami and Audus, 1976), and it has been suggested that in some, as yet undefined way, this asymmetry is an outcome of auxin gradients in the tissue (Lee et al. 1983a, 1984; de Guzman and de la Fuente, 1981).

In the last few years the question has been raised as to whether the changes in growth rate observed in a gravitropically responding organ, are consistent with the Cholodny-Went hypothesis. Digby and Firn (1979) and Hall et al. (1980) have carried out studies on the gravitropic responses of Zea coleoptiles, and they claim that the changes in the growth rates of the upper and lower sides, are incompatible with the Cholodny-Went hypothesis: that is, that they are inconsistent with merely a re-distribution of already limiting amounts of growth regulators. Furthermore, as discussed earlier, they have questioned whether the asymmetry of IAA distribution demonstrated in horizontal Zea coleoptiles, is large enough to account for the observed changes in growth rate. However, Hall et al. (1980) have based their conclusions on relationships between external concentrations of IAA, in which a segment of coleoptile is immersed, and the observed growth rates. Precisely what relevance such results have to the relationship between the amount of endogenous IAA present in an organ, and its growth rate, has yet to be established. This difficulty arises because it is not possible to measure the concentration of a compound in a cell or organ. In reality, only the amount can be determined, and without knowing precisely the distribution throughout the volume of the organ, and indeed the cell, the concentration cannot be calculated.

Thus, at the present time, knowing that IAA does undergo

downward, lateral, transport, to the lower side of an intact, horizontal, Zea or Avena coleoptile, thereby becoming distributed asymmetrically, it seems that the Cholodny-Went hypothesis is supported at least in coleoptiles. However, for reasons stated earlier, it must be recognised that this process alone may not be wholly responsible for the growth rate changes observed during gravitropic curvature; other transport or metabolic processes, or other plant growth regulators, may have a role.

Roots.

The growth-regulating mechanism involved in the gravitropic response of roots is even more unclear than that in coleoptiles. The effects of applying exogenous natural and synthetic growth regulators such as 2, 4-dichlorophenoxyacetic acid have been examined but do not assist in the elucidation of the natural mechanism controlling root growth since roots grow normally without deriving any major organic nutrients or growth regulators from the exterior. Moreover, they have a very high capacity to metabolise compounds such as IAA (Bridges et al., 1973; Feldman, 1980a) when supplied externally. All the nutrient and growth regulatory compounds required by the root are normally supplied by the transport system in the stelar core. It is now certain that IAA, cytokinins, gibberellic acid and abscisic acid (ABA), are all present in roots, although their physiological functions are as yet unclear. IAA transport in roots is highly polarised towards the tip, and occurs in the stele (Scott and Wilkins, 1968; Wilkins and Scott, 1968; Bowen et al., 1972; Shaw and Wilkins, 1974). Other inhibitory substances are also present, and at least one

inhibitor, arising in the root cap, is of particular interest with regard to the gravitropic response of primary roots.

The Cholodny-Went hypothesis, as an explanation of gravitropic curvature in roots, was supported by the results of studies carried out by Hawker (1932b); she performed similar experiments to those of Dolk (1929) and found, as in coleoptiles, that more net growth- regulating, activity, diffused from the lower half, than from the upper half, of the tip, into basally applied agar blocks. Hawker (1932b) used the Went Avena coleoptile curvature test to demonstrate the presence of growth regulator in the agar blocks, and discovered that the curvature developed towards the block. This direction of curvature is indicative of the regulating activity being inhibitory, a finding which is in contrast to the promoting influence found in the agar blocks that had been in contact with coleoptile tips (Went, 1928; Dolk, 1929). Despite these 2 different directions of curvature, induced by the diffusates from roots and coleoptiles, Boysen-Jensen (1933) presented evidence for an apparently similar growth- regulating factor being involved in the gravitropic curvature of roots and shoots. Boysen-Jensen (1933) found that decapitated roots would curve if the root tip was replaced by a coleoptile tip; in fact a greater curvature was achieved. This finding of a greater effect, indicates that there may be a greater concentration of regulator in coleoptile tips than in root tips, and supports the idea that the same growth regulator could lead to the opposite effects observed in these roots and shoots. Boysen-Jensen's findings are consistent with those of Keeble, Nelson and Snow (1931) who produced evidence which indicated that shoots and roots had different

sensitivities to endogenous growth regulators, by carrying out a series of 're-heading' experiments where root tips and coleoptile tips were placed on root stumps and different amounts of curvature were achieved.

A more recent study by Schurzman and Hild (1980) revealed that the rate of curvature was doubled when coleoptile tips were placed on root stumps, as compared with that when the root tips were replaced. Steen and Hild (1980) carried out similar experiments with detipped coleoptiles, and found that a strong gravitropic curvature was induced by retipping with root tips, but this curvature was not as great as that when other coleoptile tips were placed on the coleoptile stumps. Thus, it is obvious that some factor is produced, by root and coleoptile tips, that can induce curvature in both roots and shoots. It was also shown that this factor reproduced the effect of IAA (10^{-6} m mol.m^{-3}) application during the first 4 h of curvature (Steen and Hild, 1980).

Further evidence for a growth regulator, inhibitory in its action on root elongation, being produced in response to gravity, comes from a number of investigations (Sachs, 1882; Larsen, 1953; Bennet-Clark et al., 1959) which have shown that during gravistimulation the overall growth rate of the root is depressed. This finding supported previous studies by Cholodny (1926) who studied the growth of vertical roots and discovered that elongation was accelerated when the root cap was removed. Thus, there seems to be evidence in favour of the gravity-induced production of inhibitor. Unfortunately, results contrary to the above findings, were presented by Juniper et al. (1966): they found that removal of the root cap from

Zea roots had no effect on the growth in length, whatever the orientation of the root, but the gravitropic response was eliminated. Juniper et al. (1966) therefore concluded that the root cap had no direct influence on elongation, and was unlikely to be the source of growth regulators. However, as the root cap is the site of the gravity perception mechanism, it must in some way either provide growth regulators, or control their production in the root apex, or affect their movement from the cap to the root tip. There is support for Juniper et al.'s (1966) findings, since neither Schachar (1967) nor Pilet (1971a) could find evidence of an increase in growth rate after decapping. Pilet (1972a) carried out further experiments into the effect of decapping on growth rate, and in these studies he recorded the length of the roots from the time of decapping. In this paper the results did reveal an increase in the growth rate, but only up until the third hour. Thus, the fact that Juniper et al. (1966) did not take their first reading until 4 h after decapping, could explain why they did not observe any increase in growth rate.

Since the gravity-sensing system is in the root cap, which is 2 to 3 mm from the elongation zone where the response occurs, it is obvious that some communication mechanism exists in the overall guidance system. On the basis of the evidence cited above, there is a reasonable amount of doubt as to whether or not an inhibitor is produced by the root cap. However, the results of studies by Gibbons and Wilkins (1970) have established that the cap is the source of a net growth-inhibiting influence. In a series of experiments they removed only one half of the root cap, and roots, so treated, always developed a large curvature towards the side of the root upon which

the remaining half-cap was located. This was the same result as the direction of curvature, towards an agar block containing root diffusate, observed in Hawker's (1932b) experiments. Gibbons and Wilkins (1970) observed this direction of curvature whatever the orientation of the root with respect to gravity. Furthermore, Shaw and Wilkins (1973) using half-decapped roots and roots with small, impermeable barriers inserted horizontally, into either the root cap and the root apex, or the root cap alone, were able to confirm that it was the root cap, as distinct from the root apex, which was the source of the inhibitor. Pilet (1973b) supported this finding by showing that if the half root cap was immediately replaced no curvature developed; this also demonstrated that it is the absence of the root cap tissue, rather than surgical damage, which is causing the curvature. It also appears that the inhibitor produced is water-soluble, since when the half root cap was re-attached using Oleic oil, a curvature developed towards the side with the root cap still attached, but when the root cap was reattached with Ringer's solution, no curvature developed (Pilet, 1971a). Furthermore, if root caps from Zea are placed on the root stumps of Lens culinaris, the root elongation is decreased, demonstrating that the inhibitor is not species-specific (Pilet, 1972a).

There is, therefore, evidence that at least one inhibitor is produced in the root cap which causes a reduction in growth rate. If this inhibitor is responsible for gravitropic curvature, it must be shown that an asymmetry in its distribution occurs between the upper and lower halves of the root. As previously mentioned, Hawker (1932b) carried out experiments which showed that agar blocks which had been

in contact with the lower halves of the tips from horizontal roots, inhibited the cell extension of vertical root stumps to a greater extent than blocks that had been in contact with the tips from the upper halves. This finding is indicative of an asymmetry in inhibitor distribution in the root. Shaw and Wilkins (1973) were able to show that this asymmetry arose as a result of downward, lateral, transport, in experiments involving the removal of half the root cap, or insertion of impermeable barriers, which impeded the longitudinal transport of substances between the cap and the elongation zone. The roots were orientated vertically, and curvatures always developed towards the untreated side of the root, indicating that an inhibitory factor was moving basipetally through the root apex and inhibiting cell extension in the elongation zone. More direct evidence for the downward, lateral, transport of an inhibitor, came from inserting barriers either horizontally or vertically, into the apices of horizontal roots. When the barriers were inserted horizontally the curvature obtained was less than when they were inserted vertically. A horizontally placed barrier would be expected to impede downward, lateral, transport, and hence reduce curvature.

So far it appears that there is a certain amount of evidence which satisfies the requirements to establish the validity of the Cholodny-Went hypothesis. From this evidence it appears that the gravitropic response in roots involves the production of at least one growth inhibitor in the cap which undergoes downward lateral transport in a horizontal root. It has not yet been confirmed whether or not such a mechanism adequately accounts for the establishment of differential growth, but it appears that at least in principle, a

Cholodny-Went type of mechanism might be involved.

One of the requirements, listed earlier as necessities for proving the validity of the Cholodny-Went hypothesis, was to identify chemically the growth regulator, and much of the research in recent years has been centred on the identification of the inhibitory compounds in the root cap. When Cholodny and Went proposed their hypothesis in 1926, they believed that the compound involved in the gravitropic response was auxin (IAA). There is now, however, increasing evidence against this view. The presence of IAA in roots was established unequivocably in the early seventies using mass spectrometry (Bridges et al., 1973; Elliott and Greenwood, 1974). In Zea roots the IAA is virtually confined to the stele, although small amounts have been found in the cortex, the root apex, and the root cap (Bridges et al., 1973; Rivier and Pilet, 1974).

The first difficulty in accepting IAA as the growth regulating influence involved in the gravitropic response in roots, arose when a number of investigations revealed that the transport of IAA, in the stele, was polarised in the direction of the apex (Scott and Wilkins, 1968; Bowen et al., 1972). These findings, thus indicate that IAA transport is in the wrong direction for it to be the compound involved in the gravitropic response of roots. Shaw and Wilkins (1974) discovered that the polarity of IAA movement was greater for segments taken 1mm behind the apex and they attributed this to different capacities to transport acropetally IAA, from the cortex to the stele, in older and younger tissues; the older tissue being capable of greater IAA movement. Shaw and Wilkins (1974) therefore posed the question of whether or not the different capacity to trans-

port IAA was related to different ability to metabolise IAA. It was subsequently found that isolated cortex was able to metabolise IAA to a greater extent than isolated steles, with IAA being extracted after 8 h from intact segments, whilst none was extracted from de-steled segments (Greenwood et al., 1973). These experiments were carried out using thin-layer chromatography (TLC) and similar experiments have been performed more recently, using high-performance liquid chromatography (HPLC) techniques, which have a greater resolving power than TLC. Using this technique Nonhebel (1982) examined extracts of cortical and stelar tissue and after a 2 h incubation in aqueous solutions of IAA-2-^{14}C (10^{-3} mol m^{-3}) and extraction in methanol, 96% of the radioactivity in the stelar tissue was found to be IAA, whilst in the cortical tissue, only 8% of the radioactivity was IAA. Feldman (1980a,b) has also carried out studies on auxin synthesis and metabolism in Zea root segments. He divided the root into various segments which either included or excluded the root cap with the terminal segment; these segments thus differed from those used by Shaw and Wilkins (1974) which were all taken from behind the root cap. Feldman (1980a) found that the ability to metabolise IAA in the terminal 0.5-1mm segments was decreased by one third in the absence of the root cap. This finding implies that the root cap may play an important part in controlling the amount of IAA present in the root, and this indicates that segments taken from the apical regions, minus the root cap, may not be giving a true reflection of the actual levels of IAA present in intact roots: such studies should, therefore, be treated with caution.

The above evidence seems to indicate that IAA is present in the root cap and that it is transported there from the more basal regions of the root. However, the root cap, like all other tissues in the root, is able to synthesise IAA when supplied with tryptophan (Feldman, 1980a) and therefore, the acropetal transport does not appear to arise from the inability to synthesise IAA. Despite the amount of evidence, cited above, to the contrary, the presence of IAA in the root cap has been questioned by a number of investigators. Using a micro-bioassay technique, based on the growth inhibition of segments of seminal roots of Zea, Kundu and Audus (1974a;b) investigated the inhibitors present in the root caps of Zea. Paper chromatography of their extracts revealed that there was an inhibitor in the root cap, but it was not identifiable as IAA; a Commelina stomatal closure, bioassay, however, revealed that this inhibitor had ABA-like properties. H. Wilkins et al. (1974) were also unable to find evidence of IAA in maize roots using TLC. However, Rivier and Pilet (1974) were able to detect IAA in Zea root caps using mass spectrometry, which is a more precise technique than that used by either Kundu and Audus (1974), or H. Wilkins et al. (1974).

In a number of plant species the gravireaction does not come about merely because the root is exposed to the stimulus of gravity. In these species there is a requirement that the roots be illuminated, as well as gravistimulated. In 1961, Lake and Slack had noticed that light exposure influenced the concentration, and direction of growth, of roots, with the roots of seedlings grown in transparent pots being concentrated away from the periphery of the block of soil, along with a greater number of nearly vertical roots. The turning away from the

surface of the soil, which Lake and Slack also noted, could have been due to either a negative phototropic response, or a positive gravitropic response. In order to test which tropic response was in fact occurring, they grew a variety of seedlings (Callistephus chinensis, Matthiola incana, Calendula officinalis, Lycopersicon esculentum and Cucumis sativus) in opaque pots with transparent bottoms and illuminated them from below. Since the roots still grew downwards Lake and Slack concluded that it was a positive gravitropic response. In unilluminated, opaque pots, the direction of root growth was not predominately vertical, as it was in the transparent pots, and it, therefore, appears that light is a prerequisite for gravitropism.

There is a great deal of evidence in the literature showing that light is inhibitory in its action on root growth in Zea, Lens, Triticum, Pisum, and Oryza seedlings (Torrey, 1952; Pilet and Went, 1956; Burström, 1960; Masuda, 1962; Ohno and Fujiwara, 1967; H. Wilkins et al., 1973). Furthermore, H. Wilkins et al. (1974a) have demonstrated that the root cap is the site of perception of the light stimulus. They studied the growth rate of intact and decapped seedlings, in darkness and light, and found that removal of the root cap before illumination resulted in an elongation equal to that of dark-grown, intact, roots. If, however, dark-grown seedlings were decapped, there was no change in the growth rate of the roots. This lack of a change also indicates that the observed change in growth rate is not the result of surgical injury to the root tissues. The root cap could satisfy one of two roles in the light-induced inhibition of root growth; firstly, it could merely perceive the photostimulus, or secondly it could perform a secondary role in which

it enables the root behind the cap to perceive, and respond to, the stimulus. It is quite possible, on the basis of the data cited above (H. Wilkins et al., 1974a) that the decapped roots are still able to perceive the stimulus of light, but are unable to respond. In order to resolve this ambiguity, H. Wilkins and Wain (1974) carried out experiments in which root caps and root stumps were exposed separately to light, or kept in darkness. They then placed light-treated caps on dark roots and vice versa, and discovered that the former combination resulted in a significant inhibition, and resulted in an elongation similar to that of light-grown, control seedlings. These results, therefore, seem to indicate that it is the root cap alone that is responsible for the perception of light. This evidence has since been supported by the work of Pilet and Ney (1978) who, rather than physically separating and then rejoining the root caps and roots, utilised the availibility of optical microfibres, to give a localised exposure of light to either the cap or the elongation zone of intact roots.

There are conflicting reports in the literature as to how the light-inhibition of root growth is related to the energy of the light. From the results of experiments using Z. mays cv. Kelvedon 33, Pilet (1973a) concluded that with increasing intensity of white light, the inhibition of growth increased to a peak, and then any further increase resulted in a reduction of the inhibition. This statement was, however, contradicted by Suzuki and Fujii (1978) who examined the curvature induced by various light energies, and stated that the light-response was governed by the all-or-none law. That is, that the response was induced by light energies above a certain threshold, but

having attained that threshold, any further increase in light energy had no effect on the degree of curvature observed. Furthermore, Pilet, himself, has produced data which are more consistent with the conclusion of Suzuki and Fujii than his earlier findings (Pilet, 1979).

It appears that light perceived by the root cap induces an inhibition of root growth. H. Wilkins and Wain (1974) have been able to show that there are a number of analagous aspects of the response of Zea roots to white light and gravity: i) the root cap perceives the stimulus of gravity and white light; ii) decapped roots are unable to perceive gravity or white light stimuli but regain this ability several hours after decapping; iii) the root cap is the site of production/release of growth inhibitory factors which are transported basipetally to the growing zone where they produce the response to light and gravity; and iv) the growth inhibitors produced in response to gravity and light are both water-soluble. However, not all plant species, and indeed, not all cultivars of the same species, e.g. Zea, have roots which have a light requirement as a prerequisite for gravitropism. This variation in requirement for a single species, has provided a useful means by which the identity of growth regulators involved in the graviresponse can hopefully, be elucidated, since it is possible to compare the regulators present in dark- and light-grown root caps of both light-requiring, and non-light-requiring cultivars.

Following their discovery that the root caps from light- and dark-grown roots had different effects on root elongation, H. Wilkins and Wain (1974) analysed the extracts from the Zea variety LG11, which is a light requiring cultivar, and found that ABA and two other,

unidentified, inhibiting, compounds were present in the root caps of light-grown, but not dark-grown, seedlings. In further experiments (H. Wilkins and Wain, 1975b) investigated the response of LG11 roots to exogenous application of various concentrations of ABA. The roots were suspended vertically and held with their tips in either ABA solutions, or water, for 2 hours in darkness prior to gravistimulation. ABA solutions from 10^{-8} to 10^{-4} $mol.dm^{-3}$ were found to induce curvatures in the roots whereas no curvature developed in the roots which had had their tips immersed in water. Placing decapped roots in 10^{-4} $mol.dm^{-3}$ ABA, also induced a curvature, but it was only a quarter as large as the curvature induced in intact seedlings. A very small curvature was also observed in water treated, decapped roots, but H. Wilkins and Wain believe that this was probably due to a small amount of the cap tissue remaining after decapping. It was also found that 10^{-4} $mol.dm^{-3}$ ABA inhibited the elongation of intact roots, whereas the lower concentrations had no more effect on elongation than the water control which gave an elongation of approximately 3.5 mm/3.5 h. This concentration of ABA also inhibited the elongation of decapped seedlings, indicating that the ability to take up ABA had not been lost by cutting the apical tissues. From these results it again appears that the cap is necessary for the graviresponse, and in addition, it is noted that ABA satisfies a number of the requirements of the root cap inhibitor involved in the graviresponse.

Before ABA can be accepted as a growth regulating substance involved in the gravitropic response of roots, it must again be established that it satisfies two criteria outlined as basic

requirements for the growth regulator involved in the Cholodny-Went hypothesis. Firstly, it must be shown that there is a downward, lateral, transport of ABA in horizontal roots, and secondly, that there exists an asymmetric distribution of naturally occurring ABA in favour of the lower half of horizontal roots. As yet there are no published data to show that ABA is laterally transported in roots; there is, however, more evidence of an asymmetrical distribution of ABA. Hartung (1976, 1981) carried out experiments to ascertain the distribution of ABA and examined both horizontal roots which had developed a curvature, and those which had not. He found that there was an asymmetry in ABA distribution in the roots which had curved, but not in roots which had failed to respond to the gravitropic stimulus. Although these results appear to support the theory of asymmetric ABA distribution, closer examination of the data reveals that the differences in the ABA levels are only barely significant, and it is questionable whether or not such small differences are sufficient to cause curvature. Suzuki et al. (1979) investigated the possibility of an asymmetric distribution of ABA, in Zea cv. Golden Bantam 70, a cultivar of maize which again has a light requirement for gravitropism. These researchers found ABA was present in considerable amounts prior to the irradiation of the seedlings, a result in direct contrast to that of H. Wilkins and Wain (1974), who found ABA in the root caps only after irradiation. Suzuki et al. (1979) did, however, observe that the amount of ABA increased when the roots were irradiated with red light. When the upper and lower halves of horizontal roots were analysed, there was 1.6 times more ABA in the lower half. Despite the fact that this result appears to indicate a

redistribution of ABA, Suzuki et al. concluded that ABA was not the growth regulator involved in the gravitropic curvature, since they reported that ABA did not inhibit the elongation of the maize variety used, and no difference in growth was noted between the upper and lower halves using a root-growth assay. Furthermore, they detected an unidentified inhibitor which was asymmetrically distributed in favour of the lower halves of irradiated, horizontal, roots, but evenly distributed in roots kept in complete darkness. In addition, the absolute amount of this compound was increased when the roots were exposed to red light. It is, therefore, possible that this unidentified inhibitor has a role in the gravitropic response of roots. Close examination of Suzuki et al.'s results does, however, illustrate that there is a discrepancy between the data obtained using extraction and gas-liquid chromatography techniques, and those from bioassays, and that caution should be exercised when drawing conclusions from results obtained using a number of different analytical techniques since the data may not be compatible.

Gougler and Evans (1979) investigated the effect of ABA on primary root elongation by immersing the roots in nutrient solution in light. When ABA was added to the solution there appeared to be no effect on the root elongation. However, as mentioned previously, conclusions based on the results of experiments using external solutions of growth regulators, have to be treated with caution, since the root does not normally take in major regulatory organic ions, or growth regulators, from the outside environment. Applying ABA in buffer droplets to vertically-orientated, root tips, significantly enhanced curvature in both light and darkness, with the curvature in

light being the greater. The amplitude of the increase in curvature was found to be dependent upon the concentration of ABA, and the duration of the pretreatment (Chanson and Pilet, 1981).

A great deal of the contradictory evidence about the presence and distribution of growth inhibitors could possibly arise due to the variety of techniques used in analysing the root tissues. Another failing of the agar-diffusion techniques, and the techniques involving the distribution of radioactivity in gravireacting organs, is, that the analyses are made after the gravireaction has occurred and, thus, it is not possible to state whether the observed asymmetry is a cause, or a consequence, of the differential growth of the upper and lower halves of the organ. In addition, the fact that a compound is asymmetrically distributed in receiver blocks provides only circumstantial evidence that an asymmetry also exists in the tissue itself. Mertens and Weiler (1983) have recently carried out a study to try and answer the question of whether or not a redistribution of endogenous regulator(s) occurs before the changes in growth become established.

Using intact tissue as much as possible, to avoid complications caused by wounding, Mertens and Weiler (1983) analysed the distribution of IAA, ABA, and the gibberellins, GA_1 and GA_3, in the upper and lower halves of gravireacting maize coleoptiles, sunflower hypocotyls, and primary roots of maize and broad bean. To analyse the endogenous growth regulators they used the sensitive and selective technique of immunoassay. They found that there was no asymmetric distribution of IAA, ABA, or the gibberellins in the root tips of *V. faba*; in *Zea* there was also no asymmetric distribution of

IAA and gibberellins, and only a transient, and barely significant, asymmetry in the distribution of ABA, after 60 minutes. At 30 minutes, which is at the end of the latent period, there was a symmetrical distribution of ABA in the root tip, which indicates that a redistribution of ABA is not the cause of the differential growth, but rather a consequence of the difference in the growth rates. Exogenous, unilateral, application of ABA, to the root tips of vertical Zea roots, failed to inhibit root elongation and induce curvature, thus, supporting Suzuki et al.'s (1979) bioassay results, and Schurzmann and Hild's (1980) findings. However, in Zea coleoptile tips, there was evidence of an asymmetric IAA distribution, with more accumulating in the lower half of horizontal organs during the latent period, and the period of gravitropic curvature. Thus this very precise method is able to provide further evidence in support of the Cholodny-Went hypothesis in coleoptiles, with IAA as the growth regulator initiating the graviresponse. However, this method also provides data which confirm the reports that ABA is not the growth regulator involved in the graviresponse in roots.

Feldman (1981a,b, 1982) analysed the inhibitors in Zea root caps, and found that both acid and neutral inhibitors were formed in root caps exposed to light. The acid inhibitor appeared to be ABA and was only formed in root caps which were still attached to the root, whereas the neutral inhibitor was formed in both the cap and the more basal regions of the root. The neutral inhibitor comprised two discrete substances (Feldman, 1982). When root caps were illuminated there was an increase in the levels of both the acid and the neutral inhibitor. If, however, the root caps were removed from the root and

incubated in light, there was an increase in inhibitory activity in the neutral fraction, but not in the acid fraction (Feldman, 1981b). If these cultured caps were placed on dark-grown decapped roots, a large curvature was obtained, implicating the neutral inhibitor and not ABA in the gravitropic response (Feldman, 1981a). This finding correlates with the suggestion of Suzuki et al. (1979) that it was the asymmetric distribution of an unidentified inhibitor, rather than ABA, that was involved in the gravitropic response of roots. However, Suzuki et al.'s unidentified inhibitor was an acid inhibitor, whereas Feldman's (1982) was a neutral inhibitor.

It is possible that ABA is a precursor for the production of the neutral, as yet unidentified, inhibitor, or that ABA in some way controls the inhibitors synthesis or release (Feldman, 1982). However, such an explanation is not consistent with Feldman's earlier findings, since he found only the unidentified inhibitor, and not ABA, in the cultured caps kept in light (1981b). It may be that light has an effect on the presence of the unidentified inhibitor as well as exerting a control through ABA. It has, as mentioned earlier, been reported by Suzuki et al. (1979) that their unidentified inhibitor is distributed asymmetrically in horizontal maize roots, and it thus satisfies one of the requirements of the inhibitor in the Cholodny-Went hypothesis. However, .the chemical identity of the inhibitors found by Susuki et al. (1979) and Feldman (1981, 1982) is still unknown, and until they are identified unequivocally the findings reported in these two accounts cannot be reliably taken to formulate a single theory concerning the unidentified inhibitor in the gravitropic response of roots.

As discussed previously for shoots, an asymmetric distribution of calcium (Ca^{2+}) ions has been identified in roots, and recently Lee et al. (1983b, 1984) have proposed that calcium plays a role in linking gravity perception and curvature. Gravitropic sensitivity is lost when calcium chelating agents, such as EDTA or EGTA, are applied to the tips of maize roots. Furthermore, asymmetric application of calcium chloride to the tips of decapitated roots causes curvature towards the calcium source. Calcium is found in substantial amounts in the amyloplasts in the root cap (Chandra et al. (1982) and is also required for auxin transport (de la Fuente and Leopold, 1973). Lee et al. (1984) have considered all of these effects of calcium in root and shoot curvature and proposed a model which focuses on gravity-induced calcium movement as the trigger for auxin redistribution, and the subsequent gravicurvature. However, the reverse may also be true, and further experimentation is needed to find out whether this speculative model is the true sequence of events linking graviperception to gravicurvature.

Evidence in favour of the Cholodny-Went theory of gravitropism has come over the past few years from studies which are based on considerations of how the growth rate of organs is promoted, or inhibited, at the cellular level. In order for the growth rate to be changed there must be an alteration in the rate of cell elongation or cell differentiation.

Rayle and Cleland (1970) proposed that hormone-induced, cell wall extension, plays a role in the control of elongation of stems and coleoptiles. This proposal is based on the theory that IAA initiates rapid cell elongation by causing wall loosening (Cleland, 1971) by

acting on some site in the cytoplasm. If the site of auxin-action is in the cell cytoplasm, the need arises for some factor to communicate between the cytoplasm and the cell wall, and this is referred to as the "wall-loosening" factor. Protons (H^+) were proposed as this wall-loosening factor (Rayle and Cleland, 1970; Hager et al. 1971) and the 'acid-growth' theory was formulated. This theory states that auxin initiates acidification of the cell which results in a reduction of pH in the wall solutions; this low pH then activates enzymes which leads to wall loosening and cell enlargement (Rayle and Cleland, 1977).

Evidence that growth promoting concentrations of auxin stimulate H^+ efflux in stems (Rayle, 1973; Evans and Vespers, 1980) and that exogenous acid promotes growth, have led to the hypothesis of auxin-induced acid efflux having a causal role in the enhancement of stem elongation. In roots it appears that there is a greater acid efflux from the more rapidly growing, upper half of the elongation zone, than from the slower growing, lower half, in gravistimulated roots of maize (Mulkey and Evans, 1981) whereas in shoots the reverse is observed (Mulkey et al., 1981). Furthermore, in both roots and shoots this differential acid efflux appears to be established prior to the initiation of gravicurvature (Mulkey and Evans, 1981; Mulkey et al., 1981). Since it has been shown that root growth is promoted by an acid pH, and that the application of auxin at concentrations inhibitory to root growth causes an increase in basicity (Evans et al., 1980) it seems possible that the development of a differential acid efflux may be a requirement for gravicurvature. This differential efflux could arise in response to a redistribution of auxin in the

root, or in direct response to gravity. Mulkey and Evans (1981) studied changes in pH using agar containing bromocresol purple indicator dye, which changes colour in response to a change in pH. Roots of Zea were placed on the agar and the dye changed to red in regions of low pH and yellow in regions of high pH. The high pH regions correspond to the parts of the root where there is an uptake of H^+ by the root, and the low pH regions to those zones where H^+ efflux occurs. Using this technique, Mulkey and Evans (1981, 1982b) followed the effects of a number of auxin transport inhibitors on differential H^+ efflux, and gravitropic curvature; all of the inhibitors used were found to prevent the development of an asymmetric H^+ efflux, and the development of gravicurvature. These results, therefore, indicate that lateral movement of auxin is necessary for the development of asymmetric H^+ efflux during gravicurvature, and are, thus, consistent with the proposal that a differential acid efflux mediates gravitropic curvature in roots. Similar data to those of Mulkey and Evans (1981) have been obtained by Wright and Rayle (1983) who examined the effect of auxin inhibitors on H^+ efflux in shoots. They discovered that when Helianthus hypocotyls and coleoptiles were submerged in a solution of neutral buffers no curvature developed, and this could arise from the fact that the neutral buffers prevent the establishment of a proton gradient (Wright and Rayle, 1982, 1983). Pilet et al. (1983) used Sephadex beads soaked in bromocresol purple indicator dye to study the elongation and pH patterns along the roots of maize. By placing the beads at intervals along the roots and recording their position and colour over

time it was possible to relate the increase in length to pH. It was observed that the greatest amount of growth occurred between 2 and 4mm from the root tip, and this region also showed the maximum decrease in pH.

These results in support of the acid-growth theory also provide evidence in favour of the Cholodny-Went hypothesis, but the hypothesis needs to be extended to incorporate the induction of asymmetric acid efflux as the means by which auxin mediates the differential growth and hence curvature.

Thus, despite almost half a century of research, it has not been possible to elucidate the response mechanism involved in the gravitropic response of roots. From the results of analytical studies such as that carried out by Mertens and Weiler (1983) it seems improbable that IAA is the growth inhibitor which is asymmetrically distributed in horizontal roots, thus giving rise to differential growth. This evidence is difficult to reconcile with the proposed acid-growth theory, and it may be that a regulator which behaves in the same way as IAA is mediating the gravitropic response in roots. Alternatively, inhibitor asymmetry may affect IAA induced H^+ ion efflux. The idea that the growth inhibitor was ABA, which seemed so attractive about a decade ago, is also no longer tenable. The unidentified inhibitors of Suzuki et al. (1979) and Feldman, (1982) seem to be favourable contenders for the role of growth inhibitor in gravitropism, but only further research will show if this is the case, and whether or not, the Cholodny-Went hypothesis is the mechanism that brings about curvature in horizontal roots.

If the Cholodny-Went hypothesis is the mechanism by which

gravicurvature occurs, the asymmetric distribution of growth inhibitor should be reflected in the growth rate changes of the two sides of the organ. Digby and Firn (1979) who have seriously questioned the validity of the Cholodny-Went hypothesis as an explanation of the mechanism of shoot gravitropism, studied the growth rate changes on the upper and lower surfaces of the shoots of a number of plant species, during the initial stages of gravitropic curvature. In all of the species investigated (Zea seedlings, Cucumis sativus and Helianthus annuus hypocotyls) the upper side ceased to grow and the lower side continued to grow normally (C. sativus) or the growth rate accelerated (H. annuus). Digby and Firn (1979) argued that if the upper side ceases to grow, and the lower side does not alter in growth rate, this cannot be accounted for by a downward movement of growth regulating substance. However, as discussed earlier (page 30) if one considers the dose-response curve for IAA concentration and growth rate (Cleland, 1972) the observed growth rate changes could be explained by a redistribution of inhibitor.

It is therefore, apparent that there is disagreement as to the mechanism by which roots and shoots achieve gravitropic curvature. A particular difficulty of research in this area is that of examining the plant organs under conditions compatible with those of normal growth. This problem is especially relevant when examining roots which are normally grown in a soil environment which is damp and with limited illumination and where the root is in physical contact with soil particles. By growing and observing the roots in moist air a suitable humidity for growth can be achieved, but most of the studies reported in the literature review of this thesis, have been carried

out under controlled conditions which have excluded continuous darkness. In these studies safe-lights, usually low intensity green light of approximately 510-580 nm, were used to manipulate the seedlings. (Scott and Wilkins, 1969; H. Wilkins and Wain, 1975b; Beffa and Pilet, 1982; Feldman, 1982, 1983; Pilet et al., 1983; Suzuki et al., 1979). Light must also be used to make continuous photographic records of curvature or length of roots (e.g. Pilet et al., 1983; Ney and Pilet, 1981) or darkness can be maintained and a destructive sampling technique used to record curvature and length (e.g. Scott and Wilkins, 1969; Pilet, 1979). It was, therefore, felt necessary to reinvestigate some of the studies carried out on root growth and curvature and pay particular attention to the fact that complete darkness had never been used in conjunction with continuous recording of growth. A further criticism of these reported studies must also be that a number of them such as those of Shaw and Wilkins (1973) and Pilet (1975b, 1979) have been carried out using apical root segments. Whether such segments behave in the same way as intact roots is questionable; in fact, Beffa and Pilet have shown that the curvature of intact roots is twice that of apical root segments after 6 h gravicurvature. It is possible that nutrients, or some other factor, produced by either the caryopsis or the more basal regions of the roots, are required for maximum bending or growth of the root. It is known that a number of regulators such as ABA, IAA and gibberellins are synthesized in both the seed (Burström, 1969; River and Pilet, 1974; Pilet, 1976; Pilet et al., 1979) and the fully differentiated regions of the root (Reinhold, 1978) and are acropetally transported towards the root-tip. For this reason the studies in this thesis were

carried out on intact seedlings so that the true behaviour of the root could be ascertained.

Recent developments in infra-red, video equipment have been of especial value in making possible the study of the growth responses of roots in the complete absence of visible light. With this video-recording equipment it is possible to make continuous recordings of growth and curvature of an individual root and this removes the necessity for destructive sampling from large numbers of seedlings and basing conclusions on mean growth rates. This method of observing single roots is considered advantageous since Hillman and Wilkins (1982) have recently shown that the use of such mean data does in fact obscure the individual behaviour of roots due to the variability that exists between individuals.

The aim of this thesis is to re-assess gravitropism in roots. Using the advances in video-technology it was hoped to establish in detail the characteristics of the graviresponse under defined environmental conditions and to rationalise the conflicting reports in the literature as to the changes in growth rate and curvature exhibited by roots.

It was hoped that by carrying out the series of investigations reported in this thesis it would be possible to present a more coherent description of the behaviour of an individual root under defined conditions with particular attention being paid to:-

i) the effect of illumination on the growth rate of intact and decapped roots to investigate the possibility of light-induced production of growth regulators (H. Wilkins and Wain, 1974);

ii) the effect of the rootcap on elongation to resolve the

conflicting reports of Cholodny, 1926; Juniper et al., 1966; Schachar, 1967; Pilet, 1971a);

iii) the growth rate changes on the opposite sides of a gravitropically curving root in order to ascertain whether they are compatible with the Cholodny-Went hypothesis for gravicurvature.

CHAPTER TWO

MATERIALS AND METHODS

Plant Material

Seeds (caryopses) of Zea mays L. (cv. Fronica) (Sinclair and McGill, Ayr, U.K.) were soaked for 8h in running tap water in the laboratory and then kept for a further 16h in a beaker of water in a dark cupboard in a darkened growth room, to ensure that no light was admitted. The growth room was maintained at 25 ± 3°C throughout the study. After a total of 24h soaking the seeds were set out, in total darkness, embryo-up on slabs of 0.5% agar in plastic boxes (25 x 9 x 4.5cm). Forty-eight hours after the onset of soaking the primary roots had attained a length of between 10 and 15mm, and were suitable for use.

Equipment

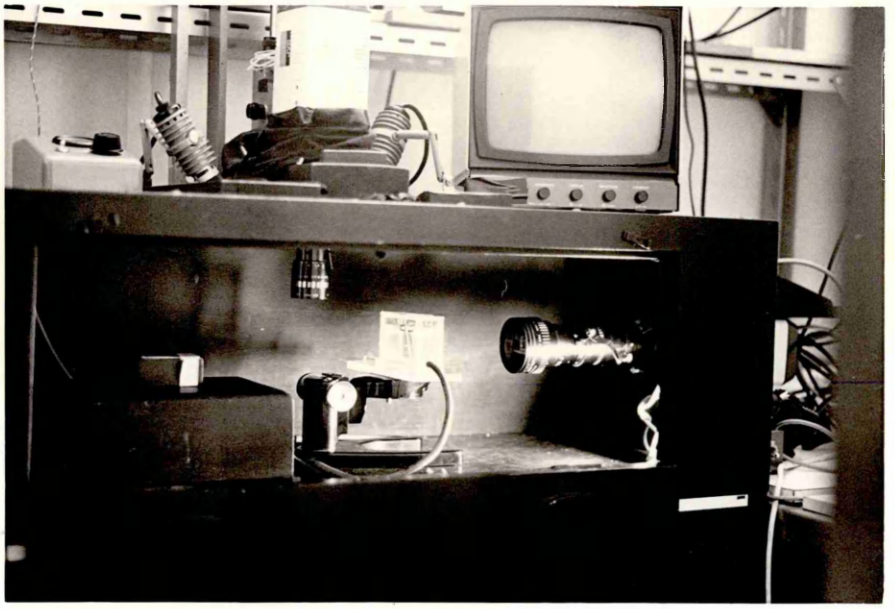
For this investigation an apparatus was designed and built to enable the growth and curvature of plant roots to be measured under defined conditions, particularly darkness, utilising the relatively newly-available infra-red-sensitive television cameras, incorporating Newvicon tubes which are highly sensitive to low fluence rates of radiation in the region 900-1000nm. This waveband is without reported effects on plant growth and development (Iino and Carr, 1981).

The apparatus (Fig. 2.1A and B) consisted of a wooden box 103cm wide, 33cm high and 48cm in depth, the front of which was hinged so that it would open for easy access. This hinged door had two

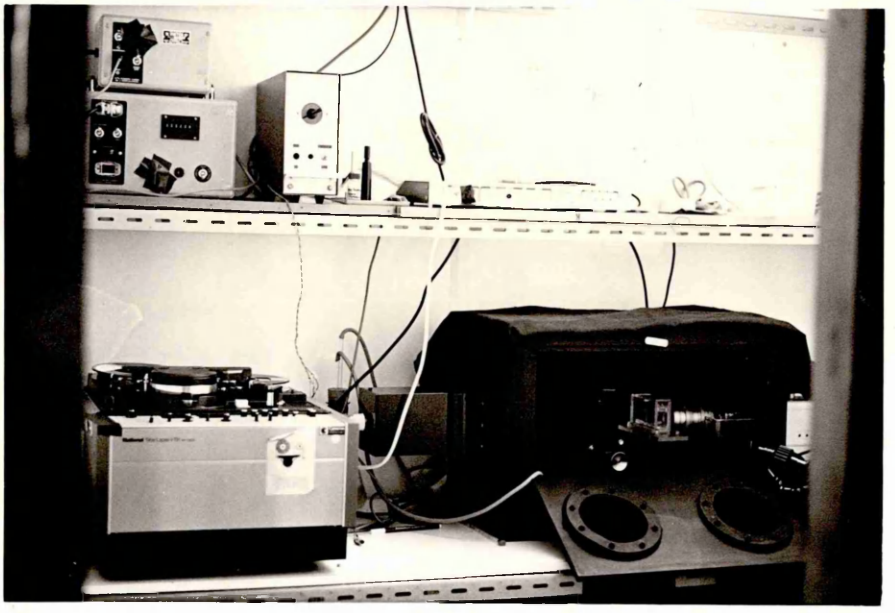
Figure 2.1 (A) Photograph showing the apparatus used for selection and treatment of roots and recording of growth rate and curvature, using infra-red radiation.

(B) Photograph showing the apparatus used for recording growth rate and curvature of roots using infra-red radiation.

A



B



large, circular holes, fitted with sleeves of black, light-tight, material, through which it was possible to insert one's hands and arms into the box and adjust the position of the plant material and the camera lens settings. Two such boxes were used, each housed in a separate controlled environment dark room maintained at 25 ± 3°C and into which access could be gained in total darkness because of a corridor which acted as a light-trap.

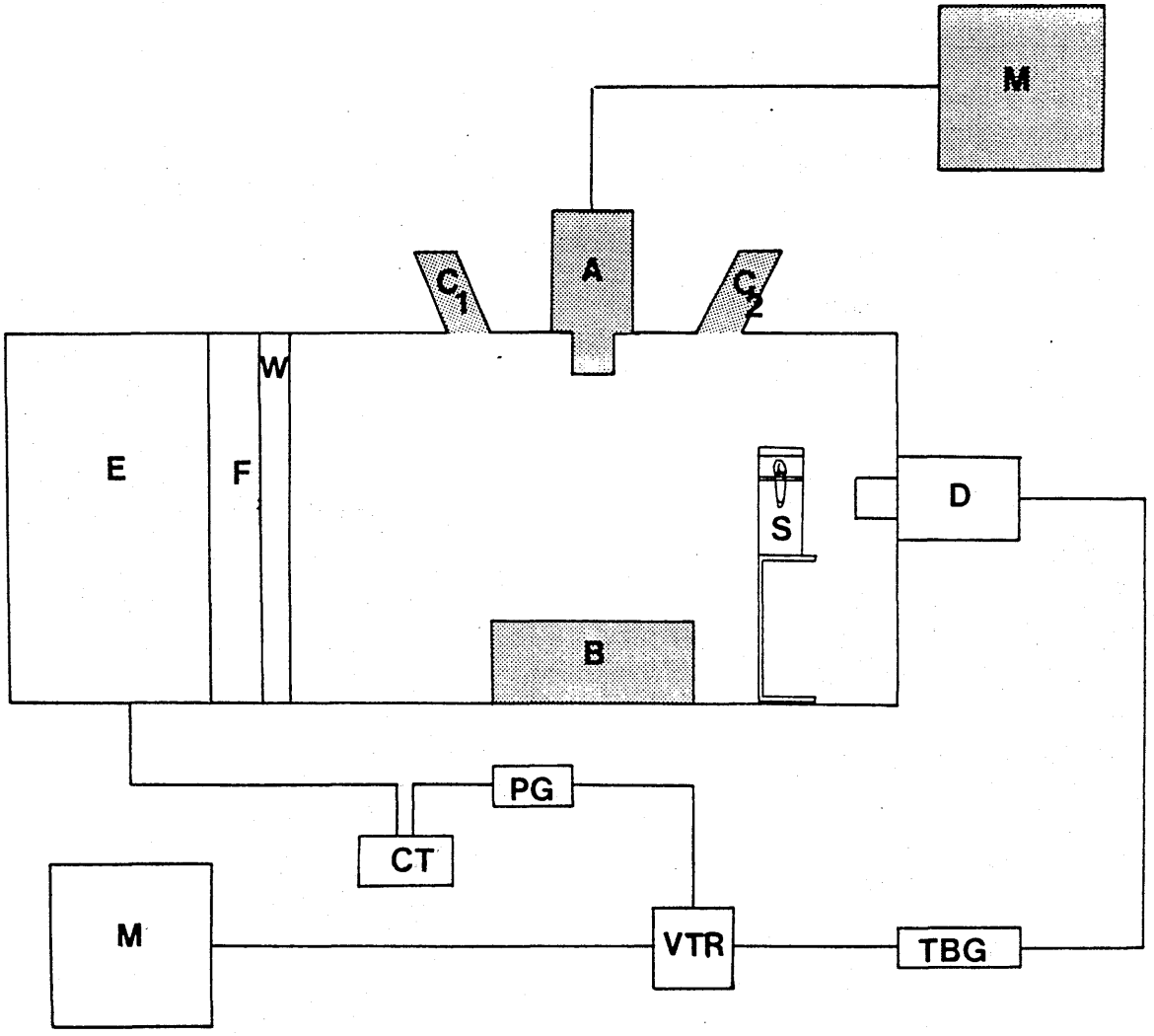
One of the boxes (Fig. 2.1A) was fitted with two separate video-cameras, one for selection and treatment of the seedlings, and the other for recording the growth of their organs. The second box (Fig. 2.1B) was fitted only with a recording camera. Each video-system will be described separately.

Manipulation System

A JVC TK 1700E video camera (A), fitted with a f 1.8, 17-85mm zoom lens (Monital) was mounted vertically above a small wooden platform at the point of focus (B), as shown in figure 2.2. This working platform was irradiated with radiation in the band 800-1000nm by means of two Watson 6 volt microscope lamps mounted outside the box. The radiation was passed through a filter system consisting of 3 layers each of Cinemoid Primary Red, Green and Blue plastic based filters (Rank Strand Electric Comp., London, G.B.). (C_1 and C_2 in Fig. 2.2). The transmission spectra (Fig. 2.3) of the filters was determined using a spectrophotometer (SP800, Unicam)). The output signal from the camera was passed to a high-resolution Electrohome monitor, on which it was possible to observe and manipulate the seedlings. The video-system provided a magnification of between 7 and

Figure 2.2 Diagrammatic representation of the apparatus used for selection, treatment and recording of growth rate and curvature.

A	-	IR video-camera for selection of seedlings.
$C_1 + C_2$	-	sources of IR radiation.
M	-	video monitors.
B	-	wooden platform.
S	-	seedling in its perspex box.
D	-	IR video-camera for recording growth and curvature.
E	-	source of IR radiation.
F	-	Cinemoid filters.
W	-	water screen.
TBG	-	time base generator.
VTR	-	video tape recorder.
PG	-	pulse generator.
CT	-	cam timer.


M
A
C1
C2
W
E
F
S
D
B
PG
CT
M
VTR
TBG

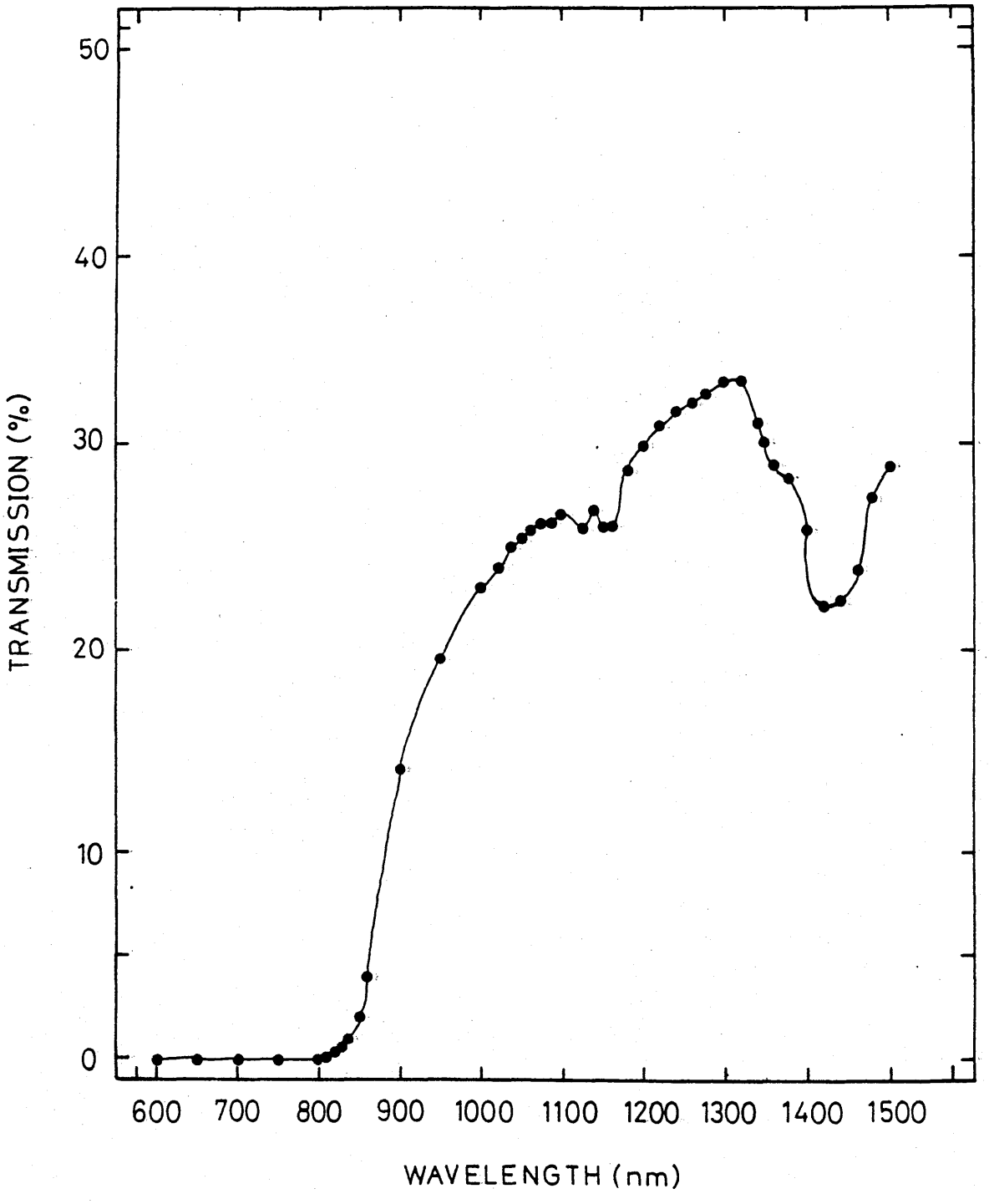


Figure 2.3 Transmission spectra of 3 layers each of primary red, green and blue plastic based filters determined using a SP800 spectrophotometer.

29 times lifesize, which was adequate for all treatments including the removal, if necessary, of one half of a root-cap.

In order to record the growth and curvature of the roots, the seedlings were placed in a plastic box. Figure 2.4A and B, show scale diagrams of the two types of box used in the experiments described in this thesis. The bottom of each box was lined with damp filter paper and during experiments the boxes were aerated with a humidified air supply (Fig. 2.5).

Recording and measurement system

For recording elongation and curvature a second JVC TK 1700 E, video camera (D) was mounted horizontally at the end of the apparatus (Fig. 2.2). This camera was fitted with a f 2.8, 15-150mm zoom lens (P. Angenieux, Paris, France) together with 3 supplementary lenses, to provide adequate magnification. The camera was directed towards the opposite end of the apparatus where an I.R. source was located (E). The camera, therefore, recorded the silhouette of the organ against a background of I.R. radiation. The output signal from the camera was passed first into a compact video display time and date generator (For-A, VTG 88) (TBG) then into a National video recorder with a single shot facility (NV 8030) (V.T.R.) and finally to a large (26 inch) television monitor.

A Wagner 12 volt car headlamp was used as the radiation source. Radiation of wavelengths greater than 1000nm was absorbed by a 4cm thick water-screen (W) and wavelengths below 800nm were absorbed by a Cinemoid filter system similar to that used in the manipulation system (F). A piece of frosted glass located on the outside of the

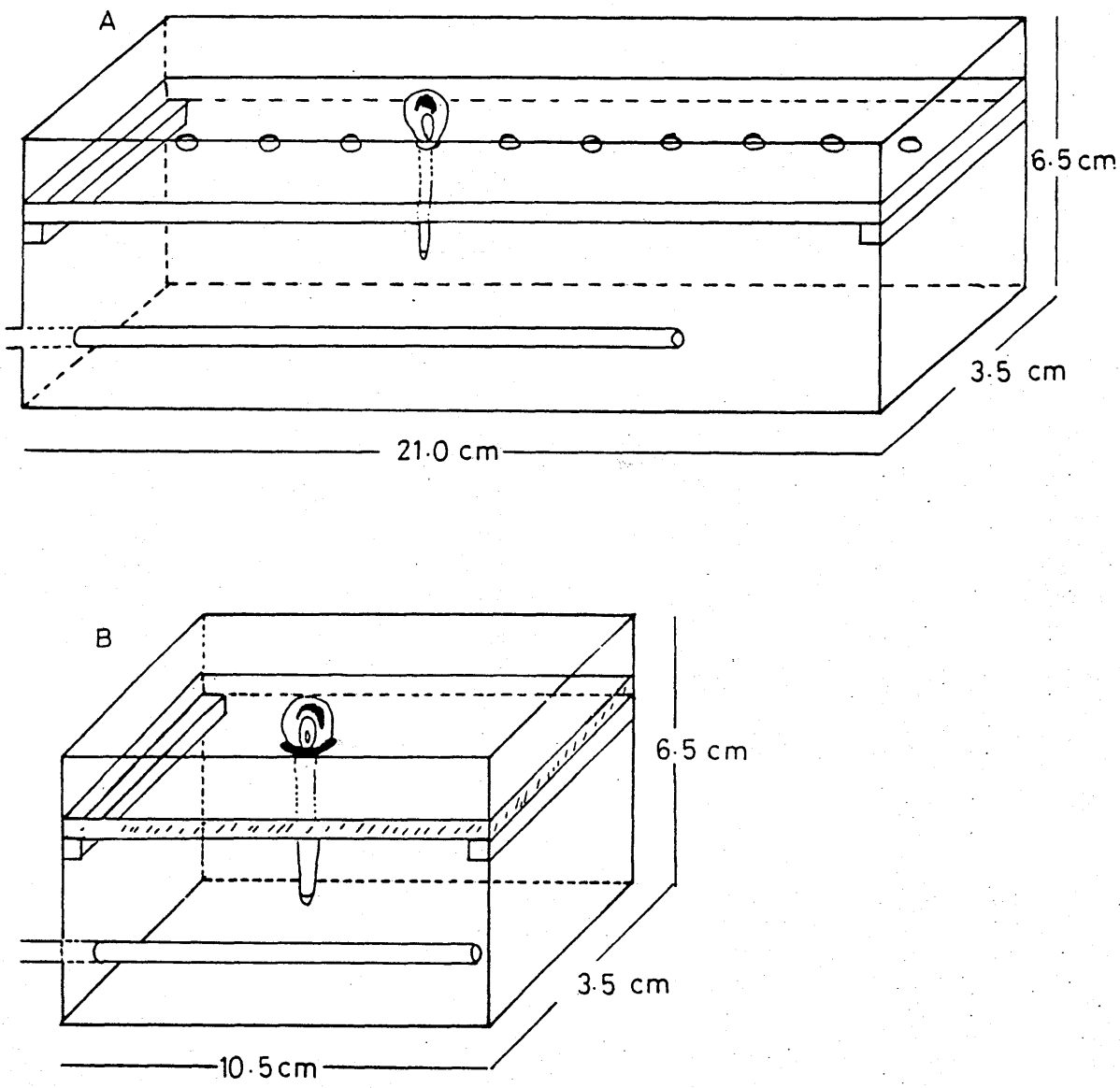


Figure 2.4 Scale diagrams of the perspex boxes in which seedlings were kept during experiments. The diagrams are 0.57 times actual size.

filters diffused the radiation beam.

The interval between pictures was, unless stated otherwise, 15 min, and this interval was timed using a Vinten intervalometer and a pulse generator (PG). Every 15 min the Vinten cam timer switched on, simultaneously, the pulse generator and the IR radiation. The pulse generator stayed on for only 15 s and as it switched off a pulse was sent to the video recorder and a single frame was taken, in addition the same pulse switched off the radiation source. This delay of 15s was used to ensure that the IR source had adequate time to reach full emission before the picture was taken and switching the IR radiation off after 15s minimised the ammount of heat generated inside the apparatus.

Before carrying out the growth rate and curvature studies the magnification and resolution of the system were determined. To check the magnification of the lens, at maximum focal length, a piece of graph paper was placed at the point of focus of the measuring camera. Twenty-three squares, at random locations on the screen, were measured and found to be the same size. When the camera lens was adjusted to its highest magnification (lowest focal length) the lines on graph paper were found to be too inaccurate to use as reference points. Therefore, in order to assess the magnification, a microscope calibration slide (100 x 0.1mm graduations) was used. At ten points over the screen the distance between adjacent 1mm marks on the slide was measured and at all locations the distance was found to be 60mm. Both the magnification and the uniformity of the magnification over the screen surface were found to be constant.

The resolution of the system was determined using the

sharpness of the image on the screen. The monitor screen has 625 horizontal lines and the screen is 370mm in height. Thus the lines are 370/625 = 0.59mm in width. When there was a sharply focused image on the screen, for example the apex of a root, it was possible to determine precisely on which line the image of the tip of the root was located. Thus, it is possible to discriminate the position of the root apex to a zone 0.59mm in depth with confidence at the magnification used. This distance is equivalent to an increase in length of 10μm in depth when the lens focal length setting was such as to give a magnification of 60x.

The radiant fluence rates of all the various radiation sources were measured using a thermopile (KIPP + ZONEN CAI - 65057) and a DC. millivolt potentiometer (404N - Time Electronics Ltd, Kent, G.B.). The thermopile was placed in the apparatus at the point where the seedlings were held for treatment and recording. The intensity measurements were calculated and quoted as Joules per meter^2 per second (J m^{-2} s^{-1}).

The second box was fitted only with a recording camera (JVC TK 1700E) having a 20-80mm zoom lens (P. Angenieux) and an extension tube. In this box a 40 watt tungsten lamp was used as the radiation source. The maximum magnification achieved was 57 times lifesize. The magnification and resolution of the system was tested as described for the system in the larger apparatus, and were found to be similar.

Tests were carried out with Avena coleoptiles to ensure that there was no red or blue light leakage occurring through the filters. Blue light leakage was tested by looking for phototropic curvature and red light by comparing mesocotyl lengths of control and experimental

shoots since red light causes a suppression of mesocotyl elongation. The results of these two tests showed that no leakage was occurring (Tables 2.1 and 2.2).

Measurements

Root lengths. The length of the image of the root was measured directly from the television monitor screen which was covered with a sheet of perspex to provide a flat surface. A ruler fitted with a cursor, with lines scored on it, in such a way that when they were aligned measurements were only made when the observers' eye was normal to the screen, was used for straight-growth measurements, which were made to an accuracy of 10μm (Fig. 2.6A). To measure the length of curved roots a flexible ruler was used. In both cases measurements were divided by 60 to convert them to lifesize.

Root curvatures. Curvatures were determined directly from the monitor screen to an accuracy of 1° using a specially adapted protractor (Fig. 2.6B).

Experimental Procedure

After selection and, in some cases, pretreatment, for example, removal of the root-cap, seedlings were placed inside one of the small perspex boxes (Fig. 2.4) and placed on an adjustable stand at the point of focus of the recording video camera (Fig. 2.5). As the roots grew it was possible to keep the root-tip in view by raising the stand. An initial picture was taken as soon as the box was placed in front of the camera and subsequent pictures were taken at 15 or 30 min intervals as specified in each experiment.

TABLE 2.1

A:	50	35	51	40	38	55	45	42	45	53
	43	38	51	53	35	52	34	49	40	24
	40	47	49	40	36	44	37	39	48	48
	40	41	51	39	48	35	33	37	40	39
B:	61	67	59	55	52	53	65	55	58	53
	56	58	58	37	60	66	62	51	53	61
	47	65	72	49	64	64	54	58	39	69
	52	68	54	53	50	49	47	54	51	52

TABLE 2.1

Mescotyl length (mm) of 50 Avena seedlings after 5 days growth in (A) continuous white light (fluorescent 5.62 $Jm^{-2}s^{-1}$) or (B) infra-red radiation. The mean lengths of (A) and (B) are significantly different at $p = 0.01$ level of probability.

TABLE 2.2 Curvature of 100 *Avena* coleoptiles after 6h in (A) fluorescent light (5.62 $Jm^{-2}s^{-1}$) or (B) infra-red radiation. The mean curvatures are signficantly different at p = 0.01 level of probability.

A:	35	28	30	35	26	30	26	36	20	19
	28	25	31	32	25	23	30	32	27	29
	31	24	19	32	28	30	30	36	21	19
	23	22	17	37	25	26	18	37	31	20
	24	25	32	29	31	31	28	26	19	33
	22	25	30	27	23	24	19	30	31	29
	28	28	35	26	19	20	25	31	28	26
	23	21	29	28	31	22	19	17	23	26
	22	25	28	30	29	35	31	26	30	27
	23	28	29	33	27	21	30	31	35	20

B:	0	2	3	1	0	0	2	1	0	0
	4	0	0	1	0	2	1	0	3	0
	0	0	1	0	0	0	1	0	0	2
	0	0	0	3	2	1	0	0	1	0
	0	1	1	0	1	2	0	0	0	0
	0	0	3	0	1	2	0	0	5	0
	0	0	0	7	0	0	0	1	0	0
	0	2	2	0	1	3	0	1	0	0
	0	1	2	0	0	0	1	0	0	0
	0	0	0	0	1	1	0	2	0	3

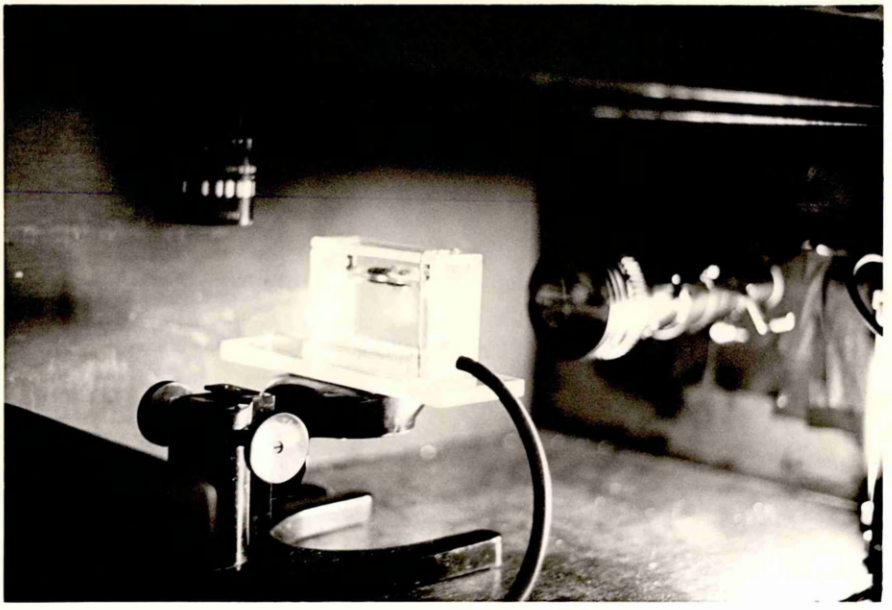
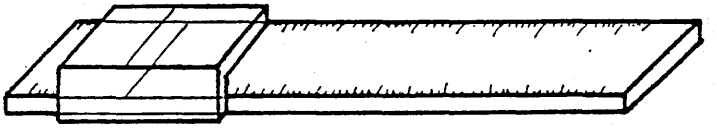
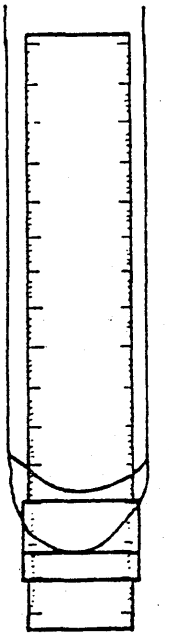


Figure 2.5 A close-up photograph of a seedling in its perspex box, showing the lenses of the recording and the manipulation cameras. The box is positioned on an adjustable stand which allows the root tip to be kept in view at all times.

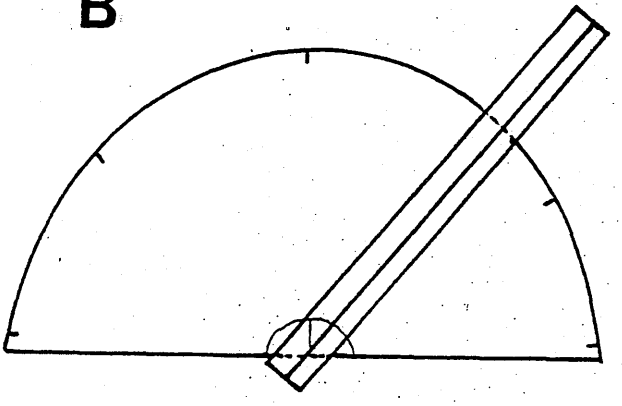
Figure 2.6 Diagrams showing (A) the ruler and cursor used to measure root length and (B) the protractor used to measure the angle of root curvature

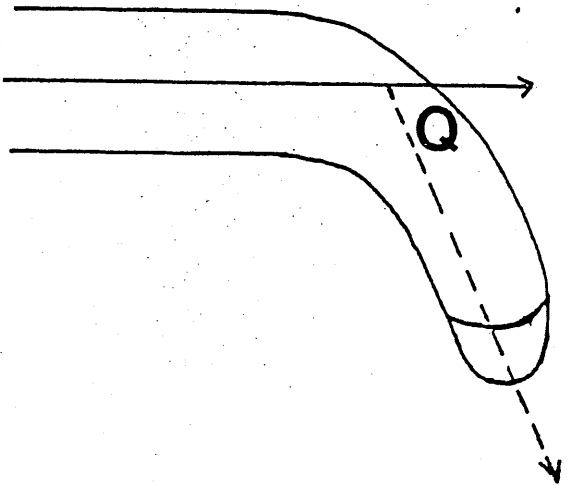
A





B





In a number of experiments the root-cap was removed. This was achieved by cutting away the root cap, under the IR-camera; using a sharp scalpel, a cut was made at the junction between the meristem and the root-cap, leaving the meristem intact with a slight "collar" of root cap tissue around it (Fig. 2.7).

For experiments requiring light the illumination was provided either by a Philips fluorescent microscope lamp, (radiant fluence rate 3.67 J m^{-2} s^{-1}) or two Nikon tungsten filament lamps used with water screens and giving a range of radiant fluence rates from 1.17-9.30 J m^{-2} s^{-1} according to the setting on a rheostat.

Statistics

All mean values quoted are the averages of individual measurements made on a number of separate occasions, as specified in each experiment.

Standard Error of the mean values was calculated using the formula:-

$$SE = \frac{SD}{\sqrt{n}}$$

Standard Deviation $$= \sqrt{\frac{\Sigma(x-\bar{x})^2}{n}}$$

Σx^2 = sum of squares of samples

$\bar{x}$ = mean value of sample $= \frac{\Sigma x}{n}$

n = number of individuals

x = the individual value of each observation.

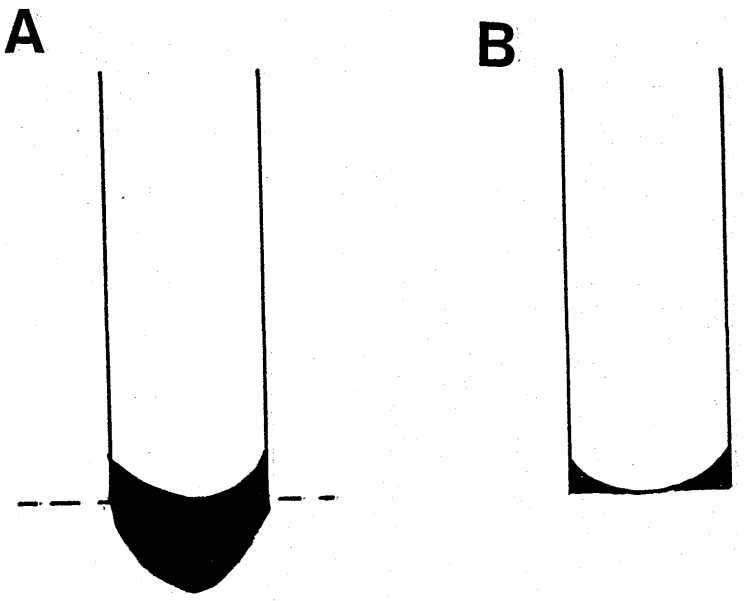


Figure 2.7 Diagram showing

(A) where the incision is made to remove the root cap (dotted line) and

(B) the small collar of root cap tissue which is left on decapped roots.

Student t-test. Used to test the difference between two means:-

$$t = \frac{\text{mean difference}}{\text{SE of the difference}}$$

$$= \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

for $n_1 + n_2 - 2$ degrees of freedom

where:

$\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

$$S^2 = \left(\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1}\right) + \left(\Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}\right) \div (n_1 + n_2 - 2)$$

The level of significance for each t value was obtained from Statistical Tables. 2nd Edition, Murdoch and Barnes, pp.16-17.
t-values were calculated at 95%, 99% and 99.9% level of probability as indicated by *, ** and ***.
NS = not significant at 95% level.

Two-way analysis of variance was used to compare the effects of different factors at the same time.

The calculation was carried out as shown below and significance levels were taken from tables (Murdoch and Barnes, pp.18-19) and significance levels indexed as shown above for t-values.

1. Calculate mean of replicates $ij = \frac{1}{r} Sij$

mean of rows $i = \frac{1}{mr} SRi$

mean of columns $j = \frac{1}{nr} SCj$

Grand mean $= \frac{1}{nmr} \Sigma x$

correction factor for sum of squares $= \frac{1}{nmr} (\Sigma x)^2 = CF$

where r = number of replicates

m = number of columns

n = number of rows

2. i) calculate total sum-of-squares, TSS, $= \Sigma x^2 - CF$

ii) calculate row sum of squares, RSS, $= \frac{1}{mr} \Sigma S_R^2 - CF$

and row mean square RMS, $= \frac{RSS}{n-1}$

iii) calculate column sum of squares CSS, $= \frac{1}{nr} \Sigma S_C^2 - CF$

and column mean square CMS, $= \frac{CSS}{m-1}$

iv) error sum of squares, ESS, = TSS - CSS - RSS or

$\Sigma x^2 - \frac{1}{r} Sij^2$

and error mean square, EMS, $= \frac{ESS}{nm(r-1)}$

v) calculate interaction sum of squares,

ISS, $= \frac{1}{n} \Sigma Sij^2 - RSS - CSS - CF$

and interaction mean square, IMS, $= \frac{ISS}{(n-1)(m-1)}$

Mean sum of squares RMS, CMS, EMS and IMS represent the degrees of freedom for rows, columns, error and interactions respectively.

vi) calculate F for rows, column and interaction by dividing the respective mean square values by the error mean square,

F rows $= \frac{RMS}{EMS}$

F columns $= \frac{CMS}{EMS}$

F interaction $= \frac{IMS}{EMS}$

Definitions and equations used in Radiation Biology.

Radiant Fluence Rate:

Measured with a black body absorber such as a thermopile. This is an intensity measurement - the power per unit area or volume.

Units: Joules m^{-2} sec^{-1} (Jm^{-2} sec^{-1}) or Wm^{-2}

Radiant Fluence - fluence rate x time:

This is a dose measurement - the amount per unit area or volume per unit time.

Units: Jm^{-2}

Light of different wavelengths have a different number of quanta.

The energy per quantum is proportional to frequency and inversely proportional to the wavelength. Thus:-

Quantum energy: $\mathcal{E} = \frac{hv}{\lambda}$

$\mathcal{E}$ - energy per quantum,

h - Planck's constant - 6.626×10^{-34} J sec

v - velocity of light - 2.998×10^{8} m sec^{-1}

λ - wavelength of light - in metres

The quantum fluence rate is the number of quanta per $metre^2$ per sec^{-1} and is calculated by dividing the radiant fluence rate by

the quantum energy.

$$\text{i.e.} \quad \frac{\text{radiant fluence rate}}{\text{quantum energy}} = \text{quanta } m^{-2} \text{ sec}^{-1}$$

As the quantum fluence rate tends to be a rather large and unwieldy number, a quantum of energy being so small a unit, it is more useful to use the molar fluence rate. That is, the fluence rate of the mole of quanta. This is calculated by dividing the quantum fluence rate by the Avagadro number.

$$= \frac{\text{quantum fluence rate}}{6.022 \times 10^{23} \text{ mol}^{-1}}$$

$$= \text{mol } m^{-2} \text{ sec}^{-1}$$

CHAPTER THREE

STRAIGHT GROWTH STUDIES

3.0.0 INTRODUCTION

Two problems that arise in studying the growth rate of plant organs are firstly, the inherent variability in the behaviour of organs, and secondly, the fact that the growth rate is generally rather low. The variability between the organs can be overcome by studying a number of individuals at any one time and using the mean growth rate as the indicator of behaviour. However, it is often forgotten that this mean behaviour may be very different from the growth pattern of the individuals on which it is based. For example, Hillman and Wilkins (1982) have shown that the mean curve for the return of gravitropic responsiveness in decapped roots of Zea mays masks the behaviour of the individual roots. When using the equipment described in Chapter 2 (which permitted the non-destructive study of growth) to observe a number of roots at a time, a rather low magnification had to be employed and this limitation meant that the accuracy with which the increase in length could be detected was reduced. Obviously, if the greatest degree of accuracy is required to measure the growth of a particular organ, the highest possible magnification must be used. With the monitoring equipment described in this thesis, utilisation of a high magnification meant that only one individual organ could be observed at a time. Despite this limitation, as to the number of organs observed at one time, a high magnification was used to study the growth rate of single roots. By

employing this technique it was hoped to obtain precise information which would provide a clear indication of the behaviour of roots growing under defined environmental conditions.

3.1.0 METHODS

3.1.1 Growth rate of single roots at high magnification (x60 lifesize).

Single roots were selected and placed in a perspex box in front of the recording camera. The growth of the roots was recorded for various lengths of time, up to a maximum of 16h, as specified in each particular experiment with video pictures taken every 15 min, unless stated otherwise. The growth of the roots was studied under the various conditions listed below; in each case only one root was studied at a time and a number of replicates carried out for each experiment. The SE of the mean was calculated for each sample and significant differences assessed by 2-way analysis of variance.

The growth was recorded for roots treated in the following ways:-

a) Dark-to-light transition. Roots were kept in darkness for the first 4h of the experiment and then exposed to white light for a further 8 to 12h;

b) Dark to light to dark transition treatment. Individual roots were kept in darkness for 4h before being exposed to white light for a further 4h. After the light treatment the roots were once again returned to darkness where they were kept for the subsequent 8h;

c) Dark to light transition: decapped roots. Roots were decapped in darkness before placing them in the perspex box and then treating them as described in a);

d) Decapping in darkness. Individual roots were kept in darkness throughout the 9h recorded time period but the rootcap being removed after 3h growth;

e) Decapping in light. The roots were given a similar treatment to that described in d) but this time they were continuously illuminated and the observation period was limited to 8h;

f) Short light exposure at 3h. Twenty roots were, on separate occasions, kept in darkness for up to 12h with a 10 min light period at 3h;

g) Short light exposure and decapping at 3h. The procedure was essentially the same as in f) except for the rootcap having been removed immediately after the 10 min light period;

h) Surgical trauma. Individual roots were kept in darkness and after 3h incisions were made in the rootcap in two planes parallel to the long axis;

i) Dark to red light transition. Roots were kept in darkness for 4h and then exposed to red light for a further 5h;

j) Dark to blue light transition. Twelve roots were, on different occasions, kept in darkness for 4h and then exposed to blue light for a further 9h.

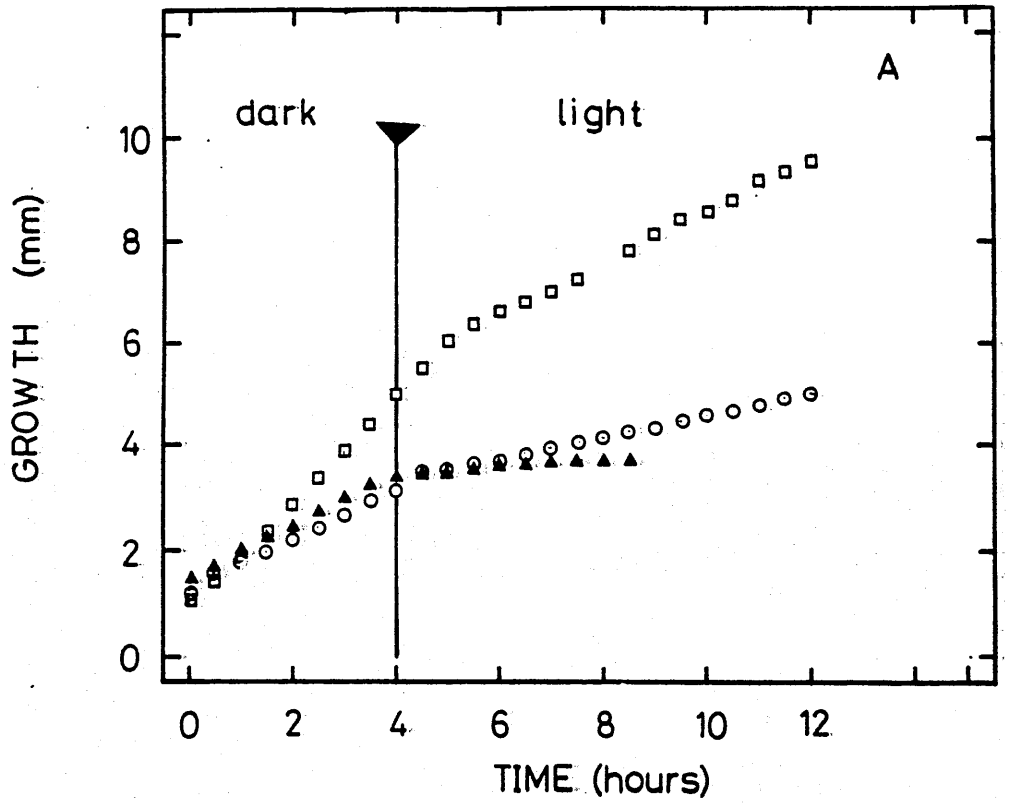
3.2.0 RESULTS

3.2.1 Dark to light transition

Data for the increase in length of roots kept in darkness for 4h prior to illumination are presented in Table 3.1 and 3 representative curves are shown in Figure 3.1A. The length of most of the roots increased steadily both in darkness and light, but within 2h

TABLE 3.1 Length of intact *Z. mays* roots kept in darkness for 4h prior to illumination with white light (3.67 $Jm^{-2}s^{-1}$).

Time (Hrs)	Sample No. 1	3	4	5	7	12/4	13/4	11	17/4	18/4	19/4	20/4	26/4	2/5	3/5
0	1.30	1.37	1.52	1.38		1.33	1.47	1.33	1.42	1.32	1.37	1.50	1.33	1.13	1.42
	1.67	1.72	1.67	1.53	1.33	1.43	1.60	1.48	1.63	1.50	1.62	1.67	1.43	1.37	1.50
	1.93	1.95	1.82	1.67	1.35	1.52	1.70	1.65	1.92	1.65	1.77	2.12	1.52	1.53	1.62
	2.12	2.23	2.00	1.92	1.48	1.67	1.78	1.83	2.08	1.82	1.92	2.55	1.62	1.73	1.82
1	2.23	2.40	2.08	2.10	1.58	1.75	1.95	2.08	2.27	2.05	2.08	2.75	1.75	1.97	2.00
	2.35	2.55	2.20	2.30	1.67	1.87	2.08	2.22	2.62	2.28	2.28	2.87	1.85	2.18	2.17
	2.45	2.67	2.28	2.53	1.73	1.95	2.23	2.42	2.92	2.47	2.48	2.95	2.00	2.37	2.35
	2.63	2.77	2.40	2.78	1.82	2.03	2.40	2.60	3.28	2.65	2.68	3.07	2.20	2.63	2.53
2	2.73	2.90	2.53	3.05	1.93	2.20	2.53	2.87	3.63	2.82	2.93	3.17	2.37	2.90	2.67
	2.87	3.02	2.68	3.35	2.02	2.30	2.68	3.10	4.00	2.95	3.18	3.28	2.52	3.17	2.78
	2.97	3.10	2.83	3.58	2.17	2.45	2.78	3.38	4.33	3.08	3.38	3.42	2.70	3.42	2.90
	3.07	3.23	2.97	3.83	2.35	2.58	2.92	3.65	4.68	3.23	3.58	3.53	2.90	3.68	3.07
3	3.13	3.35	3.17	4.08	2.48	2.70	3.03	3.88	5.00	3.33	3.75	3.67	3.03	3.95	3.23
	3.23	3.43	3.32	4.32	2.63	2.85	3.17	4.15	5.33	3.42	3.95	3.77	3.22	4.22	3.38
	3.32	3.58	3.47	4.57	2.75	2.95	3.25	4.37	5.85	3.52	4.12	3.87	3.40	4.47	3.57
	3.40	3.65	3.62	4.80	2.88	3.07	3.33	4.63	6.23	3.70	4.28	3.95	3.58	4.72	3.73
4	3.47	3.75	3.78	5.05	3.07	3.18	3.38	4.87	6.65	3.77	4.45	4.03	3.77	5.00	3.88
	3.55	3.87	3.95	5.30	3.18	3.35	3.45	5.07	6.87	3.85	4.63	4.07	3.93	5.32	4.00
	3.63	3.92	4.07	5.52	3.30	3.45	3.53	5.27	7.25	3.92	4.75	4.13	4.10	5.58	4.12
	3.65	3.98	4.20	5.68	3.37	3.53	3.55	5.47	7.65	3.95	4.87	4.20	4.23	5.85	4.20
5	3.67	4.02	4.27	5.90	3.40	3.62	3.55	5.62	7.98	4.00	4.93	4.22	4.35	6.08	4.23
	3.70	4.07	4.33	6.12	3.43	3.62	3.57	5.72	8.27	4.00	4.98	4.23	4.43	6.25	4.25
	3.73	4.08	4.37	6.25	3.45	3.68	3.58	5.85	8.57	4.02	5.02	4.25	4.50	6.37	4.30
	3.75	4.10	4.43	6.37	3.48	3.72	3.58	5.97	8.90	4.02	5.05	4.28	4.55	6.48	4.32
6	3.75	4.12	4.52	6.50	3.50	3.77	3.60	6.10	9.28	4.03	5.10	4.32	4.62	6.62	4.33
	3.75	4.15	4.53	6.60	3.55	3.82	3.62	6.27	9.65	4.05	5.15	4.33	4.67	6.72	4.35
	3.78	4.18	4.60	6.77	3.57	3.87	3.62	6.43	10.00	4.10	5.27	4.38	4.78	6.80	4.38
	3.85	4.25	4.67	6.97	3.60	3.90	3.63	6.60	10.38	4.17	5.37	4.45	4.83	6.92	4.43
7	3.90	4.28	4.75	7.13	3.63	3.95	3.65	6.80	10.67	4.23	5.45	4.48	4.90	7.03	4.48
	3.95	4.30	4.82	7.32	3.70	3.98	3.67	7.00	11.02	4.27	5.52	4.57	5.00	7.15	4.52
	3.97	4.35	4.85	7.55	3.75	4.05	3.68	7.20	11.38	4.35	5.60	4.60	5.07	7.25	4.53
	4.02	4.38	4.90	7.78	3.80	4.08	3.68	7.40	11.77	4.43	5.68	4.63	5.17	7.42	4.60
8	4.05	4.43	4.97	8.00	3.88	4.13	3.70	7.67		4.43	5.78	4.67	5.25		4.65
	4.12	4.48	5.05	8.18	3.93	4.20	3.70	7.88		4.48	5.87	4.73	7.68	4.68	
	4.15	4.52	5.13	8.43	3.98	4.25	3.72	8.07		4.52	5.97	4.77	5.43	7.80	4.73
	4.22	4.55	5.23	8.65	4.07	4.33	3.73	8.25		4.58	6.05	4.80	5.58	7.98	4.80
9	4.27	4.60	5.32	8.92	4.13	4.37		8.60		4.62	6.10	4.83	5.75	8.15	4.85
	4.30	4.68	5.45	9.12	4.20	4.42				4.67	6.15	4.87	5.87	8.23	4.88
	4.33	4.72	5.57	9.35	4.25	4.48				4.72	6.22	4.90	5.95	8.40	4.93
	4.40	4.77	5.67	9.62	4.33	4.55				4.76	6.23	4.93	6.15	8.48	4.97
10	4.45	4.83	5.80	9.70	4.40	4.60				4.83	6.28	4.95	6.32	8.65	5.02
	4.55	4.87	5.88	9.93	4.47	4.65				4.87	6.33	4.97	6.47	8.73	5.05
	4.60	4.92	5.98		4.52	4.68				4.92	6.38	5.02	6.62	8.85	5.08
	4.63	4.95	6.13		4.60	4.75				4.95	6.45	5.03	6.78	9.02	5.13
11		5.02	6.25		4.65	4.78				5.00	6.48	5.07	6.93	9.18	5.20
		5.05	6.35		4.68	4.87				5.02	6.55	5.10	7.08	9.27	5.28
		5.12	6.50		4.78	4.92				5.05	6.60	5.12	7.27	9.37	5.35
		1.15	6.65		4.82	4.97				5.08		5.15		9.47	5.38
12		5.20	6.73		4.87	5.02						5.17		9.57	5.43
					4.95										



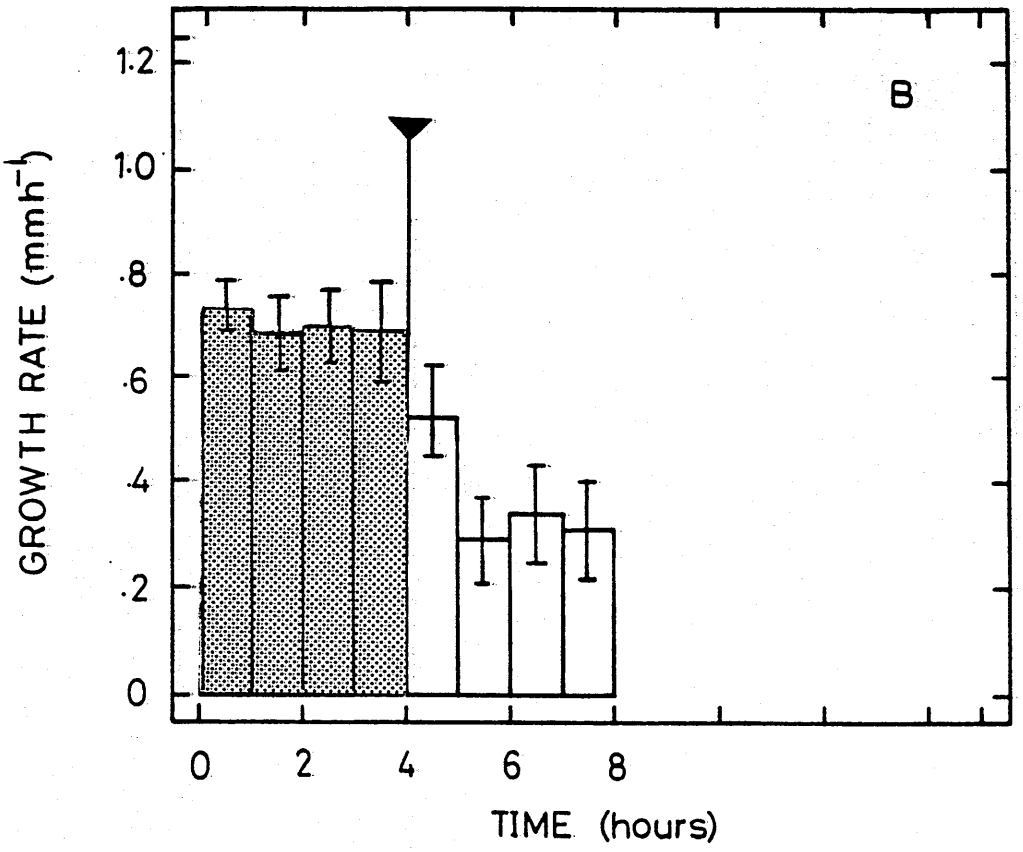


Figure 3.1 Increase in length (A) and mean growth rate (B) of intact Z. mays roots kept in darkness for 4 h prior to illumination with white light (3.67 $Jm^{-2}s^{-1}$).

of the onset of illumination the rate of increase had been reduced by approximately 50% to a new steady rate. It was noted that each root had a characteristic growth rate both before and after the light exposure.

A total of 15 roots were exposed to this dark to light change: the mean growth rate was therefore calculated and plotted against time (Fig. 3.1B and Table 3.2). The average growth rates in darkness and light were 0.7 ± 0.01 and 0.35 ± 0.03mm h^{-1} respectively. These 2 rates are clearly and significantly different (App.1, Table 1). There is a transition phase of one hours duration after the onset of illumination. The growth rate during this hour is 0.52mm h^{-1} which is significantly different to that in light, but not to that in darkness.

Thus, this transition experiment indicates that light causes a change in the growth rate of Zea roots, and this change takes the form of a reduction in growth. Having established that light inhibited the growth rate of the roots, the question arose of whether or not the growth rate would return to its original value if darkness was restored.

3.2.2 Dark to light to dark transition

The effects on the growth rate of subjecting a root to alternative periods of light and darkness are shown graphically in Figure 3.2A. The rate of increase in length changed when the roots were illuminated and again when they were returned to darkness, giving 3 definite phases to the curves. In all 3 phases the increase in length was, for the most part, constant with time. Illumination

TABLE 3.2 Growth rate of intact *Z. mays* roots kept in darkness for 4h prior to illumination with white light (3.67 $Jm^{-2}s^{-1}$).

Time (Hrs)	Sample No. 1	3	4	5	7	12/4	13/4	11	17/4	18/4	19/4	20/4	26/4	2/5	3/5	n	$\bar{x}$	SD	SE	Overall mean gr.
0-1	0.93	1.03	0.56	0.72	-	0.42	0.48	0.75	0.85	0.73	0.71	1.25	0.42	0.85	0.58	14	0.73	0.229	0.06	
1-2	0.50	0.50	0.45	0.95	0.35	0.45	0.58	0.79	1.36	0.77	0.85	0.42	0.62	0.93	0.67	15	0.68	0.259	0.07	
2-3	0.40	0.45	0.64	1.03	0.55	0.50	0.50	1.01	1.37	0.51	0.82	0.50	0.66	1.05	0.56	15	0.70	0.276	0.07	0.70 ± 0.01
3-4	0.34	0.40	0.61	0.97	0.59	0.48	0.35	0.99	1.65	0.44	0.70	0.36	0.74	1.05	0.65	15	0.69	0.344	0.09	
4-5	0.20	0.27	0.49	0.85	0.33	0.44	0.17	0.75	1.33	0.23	0.48	0.19	0.58	1.08	0.35	15	0.29	0.336	0.05	
5-6	0.08	0.10	0.25	0.60	0.10	0.15	0.05	0.48	1.30	0.05	0.17	0.10	0.27	0.54	0.10	15	0.29	0.322	0.08	
6-7	0.15	0.16	0.23	0.63	0.13	0.18	0.05	0.70	1.39	0.20	0.35	0.16	0.28	0.41	0.15	15	0.34	0.331	0.09	0.35 ± 0.03
7-8	0.15	0.15	0.22	0.87	0.25	0.18	0.05	0.87	-	0.20	0.33	0.19	0.35	-	0.17	13	0.31	0.252	0.09	

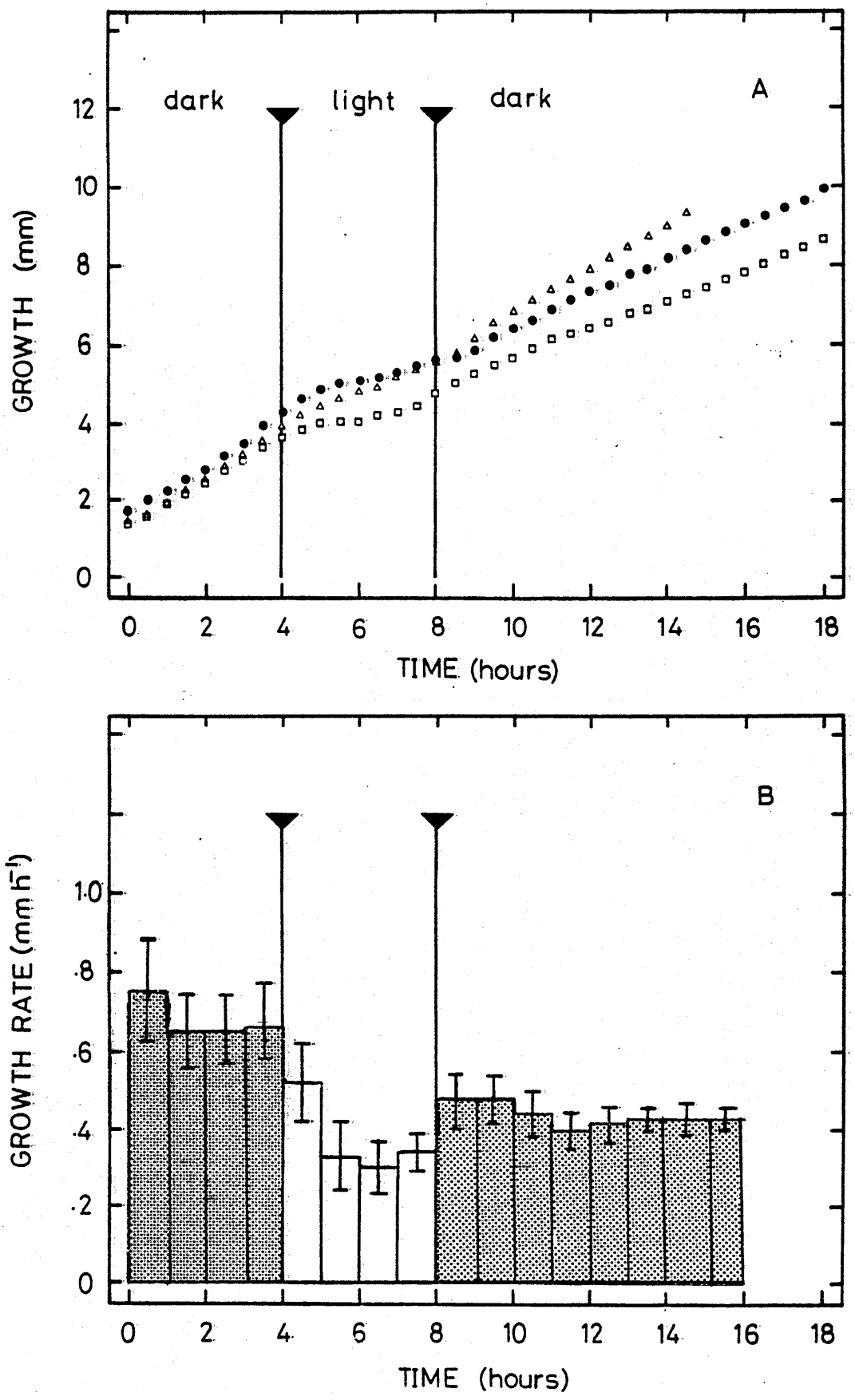


Figure 3.2 Increase in length (A) and mean growth rate (B) of Z. mays roots exposed to 4 h darkness, 4 h light and then 8 h darkness.

reduced the rate at which the roots increased in length, but the rate was increased again on returning the roots to darkness.

The mean growth rate of 9 roots was plotted against time and is shown in Figure 3.2B. The initial, mean, growth rate in darkness was 0.68 ± 0.02mm h^{-1}. On illumination the growth rate decreased over a period of one hour to 0.37mm h^{-1}, after which it remained between 0.30 and 0.34mm h^{-1}. On returning the roots to darkness the rate of growth increased within one hour to 0.48mm h^{-1} and then did not vary significantly over the next 8h. Statistical analysis revealed that the initial rate in darkness was significantly different from both the rate in light and the second dark period, but in light was not significantly different to the rate in the second dark period (App.1, Table 2).

Thus, the growth rate of roots does not increase when they are returned to darkness and therefore does not regain its original value, at least within the 8h after the roots were illuminated.

The above observations indicate that light inhibits the growth of *Zea* roots, a finding consistent with studies in the literature, for a number of plant species (Torrey, 1952; Pilet and Went, 1956; Burström, 1960; Masuda, 1962; H. Wilkins *et al.*, 1973; Pilet and Ney, 1978). A number of these publications have indicated that the light inhibition of root growth is dependent upon the presence of the root cap (H. Wilkins and Wain, 1974, 1975). The facility of being able to remove the root cap in complete darkness has enabled the validity of these conclusions to be investigated more fully.

TABLE 3.3 Length of intact *Z. mays* roots exposed to 4h darkness, 4h light and then 8h darkness.

Time (Hrs)	Sample No. 144	145	146	147	150'	150	151	152	154
0	-	1.75	2.28	-	1.42	1.40	1.82	2.00	2.05
	2.00	1.92	2.65	-	1.55	1.58	2.03	2.22	2.27
	2.03	2.03	2.92	-	1.63	1.65	2.33	2.37	2.43
	2.15	2.15	3.38	-	1.75	1.75	2.58	2.55	2.62
1	2.23	2.25	3.65	1.57	1.92	1.88	2.87	2.73	2.70
	2.33	2.33	3.87	1.58	2.05	2.03	3.17	2.92	2.82
	2.45	2.50	4.03	1.62	2.17	2.27	3.42	3.10	3.00
	2.55	2.67	4.28	1.63	2.37	2.40	3.63	3.28	3.32
2	2.67	2.87	4.57	1.67	2.48	2.55	3.82	3.43	3.62
	2.78	3.00	4.80	1.70	2.63	2.72	4.08	3.62	-
	2.87	3.20	5.00	1.77	2.78	2.93	4.33	3.78	4.03
	2.95	3.35	5.20	1.83	2.92	3.08	4.62	3.92	-
3	3.03	3.53	5.55	1.90	3.08	3.23	4.83	4.07	-
	3.07	3.77	5.82	1.97	3.22	3.42	5.12	4.20	-
	3.13	3.97	6.08	2.05	3.40	3.55	5.38	4.32	-
	3.22	4.13	6.35	2.12	3.50	3.75	5.63	4.40	5.62
4	3.25	4.33	6.62	2.25	3.67	3.90	5.97	4.52	-
	3.30	4.50	6.78	2.33	3.75	4.05	6.25	4.80	5.75
	3.33	4.67	7.00	2.40	3.88	4.22	6.58	4.92	5.97
	3.83	4.83	7.12	2.47	3.97	4.32	6.87	4.98	6.12
5	3.40	4.92	7.22	2.50	4.02	4.43	7.12	5.05	6.33
	3.42	5.02	7.40	2.53	4.07	4.52	7.33	5.13	6.63
	3.43	5.08	7.55	2.53	4.08	4.63	7.53	5.15	6.75
	3.45	5.12	7.63	2.55	4.08	4.68	7.67	5.17	6.92
6	3.45	5.17	7.72	2.62	4.08	4.80	7.83	5.20	7.10
	3.47	5.23	7.85	2.65	4.12	4.87	8.02	5.23	7.22
	3.48	5.25	7.95	2.73	4.20	4.95	8.18	5.28	7.27
	3.48	5.32	8.03	2.77	4.25	5.03	8.40	5.33	7.30
7	3.50	5.35	8.17	2.82	4.33	5.20	8.60	5.38	7.35
	3.52	5.42	8.28	2.87	4.40	5.27	8.72	5.45	7.43
	3.55	5.50	8.40	2.90	4.48	5.38	8.85	5.50	7.53
	3.57	5.57	8.48	2.97	4.62	5.50	8.97	5.55	7.68
8	3.57	5.67	8.08	3.03	4.80	5.58	9.10	5.58	7.78
	3.63	5.68	8.78	3.22	4.90	5.72	9.18	5.65	7.97
	3.67	5.73	8.95	3.32	5.03	-	9.33	5.77	8.12
	3.70	5.80	9.15	3.42	5.18	5.98	9.43	5.87	8.28

	144	145	146	147	150'	150	151	152	154
9	3.70	5.93	9.32	3.53	5.30	6.20	9.62	6.02	8.45
	3.73	6.05	9.52	3.62	5.45	6.35	9.73	6.12	8.63
	3.78	6.22	9.65	3.77	5.50	6.55	9.85	6.23	8.80
	3.78	6.33	9.78	3.83	5.58	6.68	9.97	6.35	9.00
10	3.80	6.43	9.92	3.93	5.70	6.87	10.18	6.48	9.15
	3.82	6.53	10.07	4.05	5.82	6.95	10.18	6.62	9.28
	3.82	6.70	10.20	4.15	5.92	7.12	10.30	6.70	9.43
	3.82	6.82	10.35	4.23	6.02	7.23	10.38	6.85	9.58
11	3.85	6.93	10.50	4.33	6.12	7.40	10.47	6.47	9.75
	3.85	-	10.60	4.42	6.20	7.53	10.55	7.07	9.87
	3.87	7.15	10.77	4.48	6.30	7.05	10.68	7.17	10.03
	3.87	7.25	10.93	4.57	6.33	7.78	10.73	7.27	10.15
12	3.87	7.35	11.07	4.67	6.45	7.92	10.93	7.38	10.32
	-	7.48	11.10	4.73	6.52	8.07	11.07	7.43	10.40
	-	7.58	11.10	4.82	6.62	8.20	11.13	7.55	10.57
	-	7.67	11.13	4.90	6.72	8.42	11.22	7.73	10.72
13	-	7.80	11.30	4.98	6.83	8.45	11.32	7.88	10.87
	-	7.88	11.45	5.07	6.88	8.55	11.43	7.95	10.98
	-	7.98	11.53	5.15	6.95	8.75	11.57	8.03	11.15
	-	8.10	11.67	5.20	7.07	8.90	11.63	8.13	11.27
14	-	8.22	11.82	5.32	7.12	8.98	11.77	8.25	11.40
	-	8.33	11.98	5.38	7.22	9.12	11.82	8.25	11.40
	-	8.42	12.22	5.45	7.32	9.32	11.97	8.50	11.67
	-	8.55	-	5.33	7.40	-	12.07	8.68	11.78
15	-	8.65	-	5.65	7.47	-	12.18	8.80	11.95
	-	8.73	-	5.73	7.57	-	12.27	8.90	12.08
	-	8.85	-	5.82	7.67	-	12.40	9.02	12.25
	-	8.98	-	5.90	7.78	-	-	9.12	12.37

TABLE 3.4 Growth rate of intact *Z. mays* roots exposed to 4h darkness, 4h light and then 8h darkness.

Time (Hrs)	Sample No. 144	145	146	147	150'	150	151	152	154	n	$\bar{x}$	SD	SE
0-1	-	0.50	1.37	-	0.50	0.48	1.05	0.73	0.65	7	0.75	0.312	0.12
1-2	0.44	0.62	0.92	0.10	0.56	0.67	0.95	0.70	0.92	9	0.65	0.257	0.09
2-3	0.36	0.66	0.98	0.23	0.60	0.68	1.01	0.64	-	8	0.65	0.251	0.09
3-4	0.22	0.80	1.07	0.35	0.59	0.67	1.14	0.45	-	8	0.66	0.308	0.11
4-5	0.15	0.59	0.60	0.25	0.35	0.93	1.15	0.53	-	8	0.52	0.284	0.10
5-6	0.05	0.25	0.50	0.12	0.06	0.37	0.71	0.15	0.77	9	0.33	0.259	0.09
6-7	0.05	0.18	0.45	0.20	0.35	0.40	0.77	0.18	0.25	9	0.30	0.200	0.07
7-8	0.07	0.32	0.51	0.21	0.47	0.38	0.50	0.20	0.43	9	0.34	0.146	0.05
8-9	0.13	0.26	0.64	0.50	0.50	0.62	0.52	0.44	0.67	9	0.48	0.169	0.06
9-10	0.10	0.50	0.60	0.40	0.40	0.67	0.48	0.46	0.70	9	0.48	0.169	0.06
10-11	0.05	0.50	0.58	0.40	0.42	0.53	0.37	0.49	0.60	9	0.44	0.156	0.05
11-12	0.02	0.42	0.57	0.34	0.33	0.52	0.46	0.41	0.57	9	0.40	0.158	0.05
12-13	-	0.45	0.23	0.31	0.38	0.53	0.39	0.50	0.55	8	0.42	0.104	0.04
13-14	-	0.42	0.52	0.34	0.29	0.53	0.45	0.37	0.53	8	0.43	0.09	0.03
14-15	-	0.43	-	0.33	0.35	-	0.41	0.55	0.55	6	0.43	0.09	0.04
15-16	-	0.41	-	0.38	0.38	-	-	0.43	0.57	5	0.43	0.07	0.03

3.2.3 Dark to light transiton: decapped roots

The data in Figure 3.3A show that illumination had little, if any, measurable effect on the increase in length of decapped Zea roots. A total of 15 roots were studied (Table 3.5) and the mean growth rate of these roots is shown in Figure 3.3B. In darkness the growth rate increased from 0.51 to 0.77mm h^{-1} with a mean rate of 0.64mm h^{-1}. On illumination there was a transient but insignificant, decrease in the growth rate to 0.51mm h 2h after the onset of the light period, after which the growth rate increased to 0.70mm h^{-1}. The average growth rate in light (0.59mm h^{-1}) was not significantly different from the growth rate in darkness (0.65mm h^{-1}) at the 0.05 level of probability, however the variation in the growth rate from root to root was significant as was the magnitude of their response to the transition (App.1, Table 3).

It is possible to conclude from these data that when decapped roots are transferred from darkness to light there is no significant change in the growth rate. This conclusion supports the reports of H. Wilkins and Wain (1974, 1975) which state that the presence of the root cap is required for the light inhibition of root growth. To investigate further the effect of the root cap on root growth, decapping experiments were carried out on roots maintained in either continuous darkness or continuous light.

3.2.4 Decapping after 3 hours: continuous darkness

Figure 3.4A shows the growth curves of 4 of a total of 11 roots examined and decapped in darkness (Table 3.7). The rate of increase in length was relatively uniform both before and after

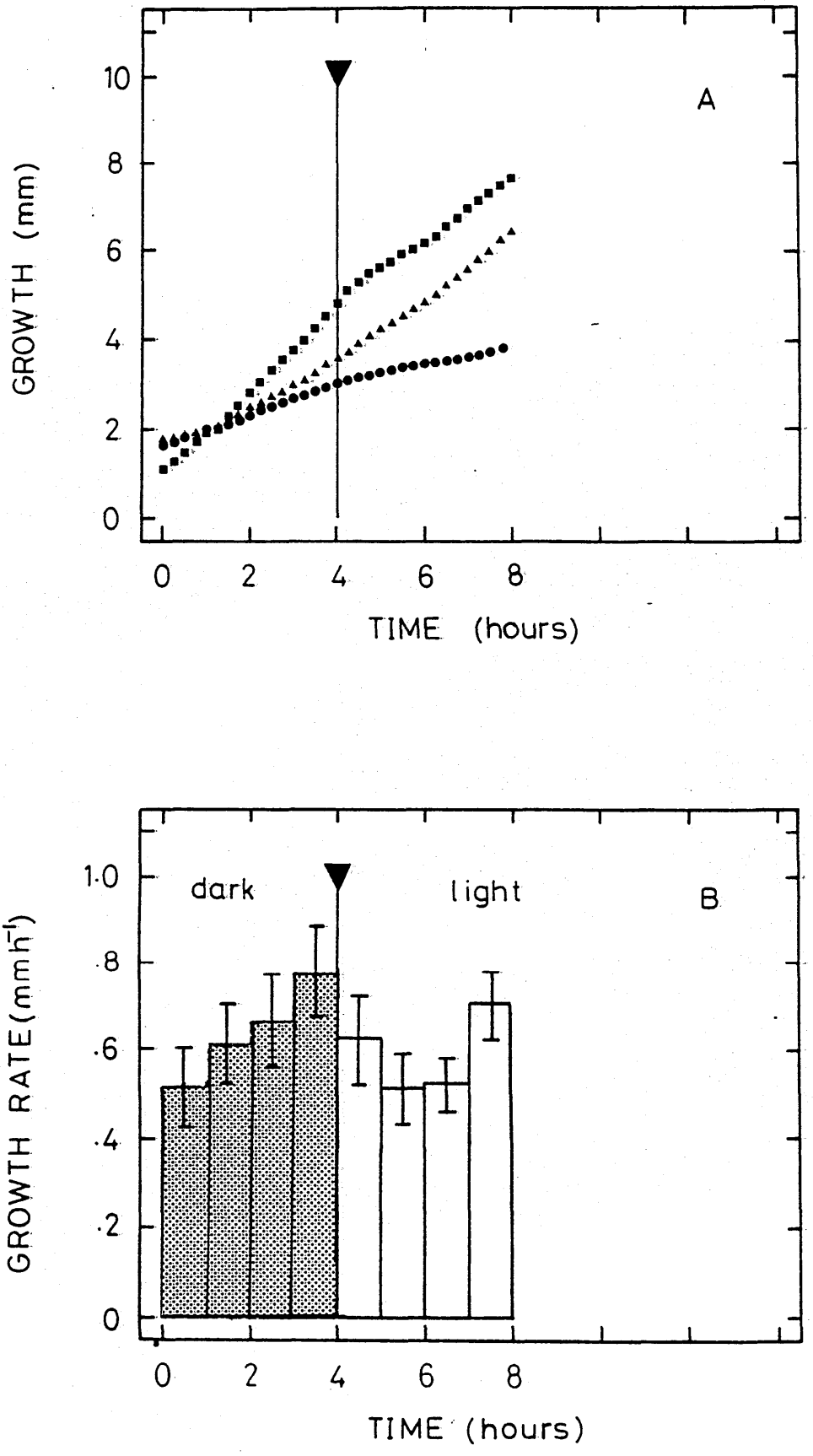


Figure 3.3 Increase in length (A) and mean growth rate (B) of decapped *Z. mays* roots exposed to 4 h darkness followed by 4 h white light (3.67 $Jm^{-2}s^{-1}$).

TABLE 3.5 Length of decapped *Z. mays* roots exposed to 4h darkness followed by 4h white light (3.67 $Jm^{-2}s^{-1}$).

Time (Hrs)	Sample No. 79	80	81	82	83	84	85	86	87	88	91	92	93	96	97
0	1.48	1.11	-	1.82	-	2.00	1.62	1.67	1.87	2.33	1.83	2.00	1.75	1.47	1.98
	1.55	1.28	-	1.83	-	2.25	2.07	1.75	1.90	2.37	1.92	2.08	1.78		2.08
	1.58	1.28	-	1.87	-	2.50	2.25	2.08	1.98	3.47	2.02	2.15	1.85	1.50	2.23
	1.62	1.70	-	1.95	-	2.77	2.45	2.35	2.07	2.53	2.18	2.20	1.93	1.53	2.33
1	1.72	1.92	-	2.07	-	3.02	2.82	2.58	2.15	2.67	2.27	2.25	2.02	1.58	2.43
	1.75	2.02	0.62	2.13	1.58	3.22	3.27	2.80	2.25	2.75	2.40	2.37	2.08	1.65	2.58
	1.80	2.35	0.68	2.28	1.60	3.53	3.53	2.98	2.47	2.83	2.52	2.45	2.17	1.73	2.73
	1.83	2.58	0.75	2.40	1.68	3.75	3.85	3.18	2.62	2.92	2.63	2.50	2.25	1.82	2.90
2	1.88	2.83	0.87	2.57	1.75	4.05	4.12	3.45	2.78	3.00	2.77	2.62	2.35	1.90	3.07
	-	3.08	1.05	2.62	1.77	4.33	4.43	3.60	2.95	3.13	2.92	2.67	2.43	2.00	3.23
	1.93	3.30	1.20	2.75	1.83	4.65	4.83	3.85	3.12	3.25	3.03	2.75	2.57	2.08	3.40
	1.95	3.57	1.37	2.88	1.88	4.92	5.30	4.18	3.33	3.42	3.17	2.83	2.65	2.22	3.58
3	1.97	3.82	1.50	3.00	1.95	5.25	5.75	4.48	3.50	-	3.28	2.87	2.75	2.35	3.73
	2.02	4.08	1.70	3.17	2.00	5.55	6.13	4.80	3.67	-	3.50	2.92	2.82	2.48	3.88
	2.02	4.33	1.80	3.32	2.08	5.83	6.55	5.08	3.87	3.92	3.65	2.97	2.92	2.57	4.03
	2.05	4.58	2.00	3.48	2.17	6.17	6.97	5.38	4.08	4.08	3.80	3.00	3.00	2.80	4.17
4	-	4.85	2.25	3.57	2.27	6.42	7.38	5.67	4.30	4.23	3.95	3.03	3.10	2.93	4.33
	-	5.15	2.45	3.73	2.30	6.55	7.80	5.85	4.48	4.27	4.03	3.03	3.10	-	4.37
	-	5.32	2.67	3.90	2.42	6.77	8.27	6.10	4.70	4.43	-	3.08	3.20	2.87	4.52
	2.17	5.48	2.87	4.05	2.52	7.05	8.68	6.35	4.92	4.53	4.33	3.12	3.27	3.03	4.67
5	2.20	5.65	3.10	4.23	2.60	7.25	8.98	6.57	5.10	4.62	4.47	3.17	3.33	3.12	4.72
	2.25	5.78	3.22	4.37	2.70	7.43	9.32	6.77	5.28	4.70	4.58	3.27	3.35	3.20	4.78
	2.25	5.90	3.40	4.52	2.78	7.62	9.68	6.93	5.45	4.75	4.70	3.33	3.40	3.28	4.88
	2.25	6.08	3.57	4.68	2.88	7.77	9.97	7.10	5.62	4.83	4.78	3.42	3.45	3.42	4.98
6	2.28	6.15	3.75	4.87	2.97	7.97	10.33	7.27	5.85	4.88	4.88	3.55	3.50	3.53	5.05
	2.30	6.43	3.93	5.02	3.12	8.17	10.60	7.43	6.02	4.97	4.98	3.67	3.53	3.62	5.13
	2.32	6.08	4.10	5.20	3.20	8.37	10.87	7.60	6.17	5.03	5.07	3.78	3.58	3.75	5.20
	2.33	6.77	4.32	5.40	3.27	8.58		7.77	6.33	5.12	5.17	3.92	3.62	3.87	5.27
7	2.35	6.97	4.53	5.57	3.38	8.80	-	7.93	6.50	5.22	5.27	4.07	3.67	4.00	-
	2.35	7.17	4.80	5.77	3.45	9.03	-	8.10	6.68	5.38	5.40	4.20	3.73	-	-
	2.38	7.33	5.03	5.98	3.62	9.33	-	8.33	6.88	5.43	5.53	4.33	3.80	-	-
	2.42	7.50	5.30	6.25	3.72	9.58	-	8.72	7.08	5.52	5.68	4.53	3.87	-	-
8	2.45	7.67	5.58	6.45	3.85	9.87	-	8.77	7.30	5.62	5.85	4.65	-	-	-

TABLE 3.6 Growth rate of decapped *Z. mays* roots exposed to 4h darkness followed by 4h white light (3.67 $Jm^{-2}s^{-1}$)

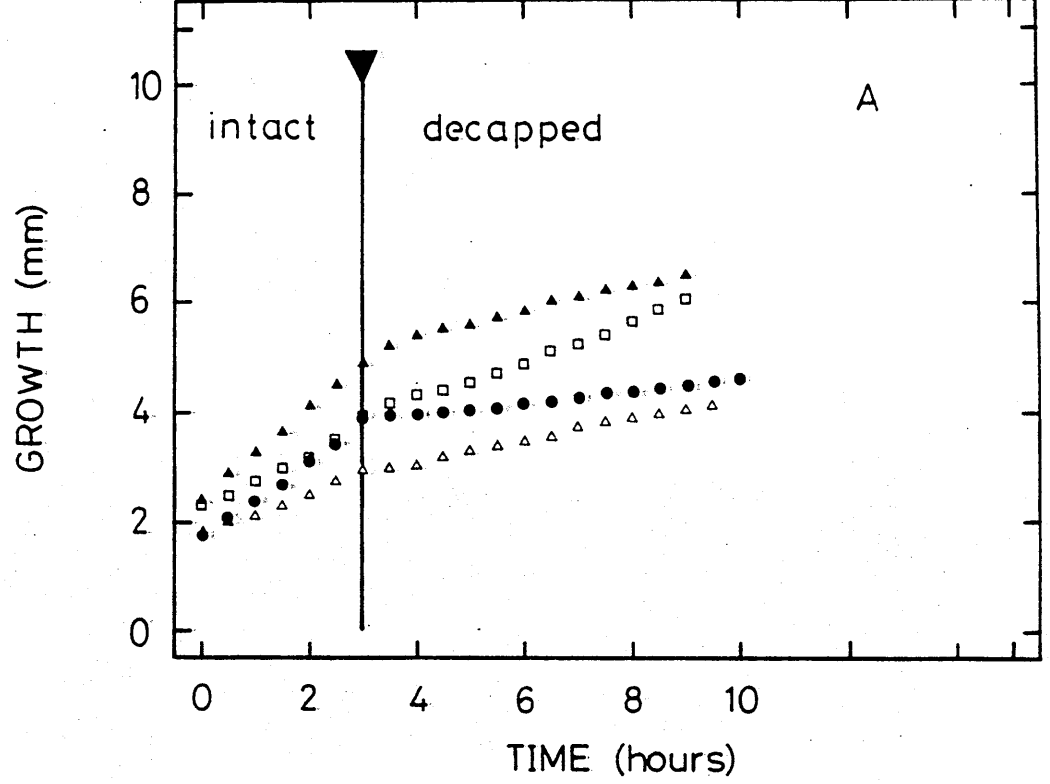
	Sample No.																			
Time (Hrs)	79	80	81	82	83	84	85	86	87	88	91	92	93	96	97	n	$\bar{x}$	SD	SE	Overall mean gr.
0-1	0.24	0.81	-	0.25	-	1.02	1.20	0.91	0.28	0.34	0.44	0.25	0.27	0.11	0.45	13	0.51	0.340	0.09	
1-2	0.16	0.91	-	0.50	-	1.03	1.30	0.87	0.63	0.33	0.50	0.37	0.33	0.32	0.64	13	0.61	0.321	0.09	0.64 ± 0.05
2-3	0.09	0.99	0.63	0.43	0.20	1.20	1.63	1.03	0.72	-	0.51	0.25	0.40	0.45	0.66	14	0.66	0.412	0.11	
3-4	-	1.03	0.75	0.57	0.32	1.17	1.63	1.19	0.80	-	0.67	0.16	0.35	0.48	0.60	13	0.77	0.397	0.11	
4-5	-	0.80	0.85	0.66	0.33	0.83	1.60	0.90	0.80	0.39	0.52	0.14	0.23	0.29	0.39	14	0.62	0.368	0.10	
5-6	0.08	0.50	0.65	0.64	0.37	0.72	1.35	0.70	0.75	0.26	0.42	0.38	0.17	0.41	0.33	15	0.51	0.298	0.08	0.59 ± 0.04
6-7	0.07	0.82	0.78	0.70	0.41	0.83	-	0.66	0.65	0.34	0.39	0.52	0.17	0.47	-	13	0.52	0.234	0.06	
7-8	0.10	0.70	1.05	0.88	0.47	1.07	-	0.84	0.80	0.40	0.58	0.58	-	-	-	11	0.70	0.278	0.08	

decapping. When the root cap was removed, the growth rate was clearly reduced. The magnitude of the decrease in growth rate was revealed by the mean curve for all 11 roots, shown in figure 3.4B. When intact the growth rate increased steadily, from 0.62 to 0.74mm h^{-1} at 3h, when the cap was removed. Within an hour of decapping the rate decreased by about 50% to 0.31mm h^{-1}, after which it again increased to 0.43mm h^{-1} at 7h. In the final 2h the rate once again decreased to 0.33mm h^{-1}. Statistical analysis revealed that removing the root cap significantly reduces the mean growth rate of Zea roots in darkness (App.1, Table 4) and also that there was a significant difference between the treatments. That is, that whilst every root was behaving the same way qualitatively, there was a quantitative difference between them.

These results indicate that removal of the root cap causes an inhibition of the growth rate of non-illuminated roots. There are no other reports in the literature with which to compare these findings since previously it has not been possible to study the growth rate of roots in darkness without the use of safelights. Such studies with safelights revealed that the growth rate of dark-grown roots was not altered by decapping (H. Wilkins et al., 1974; Baehler and Pilet, 1981) a finding at variance with the results presented here. An explanation for the observed reduction in growth rate upon decapping will be given at the end of this chapter.

3.2.5 Decapping after 3 hours: continuous light

The effect on the growth rate of removing the cap from roots elongating in continuous light is shown by the representative curves



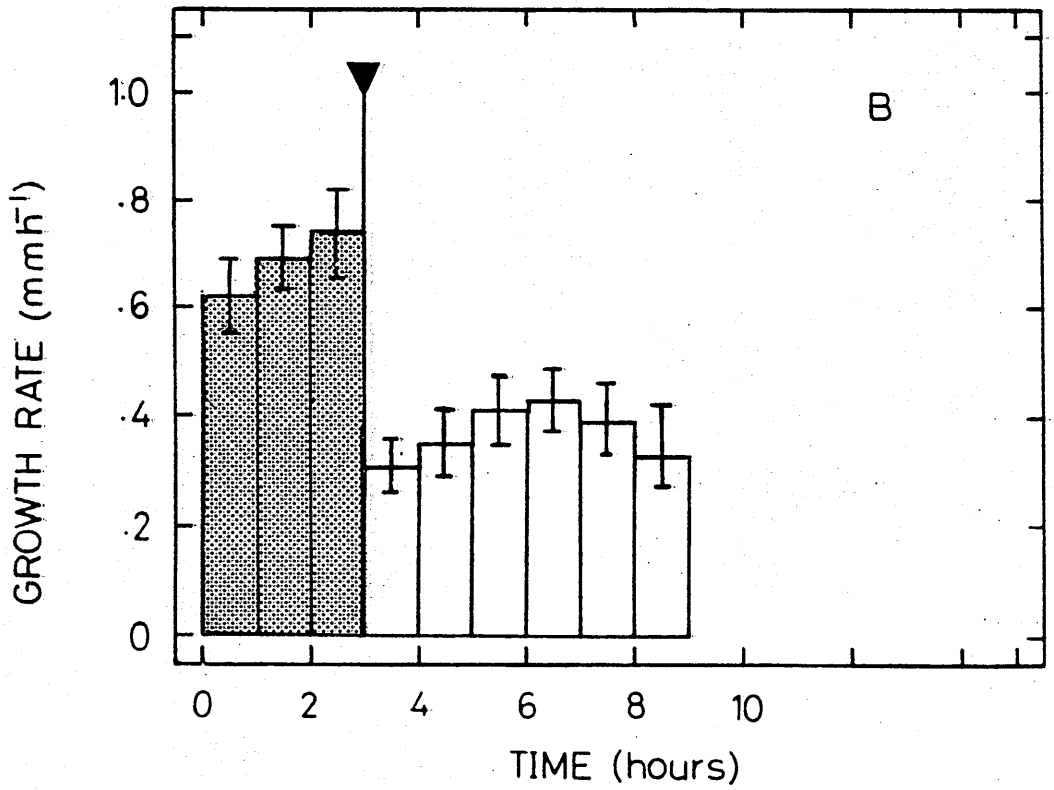


Figure 3.4 Increase in length (A) and mean growth rate (B) of Z. mays roots kept in darkness with the root cap removed at 3 h.

TABLE 3.7 Length of *Z. mays* roots kept in darkness with the root cap removed at 3h.

Time (Hrs)	124	125	126	127	128	128	129	130	131	132	133
0	1.75	2.00	2.30	1.33	1.82	2.50	2.37	1.95	2.67	1.83	1.67
	1.90	2.05	2.67	1.43	1.88	2.57	2.42	2.25	2.75	1.95	1.82
	2.12	2.42	2.92	1.62	2.00	2.68	2.52	2.50	2.88	2.03	2.00
	2.25	2.62	3.13	1.75	2.12	2.80	2.65	-	3.02	2.08	2.17
1	2.42	2.80	3.32	1.95	2.18	2.92	2.75	3.00	3.17	2.12	2.33
	2.58	3.00	3.53	2.23	2.32	3.02	2.95	3.17	-	2.22	2.50
	2.73	3.18	3.70	2.43	2.47	3.18	-	3.27	3.45	2.32	2.70
	2.92	3.37	3.93	2.50	2.58	3.28	3.10	3.53	3.57	2.42	2.88
2	3.20	3.57	4.17	2.92	2.75	3.40	3.23	4.00	3.70	2.52	3.07
	3.43	3.72	4.35	3.17	2.90	3.52	3.37	4.42	3.87	2.62	3.28
	3.53	3.92	4.58	3.40	3.00	3.62	3.53	4.80	4.00	2.75	3.62
	3.73	4.08	4.75	3.62	3.08	3.70	3.67	5.03	4.13	2.83	3.92
3	3.98	4.23	4.92	3.87	3.23	3.82	3.92	5.28	4.28	2.95	4.20
	4.10	4.30	5.12	4.18	3.20	3.82	3.95	5.40	4.33	2.97	4.32
	4.18	4.35	5.22	4.18	3.33	3.85	3.97	5.60	4.40	3.00	4.43
	4.25	4.40	5.32	4.22	3.47	3.88	3.98	5.75	4.50	3.05	4.53
4	4.30	4.45	5.38	4.27	3.57	3.93	3.98	5.85	4.58	3.10	4.70
	4.33	4.50	5.47	4.32	3.72	3.98	4.00	6.02	4.67	3.15	4.82
	4.38	4.57	5.50	4.38	3.88	4.02	4.02	6.20	4.77	3.22	4.92
	4.47	4.62	5.58	4.45	4.05	4.03	4.07	6.43	-	3.27	5.08
5	4.55	4.70	5.63	4.52	4.23	4.07	4.07	6.65	4.95	3.32	5.22
	4.63	4.82	5.68	4.63	4.40	4.15	4.12	6.85	5.08	3.35	5.37
	4.72	4.90	5.75	4.72	4.58	4.18	4.13	7.02	5.20	3.42	5.52
	4.82	5.02	5.83	4.80	4.77	4.25	4.15	7.20	5.30	3.47	5.73
6	4.50	5.10	5.88	4.92	4.93	4.28	4.18	7.38	5.42	3.52	5.90
	5.00	5.22	5.95	5.00	5.08	4.35	4.20	7.57	5.52	3.58	6.10
	5.10	5.32	6.00	5.10	5.23	4.42	-	7.72	5.65	3.63	6.25
	5.18	5.47	6.05	5.25	5.40	4.45	4.27	7.90	5.75	3.68	6.43
7	5.27	5.57	6.12	5.38	5.57	4.50	4.32	8.03	5.87	4.72	6.60
	5.37	5.72	6.17	5.52	5.73	4.57	4.35	8.20	5.98	3.80	6.75
	5.43	5.82	6.25	5.63	5.85	4.60	4.38	8.30	6.12	3.83	6.92
	5.55	5.98	6.28	5.75	6.07	4.65	4.40	8.48	6.25	3.88	7.03
8	5.67	6.15	6.33	5.88	6.23	4.68	4.43	8.60	6.40	3.93	7.20
	5.77	-	6.38	6.02	6.42	4.73	4.45	8.82	-	3.97	7.35
	5.85	-	6.42	6.13	6.58	4.77	4.48	8.97	6.67	4.02	7.48
	5.98	-	6.47	6.25	6.75	4.80	4.52	9.08	6.77	4.05	7.62
9	6.10	-	6.53	6.35	6.87	4.82	4.53	9.23	6.92	4.08	7.77
	6.20	-	6.58	6.47	7.05	4.86	4.58	9.35	7.08	4.13	7.87
	-	-	-	6.55	7.22	4.90	4.62	-	7.22	4.17	8.05
	-	-	-	6.65	-	4.93	4.63	-	-	4.22	8.17
10	-	-	-	6.80	-	4.98	4.67	-	-	-	8.35

TABLE 3.8 Growth rate of *Z. mays* roots kept in darkness with the root cap removed at 3h.

Time (Hrs)	Sample No. 124	125	126	127	128	128	129	130	131	132	133	n	$\bar{x}$	SD	SE	Overall mean
0-1	0.67	0.80	1.02	0.62	0.36	0.42	0.38	1.05	0.50	0.29	0.66	11	0.62	0.247	0.07	
1-2	0.78	0.77	0.85	0.97	0.57	0.48	0.48	1.00	0.53	0.40	0.74	11	0.69	0.198	0.06	0.68 ± 0.03
2-3	0.78	0.66	0.75	0.95	0.48	0.42	0.69	1.28	0.58	0.43	1.13	11	0.74	0.268	0.08	
3-4	0.32	0.22	0.45	0.40	0.34	0.11	0.06	0.57	0.30	0.15	0.50	11	0.31	0.157	0.05	
4-5	0.25	0.25	0.25	0.25	0.66	0.14	0.09	0.80	0.37	0.22	0.52	11	0.35	0.212	0.06	
5-6	0.35	0.40	0.25	0.40	0.70	0.21	0.11	0.73	0.47	0.20	0.68	11	0.41	0.206	0.06	
6-7	0.37	0.67	0.24	0.46	0.64	0.22	0.14	0.65	0.45	0.20	0.70	11	0.43	0.201	0.06	0.38 ± 0.02
7-8	0.40	0.58	0.21	0.50	0.66	0.18	0.11	0.57	0.53	0.21	0.60	11	0.41	0.190	0.06	
8-9	0.43	-	0.20	0.47	0.64	0.14	0.10	0.63	0.52	0.15	0.57	10	0.39	0.204	0.06	
9-10	-	-	-	0.45	-	0.16	0.14	-	-	-	0.58	4	0.33	0.188	0.09	

in Figure 3.5A. The roots exhibited a relatively constant increase in length over the whole of the recorded time period; decapping appeared to have no effect on the increase in length of these illuminated roots. The mean growth histogram (Fig. 3.5B) for a total of 11 roots, revealed that the growth rate fell from 1.05 to 0.74mm h^{-1} and then rose again to 0.83mm h^{-1} in the first 3h when the roots were intact. Within one hour of decapping the growth rate had decreased to 0.50mm h^{-1}, but in the next hour the rate increased to 0.84mm h^{-1} which was approximately the average growth rate of the roots when intact. Thereafter, there were only small hourly variations in the growth rate, none of which reached significance at the 0.05 level of probability. The growth rate of the roots when intact was not significantly different to that of the decapped roots (App.1, Table 5). Whether the decrease in growth rate during the hour after decapping was attributable to surgical trauma has yet to be elucidated.

A number of the investigations reported in the literature have led to the conclusion that the root cap is the source of at least one growth inhibiting substance (Gibbons and Wilkins, 1970; H. Wilkins and Wain, 1974, 1975); it would therefore seem likely that the effect of removing the cap from illuminated roots would appear as an overall increase in the growth rate. However, such an increase in rate was not observed in the studies reported in this thesis. During the 3h illumination prior to removal of the root cap it is possible that saturating levels of inhibitor have accumulated in the elongation zone. If such an accumulation did occur decapitation at 3h would stop any more inhibitor moving back from the root cap but the inhibitor

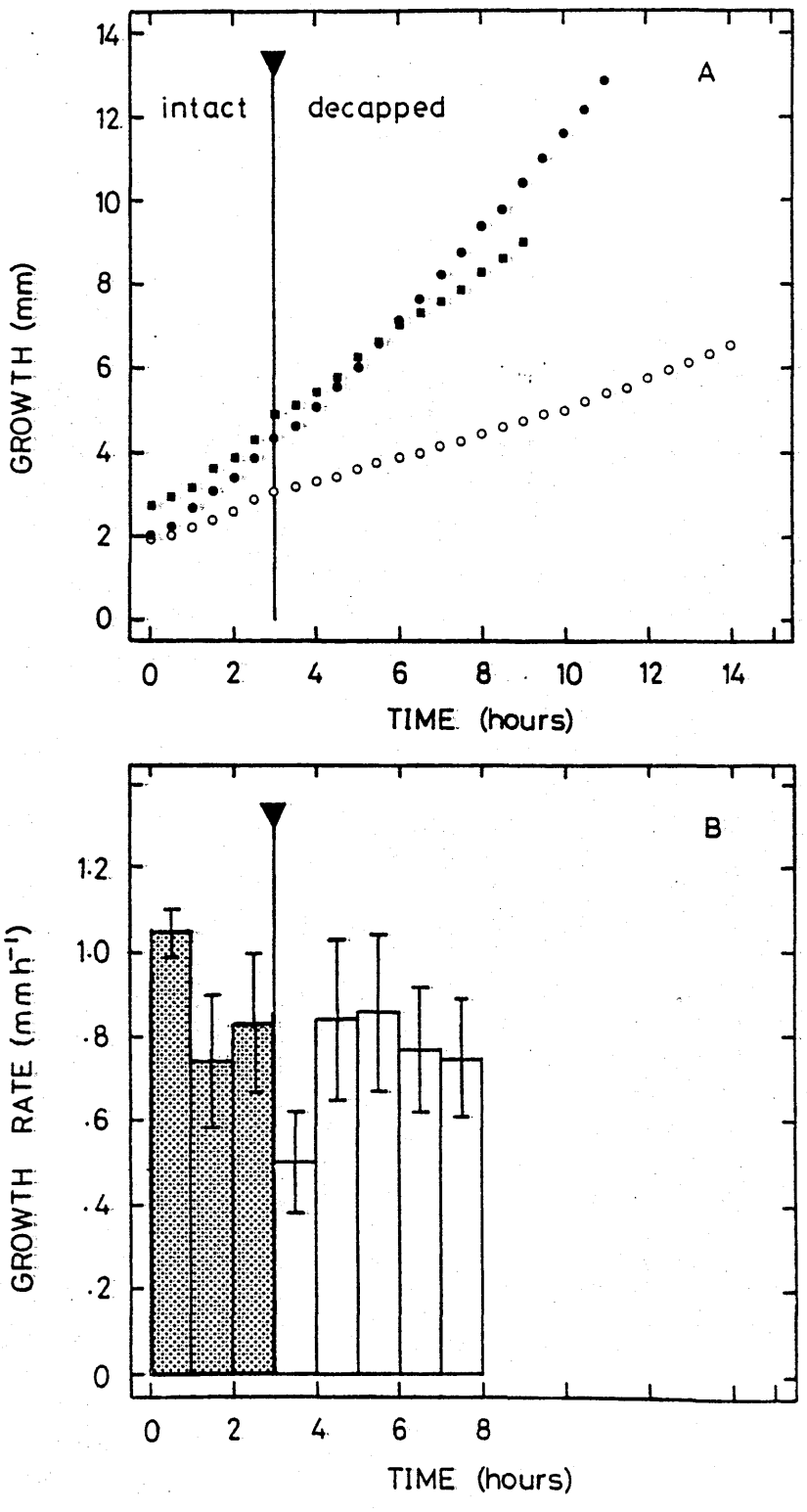


Figure 3.5 Increase in length (A) and mean growth rate (B) of *Z. mays* roots kept in white light (3.67 $Jm^{-2}s^{-1}$) with the root cap removed at 3 h.

TABLE 3.9 Length of *Z. mays* roots kept in white light (3.67 $Jm^{-2}s^{-1}$) with the root cap removed at 3h.

Time (Hrs)	Sample No. 140	141	141	CL2	CL3	CL4	CL 104	CLT 105	CLT 106	CL7	CL8
0	1.92	-	2.12	2.10	2.02	2.18	1.82	1.70	1.77	1.92	1.35
	1.97	-	2.50	2.67	2.02	2.67	1.89	2.09	1.84	2.50	1.50
	2.08	1.87	2.95	3.12	2.08	3.05	2.02	2.33	1.93	3.85	1.85
	2.17	1.88	3.03	3.33	2.27	3.43	2.26	2.54	2.02	4.35	2.12
1	2.25	1.92	3.17	3.60	2.52	3.65	2.63	2.67	2.12	4.50	2.32
	2.33	1.97	3.43	3.98	2.73	4.02	2.84	2.81	2.21	4.60	2.43
	2.40	2.05	3.67	4.45	2.92	4.37	3.14	2.98	2.37	4.77	2.53
	2.48	2.08	3.82	4.92	3.13	4.65	3.33	3.21	2.47	4.97	2.65
2	2.62	2.12	3.92	5.47	3.15	5.03	-	3.47	2.54	5.03	2.77
	2.77	2.17	4.15	4.90	3.42	5.55	3.67	3.56	2.65	5.17	2.92
	2.92	2.25	4.38	6.55	3.65	5.97	3.86	4.00	2.81	5.30	3.05
	3.00	2.33	4.65	-	3.90	6.38	4.05	4.35	2.84	5.43	3.18
3	3.08	2.42	5.00	-	4.18	6.92	4.21	4.70	2.91	5.60	3.30
	3.18	2.38	5.08	-	4.37	7.38	4.21	5.09	2.98	5.78	3.43
	3.22	-	5.18	12.46	4.67	7.67	4.21	5.37	2.98	6.00	3.52
	3.28	2.47	5.30	12.67	4.68	7.97	4.21	5.40	3.02	6.13	3.60
4	3.33	2.50	5.45	13.00	4.82	8.28	4.23	5.51	3.12	6.33	3.70
	3.38	2.60	5.62	13.38	5.10	8.62	4.37	5.72	3.12	6.55	3.87
	3.48	2.72	5.82	13.73	5.33	9.03	4.40	5.82	3.16	6.65	4.02
	3.55	2.80	5.87	14.00	5.62	10.00	4.47	5.96	3.23	6.80	4.18
5	3.62	2.92	6.25	14.57	5.72	10.63	4.58	6.05	-	6.88	4.37
	3.68	3.00	6.47	15.30	6.12	10.78	4.67	6.26	3.35	7.05	4.53
	3.75	3.10	6.65	15.85	6.32	11.30	4.72	6.51	3.44	7.22	4.68
	3.82	3.22	6.85	16.20	6.63	11.87	4.75	6.67	3.47	7.45	4.85
6	3.87	3.30	7.00	16.50	6.85	12.28	4.79	6.95	3.54	7.63	5.05
	3.92	3.38	7.18	16.73	7.10	12.72	4.88	6.98	3.58	7.83	5.23
	4.00	3.47	7.33	17.02	7.37	13.25	-	7.00	3.67	8.07	5.48
	4.07	3.55	7.48	17.20	7.65	13.75	-	7.23	3.74	8.30	5.77
7	4.15	3.63	7.62	17.38	7.92	14.13	-	7.49	3.86	8.48	6.00
	4.22	3.70	7.78	17.57	8.20	14.57	-	7.58	3.96	8.78	6.18
	4.28	-	7.93	17.75	8.48	15.05	-	7.70	4.11	9.28	6.52
8	4.45	-	8.32	18.05	9.03	15.72	-	7.74	4.18	9.52	6.73

TABLE 3.10 Growth rate of *Z. mays* roots kept in white light (3.67 $Jm^{-2}s^{-1}$) with the root cap removed at 3h.

Time (Hrs)	Sample No. 140	141	142	CL2	CL3	CL4	CLT 104	CLT 105	CLT 106	CL7	CL8	n	$\bar{x}$	SD	SE	Overall mean g
0-1	0.33	-	1.05	1.50	0.50	1.47	0.87	0.97	0.35	2.58	0.97	10	1.05	0.64	0.20	
1-2	0.37	0.20	0.75	1.87	0.63	1.38	-	0.80	0.42	0.53	0.45	10	0.74	0.486	0.15	0.87 ± 0.08
2-3	0.46	0.30	1.08	-	1.03	1.89	-	1.23	0.37	0.57	0.53	9	0.83	0.491	0.15	
3-4	0.25	0.08	0.45	-	0.64	1.36	0.02	0.81	0.21	0.73	0.40	10	0.50	0.384	0.12	
4-5	0.29	0.42	0.80	1.57	0.90	2.35	0.35	0.54	-	0.55	0.67	10	0.84	0.612	0.19	
5-6	0.25	0.38	0.75	1.93	1.13	1.65	0.21	0.90	-	0.75	0.68	10	0.86	0.54	0.17	0.74 ± 0.06
6-7	0.28	0.33	0.62	0.88	1.07	1.85	-	0.54	0.32	0.85	0.95	10	0.77	0.450	0.14	
7-8	0.30	-	0.70	0.67	1.11	1.59	-	0.25	0.32	1.04	0.73	9	0.75	0.417	0.14	

already accumulated in the elongation zone would have to decrease before a change in the growth rate was observed. It may be that the fall in the level of inhibitor in the roots in the experiment described above was not of sufficient magnitude to be reflected as a change in the growth rate. In order to examine this possibility further, investigations of the effect of decapping on the growth rate of the roots were carried out using much shorter light periods.

3.2.6 Darkness with 10 minutes light at 3 hours

Growth data for 3 roots exposed to 10 min light at 3h are shown in Figure 3.6A. The increase in length was fairly constant with time both before and after the 10 min light, although the increase was faster prior to illumination. This pattern of growth was also revealed by the mean growth rate histogram (Fig. 3.6B) which was plotted using the data from 20 roots (Table 3.11 and 3.12). During the first 3h the growth rate increased slightly from 0.75 to 0.79mm h^{-1}.

After the light period the growth rate decreased over 3h to 0.42mm h^{-1} and then it remained between 0.51 and 0.40mm h^{-1} for the last 5h of the observation period. The growth rate after the light period was significantly less than the rate prior to illumination. Thus, as little as 10 min light can significantly reduce the growth rate of *Zea* roots ($p = 0.05$) (App.1, Table 6).

The change in the growth rate of roots upon illumination is believed to be caused by inhibitors produced by the root cap moving to the elongation zone and inhibiting elongation (Gibbons and Wilkins, 1970). Unless this movement is very rapid it ought to be possible to prevent this light-induced inhibition by removing the root cap

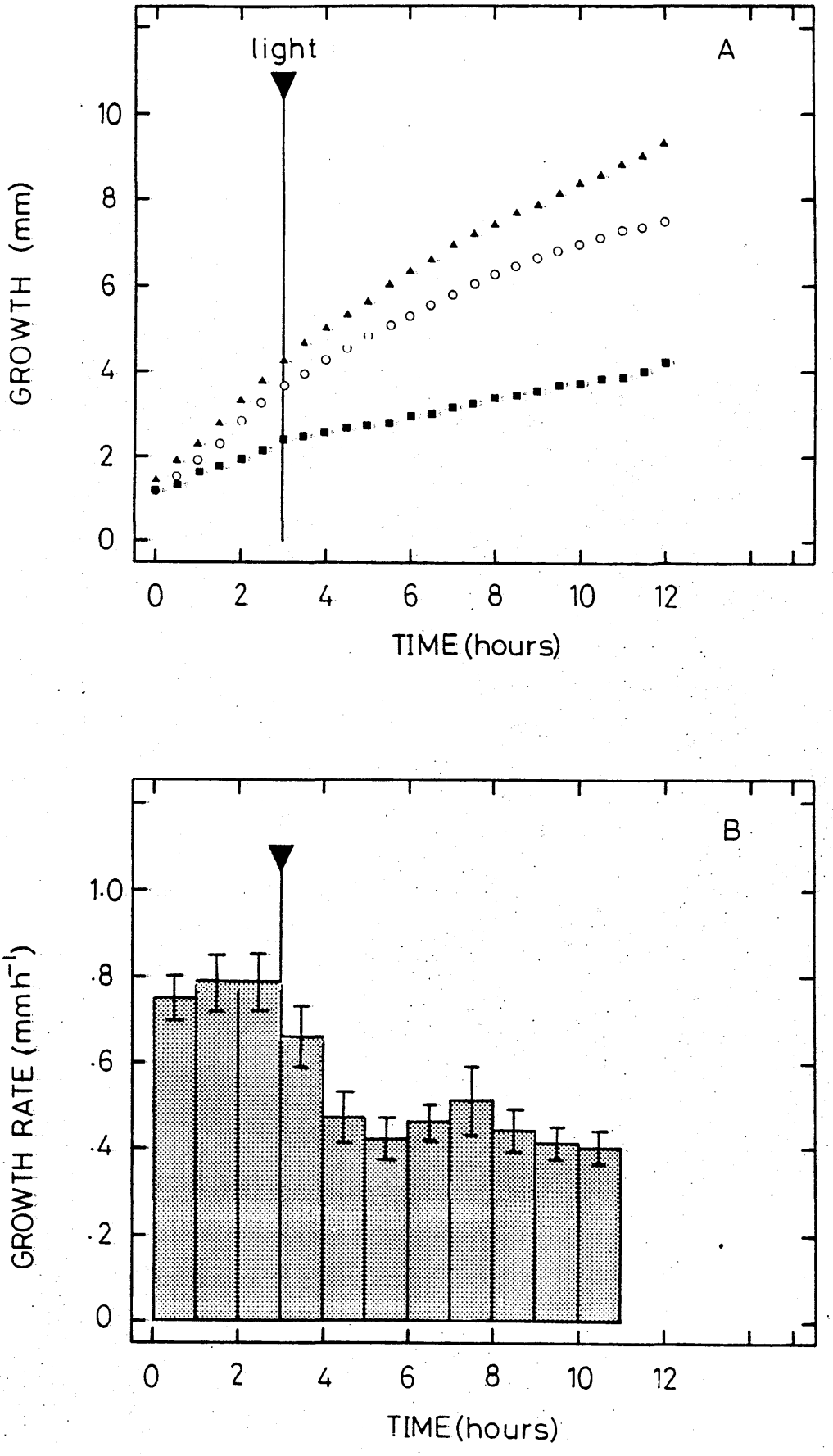


Figure 3.6 Increase in length (A) and mean growth rate (B) of *Z. mays* roots growing in darkness with a 10 min pulse of white light (3.67 Jm^{-2} s^{-1}) at 3 h.

TABLE 3.11 Length of intact *Z. mays* roots growing in darkness with a 10 min pulse of white light (3.67 $Jm^{-2}s^{-1}$) at 3h.

Time (Hrs)	Sample No. 125	121	127	128	223	PL1	PL2	157	PL3	PL4	159	PL6	PL7	PL8	PL9	PL10	PL13	PL14	PL15	PL16
0	0.70	1.07	1.58	1.75	1.67	1.18	1.53	0.82	1.70	1.28	0.91	1.47	1.48	1.20	1.42	1.12	1.25	1.58	1.27	1.42
	0.70	1.28	1.61	2.00	2.13	1.27	1.75	1.14	1.85	1.37	1.05	1.55	1.70	1.33	1.57	1.27	1.28	1.73	1.47	1.67
	0.79	1.40	1.74	2.12	2.43	1.37	1.98	1.46	2.07	1.42	1.12	1.65	1.97	1.55	1.70	1.52	1.38	1.92	1.62	2.02
	1.02	1.70	1.82	2.32	2.67	1.50	2.23	1.68	2.20	1.52	1.32	1.88	2.12	1.75	1.88	1.72	1.50	2.07	1.85	2.23
1	1.18	1.72	2.26	2.54	2.85	1.62	2.47	1.95	2.35	1.68	1.61	2.23	2.37	1.95	2.10	1.90	1.67	2.27	2.10	2.53
	1.26	1.91	2.42	2.72	3.18	1.73	2.70	2.21	2.50	1.93	1.91	2.50	2.60	2.15	2.30	2.08	1.70	2.42	2.38	2.87
	1.40	2.04	2.54	2.81	3.48	1.83	2.92	2.46	2.78	2.23	2.16	2.73	2.85	2.38	2.52	2.23	1.82	2.57	2.73	3.17
	1.56	2.18	2.81	2.93	3.77	1.92	3.15	2.70	2.93	2.37	2.32	2.93	3.08	2.62	2.70	2.28	1.87	2.73	3.02	3.48
2	1.75	2.35	3.07	2.98	4.12	1.97	3.40	2.96	3.03	2.48	2.53	3.12	3.33	2.80	2.85	2.38	1.97	2.92	3.30	3.82
	1.88	2.54	3.33	3.07	4.48	2.07	3.65	3.14	3.10	2.60	2.47	3.27	3.58	3.03	3.08	-	2.07	3.10	3.58	4.10
	2.12	2.72	3.60	3.23	4.85	2.10	3.87	3.25	3.25	2.73	3.02	3.53	3.80	3.25	3.25	2.78	2.20	3.25	3.90	4.42
	2.33	2.89	3.79	3.39	5.13	2.12	4.18	3.35	3.37	2.88	3.18	-	4.07	3.45	3.45	2.92	2.32	3.43	4.22	4.75
3	2.58	3.16	4.05	3.54	5.43	2.18	4.42	3.42	3.50	2.98	3.39	4.00	4.32	3.67	3.60	3.10	2.40	3.63	4.50	5.03
	2.68	3.35	4.35	3.75	5.83	2.23	4.80	3.56	3.55	3.10	3.40	4.25	4.53	3.80	3.67	3.20	2.43	3.80	4.83	5.40
	2.95	3.54	4.56	3.91	6.10	2.27	5.08	3.63	3.62	3.17	3.46	4.47	4.73	3.98	3.82	3.35	2.50	3.98	5.00	5.62
	3.05	3.77	4.93	4.07	6.42	2.30	5.33	3.72	3.72	3.30	3.56	4.60	4.90	4.12	3.95	3.45	2.57	4.13	5.22	5.90
4	3.25	3.95	5.16	4.18	6.68	2.33	5.58	3.75	3.78	3.40	3.70	4.80	5.07	4.30	4.05	3.52	2.62	4.28	5.43	6.17
	3.49	4.21	5.33	4.32	6.92	2.35	5.80	3.71	3.85	3.47	3.72	5.00	5.22	4.43	4.08	3.60	2.67	4.40	5.58	6.40
	3.61	4.30	5.49	4.40	7.12	2.37	5.98	3.81	3.90	3.48	3.75	5.25	5.38	4.57	4.13	3.70	2.70	4.52	5.77	6.63
	3.74	4.42	5.70	4.49	7.32	2.40	6.17	3.84	3.97	3.52	3.82	5.48	5.52	4.72	4.18	3.82	7.72	4.65	5.97	6.85
5	3.82	4.57	4.93	4.65	7.45	2.42	6.30	3.89	4.05	3.57	3.86	5.62	5.67	4.83	4.25	3.90	2.73	4.67	6.13	7.08
	3.93	4.60	6.11	4.75	7.58	2.45	6.42	3.93	4.10	3.63	3.93	5.77	5.87	4.98	4.35	3.93	2.77	4.73	6.30	7.30
	4.07	4.68	6.30	4.89	7.62	2.52	6.50	3.95	4.17	3.70	3.96	5.90	6.02	5.10	4.45	4.00	2.83	4.82	6.48	7.52
	4.19	4.72	6.49	5.02	7.75	2.56	6.63	3.98	4.25	3.78	4.00	6.05	6.22	5.22	4.55	4.10	2.87	4.85	6.63	7.67
6	4.33	4.77	6.72	5.16	7.85	2.62	6.73	4.07	4.33	3.85	4.04	6.20	6.37	5.33	4.63	4.17	2.95	4.92	6.75	7.90
	4.47	4.81	6.89	5.28	8.07	2.67	6.83	4.14	4.43	3.93	4.11	6.35	6.53	5.47	4.75	4.25	2.98	4.98	6.90	8.08
	4.61	4.88	7.14	5.40	8.23	2.75	6.88	4.18	4.50	4.02	4.14	6.52	6.67	5.58	4.87	4.32	3.03	5.10	7.05	8.25
	4.74	5.02	7.32	5.58	8.45	2.77	7.02	4.26	4.60	4.10	4.19	6.67	6.78	5.70	4.92	4.38	3.08	5.18	7.20	8.50
7	4.86	5.11	7.51	5.70	8.65	2.83	7.17	4.33	4.65	4.17	4.23	6.85	6.95	5.82	5.02	4.47	3.15	5.27	7.37	8.73
	5.25	5.21	7.72	5.81	9.00	2.87	7.28	4.39	4.72	4.25	4.25	7.00	7.12	5.93	5.07	4.68	3.20	5.37	7.57	8.95
	5.61	5.30	8.00	5.93	9.18	2.19	7.38	4.46	4.80	4.32	4.32	7.13	7.23	6.05	5.12	-	3.23	5.43	7.73	9.15
	6.04	5.46	8.19	6.03	9.38	2.93	7.50	4.51	4.87	4.47	4.35	7.25	7.33	6.17	5.17	4.78	3.33	5.33	7.85	9.40
8	6.61	5.56	8.37	6.16	9.47	2.97	7.70	4.51	4.93	4.58	4.37	7.38	7.48	6.25	5.25	4.87	3.35	5.60	7.92	9.63
	7.18	5.65	8.54	6.26	9.65	3.05	7.88	4.58	4.97	4.67	4.39	7.53	7.58	6.37	5.32	4.97	3.42	5.68	8.02	9.95
	7.56	5.75	8.74	6.42	9.88	3.12	8.05	4.61	5.05	4.72	-	7.70	7.72	6.45	5.38	5.10	3.45	5.77	-	10.01
	-	5.86	8.95	6.54	10.12	3.18	8.12	4.68	5.12	4.77	-	7.83	7.83	6.53	5.43	5.25	3.52	5.85	-	10.30
9	-	6.00	9.18	6.67	10.25	3.22	8.15	4.72	5.17	4.85	-	7.92	7.93	6.65	5.53	5.35	3.55	5.90	-	10.45
	-	6.05	9.30	6.79	10.43	3.27	-	-	5.22	4.97	-	8.02	8.03	6.72	5.58	5.50	3.60	5.93	-	-
	-	6.18	9.44	6.93	10.57	3.30	-	-	5.28	5.07	-	8.12	8.20	6.78	5.67	5.63	3.65	6.00	-	-
	-	6.26	9.63	7.07	10.78	-	-	-	5.35	5.18	-	8.23	8.28	6.85	5.70	5.75	3.70	6.08	-	-

TABLE 3.11 (continued)

10	-	6.39	9.75	7.21	10.93	-	-	-	-	5.27	-	8.32	8.43	6.95	5.75	5.87	3.73	6.10
	-	6.53	9.89	7.32	11.07	-	-	-	-	5.35		8.45	8.50	7.02	5.80	6.00	3.78	6.18
	-	6.67	10.04	7.42	11.20	-	-	-	-	5.48		8.55	8.62	7.10	5.83	6.13	3.85	6.22
	-	6.77	10.14	7.56	11.38	-	-	-	-	-		8.68	8.70	7.17	5.90	6.25	3.88	6.27
11	-	6.86	10.23	7.67	11.57	-	-	-	-	-		8.80	8.85	7.27	5.93	6.38	3.92	6.35

TABLE 3.12 Growth rate of intact *Z. mays* roots growing in darkness with a 10 min pulse of white light (3.67 $Jm^{-2}s^{-1}$) at 3h.

Time (Hrs)	Sample No. 125	121	127	128	225	PL1	PL2	157	PL3	PL4	159	PL6	PL7	PL8	PL9	PL10	P43	PL14	PL15	P46	n	$\bar{x}$	SD	SE	Overall mean gr
0-1	0.48	0.65	0.68	0.79	1.18	0.44	0.94	1.13	0.65	0.40	0.70	0.76	0.89	0.75	0.68	0.78	0.42	0.69	0.83	1.11	20	0.75	0.219	0.05	
1-2	0.57	0.63	0.81	0.44	1.27	0.35	0.93	1.01	0.68	0.80	0.92	0.89	0.96	0.85	0.75	0.48	0.30	0.65	1.20	1.29	20	0.79	0.277	0.06	0.78 ± 0.01
2-3	0.83	0.87	0.98	0.56	1.31	0.21	1.02	0.46	0.47	0.50	0.86	0.88	0.99	0.87	0.75	0.72	0.43	0.71	1.20	1.21	20	0.79	0.28	0.06	
3-4	0.67	0.79	1.11	0.64	1.25	0.15	1.16	0.33	0.28	0.42	0.31	0.80	0.75	0.63	0.45	0.42	0.22	0.65	0.93	1.14	20	0.66	0.327	0.07	
4-5	0.57	0.56	0.77	0.47	0.77	0.09	0.72	0.14	0.27	0.17	0.16	0.82	0.60	0.53	0.20	0.38	0.11	0.39	0.70	0.91	20	0.47	0.259	0.06	
5-6	0.57	0.26	0.79	0.51	0.40	0.20	0.43	0.18	0.28	0.28	0.18	0.58	0.70	0.50	0.38	0.27	0.22	0.25	0.62	0.82	20	0.42	0.253	0.05	
6-7	0.53	0.34	0.79	0.54	0.80	0.21	0.44	0.26	0.32	0.32	0.19	0.65	0.58	0.49	0.39	0.30	0.20	0.35	0.62	0.83	20	0.46	0.199	0.04	0.47 ± 0.03
7-8	1.75	0.45	0.86	0.46	0.82	0.14	0.53	0.18	0.28	0.41	0.14	0.53	0.53	0.43	0.23	0.40	0.20	0.33	0.55	0.90	20	0.51	0.36	0.08	
8-9	-	0.44	0.81	0.51	0.78	0.25	0.45	0.21	0.24	0.27	-	0.54	0.45	0.40	0.28	0.48	0.20	0.30	-	0.82	17	0.44	0.199	0.05	
9-10	-	0.39	0.57	0.54	0.68	-	-	-	-	0.42	-	0.40	0.50	0.30	0.22	0.52	0.18	0.20	-	-	12	0.41	0.153	0.04	
10-11	-	0.47	0.48	0.46	0.64	-	-	-	-	-	-	0.45	0.42	0.32	0.18	0.57	0.19	0.25	-	-	11	0.40	0.148	0.04	

immediately after the 10 min light period.

3.2.7 Darkness with 10 minutes light and decapping at 3 hours

The effects on the growth rate of four roots, which had been maintained for 3h in darkness before being given 10 min light and then immediately decapped, are shown in Figure 3.7A. Each root exhibited a relatively steady increase in length over the first 3h of the observation period but after the light and decapping treatment the gradients of the growth curves decreased indicating a reaction of the growth rate of the roots. This decrease in growth rate is also illustrated in the mean growth rate histogram (Fig. 3.7B). The rate during the first 3h was between 0.91 and 0.84mm h^{-1} and within 2h of illumination and decapping, the rate decreased to 0.39mm h^{-1}, after which there was no significant change in the growth rate for the rest of the observation period. Thus, even when the roots are decapped immediately after the light period there is still a significant ($p = 0.05$) reduction in the growth rate (App.1, Table 7).

The inhibition of growth rate following decapping could indicate either that movement of inhibitor is very rapid or, that there is an electrical signal transmitting information from the root cap to the elongation zone which in some way controls the growth rate of the roots.

3.2.8 Surgical trauma

A number of the experiments reported above have involved the removal of the root cap, and it was therefore essential to establish whether or not removing the rootcap initiated wounding responses which

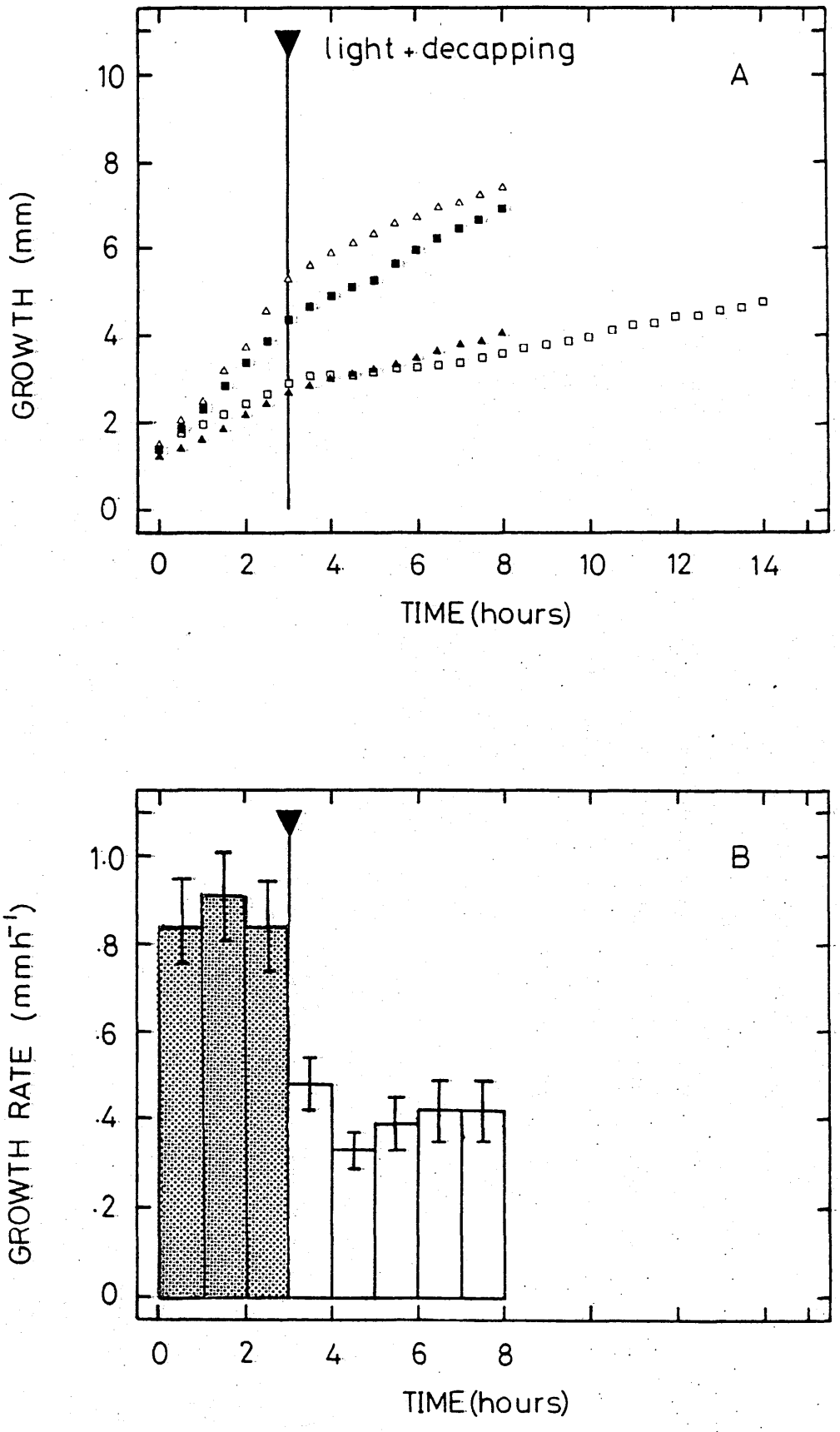


Figure 3.7 Increase in length (A) and mean growth rate (B) of *Z. mays* roots growing in darkness with the root cap immediately removed after a 10 min pulse of white light (3.67 $Jm^{-2}s-1$) at 3 h.

TABLE 3.13 Length of *Z. mays* roots growing in darkness with the root cap immediately removed after a 10 min pulse of white light ($3.67\ Jm^{-2}s^{-1}$) at 3h.

Time (Hrs)	Sample No. 120	7/83	PL219	PL5	PL6	129	PL0	PL4	PL5	PL6	X	Z
0	1.58	1.05	0.83	1.30	1.42	1.58	1.70	1.23	1.28	1.52	1.27	1.42
	1.75	1.14	1.13	1.58	1.55	2.21	1.83	1.35	1.42	1.63	1.50	1.62
	1.84	1.23	1.50	1.82	1.75	2.60	2.03	1.40	1.43	2.05	1.77	1.85
	2.05	1.40	1.83	1.98	1.83	2.89	2.18	1.47	1.50	2.25	2.08	2.10
1	2.19	1.61	2.18	2.20	1.97	3.30	2.43	1.53	1.65	2.52	2.32	2.37
	2.33	1.84	2.58	2.50	2.10	3.68	2.70	1.60	1.77	2.87	2.58	2.60
	2.53	2.11	2.90	2.72	2.22	4.05	3.03	1.72	1.92	3.18	2.83	2.87
	2.63	2.35	3.08	2.92	2.30	4.26	3.25	1.78	2.08	3.40	3.08	3.12
2	2.72	2.58	3.33	3.25	2.42	4.91	3.38	1.83	2.23	3.78	3.38	3.35
	2.86	2.72	3.58	3.50	2.52	5.28	3.50	1.88	2.35	4.13	3.62	3.62
	2.93	2.98	3.83	3.72	2.67	5.67	3.63	1.95	2.45	4.55	3.83	3.87
	2.96	3.16	4.07	3.90	2.80	6.04	3.97	2.00	2.60	4.93	4.05	4.12
3	2.96	3.42	4.32	4.20	2.92	6.49	4.23	2.05	2.70	5.27	4.30	4.35
	2.98	3.72	4.53	4.42	3.08	6.72		2.08	2.83	5.53	4.53	4.57
	3.02	3.91	4.77	4.58	3.08	6.88	4.27	2.13	2.88	5.62		4.68
	4.07	4.00	4.95	4.80	4.08	7.04	4.33	2.22	2.98	5.75	4.83	4.83
4	3.11	4.11	5.05	4.90	3.08	7.19	4.53	2.23	3.03	5.90	4.93	4.95
	3.14	4.26	5.20	5.02	3.08	7.33	4.65	2.27	3.10	6.00	5.07	5.05
	3.23	4.35	5.33	5.05	3.12	7.49	4.77	2.30	3.13	6.10	5.17	5.13
	3.28	4.42	5.43	5.12	3.15	7.70	4.92	2.30	3.18	6.23	5.25	5.23
5	3.32	4.44	5.53	5.15	3.18	7.79	5.05	2.32	3.23	6.32	5.38	5.32
	3.39	4.53	5.67	5.20	3.22	7.84	5.18	2.33	3.32	6.43	5.52	5.48
	3.42	4.62	5.82	5.27	3.27	7.98	5.38	2.33	3.38	6.55	5.65	5.63
	3.46	4.67	5.98	5.32	3.30	8.11	5.57	2.35	3.47	6.63	5.78	5.80
6	3.51	4.70	6.20	5.40	3.33	8.37	5.95	2.40	3.58	6.83	6.08	6.12
	3.54	-	6.37	5.48	3.33	8.37	5.95	2.40	3.58	6.83	6.08	6.12
	3.58	-	6.55	5.55	3.35	8.53	6.12	2.47	3.67	6.92	6.23	6.23
	3.63	-	6.70	5.63	3.38	8.70	6.35	2.48	3.70	6.97	6.40	6.35
7	3.67	-	6.87	5.72	3.42	8.89	6.57	2.55	3.78	7.07	6.53	6.48
	3.67	-	7.03	5.78	3.47	9.05	6.77	2.58	3.87	7.13	6.67	6.58
	3.68	-	7.20	5.82	3.50	9.21	6.97	2.65	3.93	7.25	6.83	6.72
	3.72	-	7.32	5.85	3.55	9.35	7.18	2.67	3.98	7.33	7.00	6.80
8	3.75	-	7.50	5.92	3.60	-	7.33	2.80	4.08	7.42	7.17	6.97

TABLE 3.14 Growth rate of Z. mays roots growing in darkness with the root cap immediately removed after a 10 min pulse of white light (3.67 $Jm^{-2}s^{-1}$) at 3h.

	Sample No.																
Time (Hrs)	120	7/83	PL219	PL5	PL6	129	PL1	PL4	PL5	PL6	X	Z	n	$\bar{x}$	SD	SE	Overall mea
0-1	0.61	0.56	1.35	0.90	0.55	1.72	0.73	0.30	0.37	1.00	1.05	0.95	12	0.84	0.394	0.114	
1-2	0.53	0.97	1.15	1.05	0.45	1.61	0.95	0.30	0.58	1.26	1.06	0.98	12	0.91	0.360	0.104	0.86 ± 0.01
2-3	0.24	0.84	0.99	0.95	0.50	1.58	0.85	0.22	0.47	1.49	0.92	1.00	12	0.84	0.412	0.119	
3-4	0.15	0.69	0.73	0.70	0.16	0.70	0.30	0.18	0.33	0.63	0.63	0.60	12	0.48	0.227	0.063	
4-5	0.21	0.33	0.48	0.25	0.10	0.60	0.52	0.09	0.20	0.42	0.45	0.37	12	0.33	0.159	0.044	
5-6	0.19	0.26	0.67	0.25	0.14	0.44	0.70	0.06	0.29	0.41	0.57	0.65	12	0.39	0.211	0.059	0.41 ± 0.02
6-7	0.16	-	0.67	0.32	0.10	0.66	0.82	0.17	0.26	0.34	0.58	0.57	11	0.42	0.231	0.070	
7-8	0.08	-	0.63	0.30	0.18	0.70	0.76	0.25	0.30	0.35	0.64	0.49	11	0.42	0.226	0.068	

manifest themselves as changes in the growth rate of the root.

The typical response of Zea roots to incisions made in the root cap is shown for several representative roots in Figure 3.8A. The growth rate of these organs was not significantly ($p = 0.05$) affected by this incision treatment. Figure 3.8B shows the mean growth rate of 10 roots treated in this manner. There were slight changes between 0.63 and 0.69mm h^{-1} and 0.49 and 0.64mm h^{-1}, before and after treatment respectively, but none of these changes ere significant ($p = 0.05$) (App.1, Table 8). It therefore seems safe to conclude that any wounding responses, caused by cutting the root cap, are either non-existent, or so small that they do not affect the interpretation of the experiments reported in this thesis.

3.2.9 Dark to red light transition (peak 660nm)

Whilst carrying out a number of the experiments described in this chapter it was found that the magnitude of the response differed depending on whether a tungsten or a fluorescent lamp was used to illuminate the seedlings. Since fluorescent lamps are a richer source of blue light than red and far-red light, and tungsten lamps a richer source of red and far-red light than blue light, the question arose of whether or not the magnitude of the inhibition of the growth rate was dependent upon the wavelength of light used.

The increase in length of 3 roots illuminated with red light after 4h darkness is shown in Figure 3.9A. The increase in length was reduced by the exposure to red light. The rate of increase in length was found to be constant in both darkness and red light, and thus the response is similar to that when the roots were illuminated with white

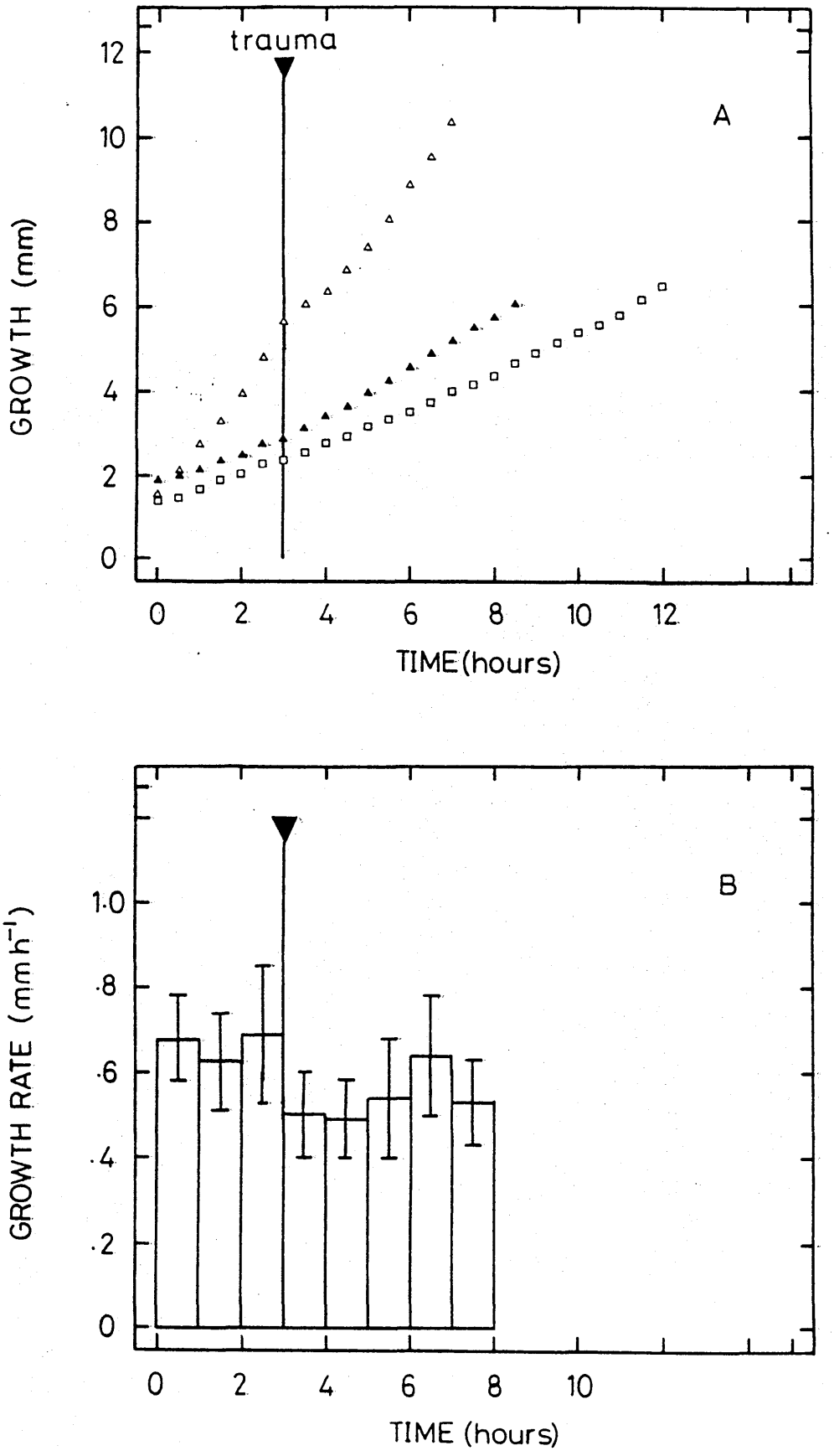


Figure 3.8 Increase in length (A) and mean growth rate (B) of Z. mays roots growing in darkness with incisions made in the root cap at 4 h.

TABLE 3.15 Length of *Z. mays* roots growing in darkness with incisions made in the root cap at 4h.

Time (Hrs)	Sample No. T1	131	134	T230	T231	139	141	144	T236	149
0	2.00	1.58	1.46	1.77	1.92	1.81	2.11	1.79	1.75	1.56
	2.17	1.63	1.53	1.90	1.97	1.89	2.37	1.95	2.07	1.81
	2.38	1.84	1.53	2.03	2.03	1.93	2.49	2.16	2.38	2.14
	2.62	2.11	1.58	2.08	2.13	2.18	2.63	2.37	2.67	2.42
1	2.80	2.28	1.75	2.22	2.18	2.30	2.74	2.51	2.93	2.72
	3.00	2.46	1.84	2.40	2.27	2.37	2.89	2.63	3.18	3.05
	3.18	2.63	1.93	2.58	2.37	2.42	2.98	2.72	3.52	3.33
	3.33	2.74	1.98	2.77	2.42	2.47	3.14	2.81	3.85	3.67
2	3.50	2.89	2.11	2.92	2.52	2.51	3.23	2.88	4.17	3.95
	3.78	3.07	2.21	3.08	2.67	2.60	3.33	2.98	4.60	4.39
	4.00	3.25	2.28	3.17	2.77	2.67	3.40	3.11	5.00	4.79
	4.22	3.40	2.39	3.32	2.85	2.72	3.47	3.23	5.42	5.16
3	4.33	3.54	2.39	3.42	2.90	2.74	3.54	3.28	5.73	5.61
	4.53	3.68	2.39	3.53	3.00	2.77	3.54	3.46	5.93	5.79
	4.67	3.77	2.58	3.95	3.15	2.81	3.63	3.54	6.12	6.05
	4.75	3.81	2.68	4.43	3.28	2.86	3.63	3.63	6.30	6.25
4	4.75	3.86	2.77	4.62	3.38	2.88	3.67	3.72	6.52	6.39
	4.83	3.96	2.89	4.82	3.55	2.96	3.70	3.81	6.68	6.60
	5.00	4.05	2.96	4.98	3.65	3.02	2.72	3.89	6.85	6.86
	5.10	4.16	3.09	5.13	3.80	3.05	3.72	3.98	7.02	7.11
5	5.20	4.25	3.21	5.28	4.00	3.07	3.75	4.02	7.25	7.42
	5.33	4.33	3.30	5.42	4.12	3.11	3.75	4.05	7.50	7.75
	5.50	4.40	3.39	5.58	4.25	3.12	3.75	4.12	7.77	8.07
	5.63	4.47	3.47	5.72	4.40	3.16	3.77	4.19	8.07	8.49
6	5.70	4.54	3.60	5.87	4.60	3.25	3.79	4.21	8.42	8.86
	5.83	4.61	3.70	6.02	4.72	-	3.81	4.25	8.77	9.26
	5.92	4.79	3.79	6.18	4.90	-	3.82	4.30	9.20	9.56
	6.08	4.86	3.89	-	5.07	-	3.88	4.33	9.48	9.96
7	6.30	5.00	4.00	6.53	5.17	-	3.89	4.40	9.67	10.35
	6.40	5.11	4.07	6.75	5.42	-	3.91	4.44	10.03	-
	6.67	5.23	4.23	6.90	5.53	-	3.93	4.47	10.25	-
	6.88	5.33	4.32	7.03	5.72	-	3.98	4.51	10.50	-
8	7.05	5.49	4.40	7.23	5.83	-	4.00	4.58	10.60	-

TABLE 3.16 Growth rate of *Z. mays* roots growing in darkness with incisions made in the root cap at 4h.

	Sample No.														
Time (Hrs)	T1	131	134	T230	T231	139	141	144	T236	149	n	$\bar{x}$	SD	SE	Overall mean gr
0-1	0.80	0.70	0.29	0.45	0.26	0.49	0.63	0.72	1.18	1.16	10	0.67	0.303	0.10	
1-2	0.70	0.61	0.36	0.70	0.34	0.21	0.49	0.37	1.24	1.23	10	0.63	0.342	0.11	0.66 ± 0.014
2-3	0.88	0.65	0.28	0.50	0.38	0.23	0.31	0.40	1.56	1.66	10	0.69	0.498	0.16	
3-4	0.37	0.32	0.38	1.20	0.48	0.14	0.13	0.44	0.79	0.78	10	0.50	0.314	0.10	
4-5	0.45	0.39	0.44	0.66	0.62	0.19	0.08	0.30	0.73	1.03	10	0.49	0.27	0.09	
5-6	0.50	0.29	0.39	0.59	0.60	0.18	0.04	0.19	1.17	1.44	10	0.54	0.424	0.13	0.54 ± 0.024
6-7	0.60	0.46	0.40	0.66	0.57	-	1.10	0.19	1.25	1.49	9	0.64	0.433	0.14	
7-8	0.75	0.49	0.40	0.70	0.66	-	0.11	0.18	0.93	-	8	0.53	0.267	0.09	

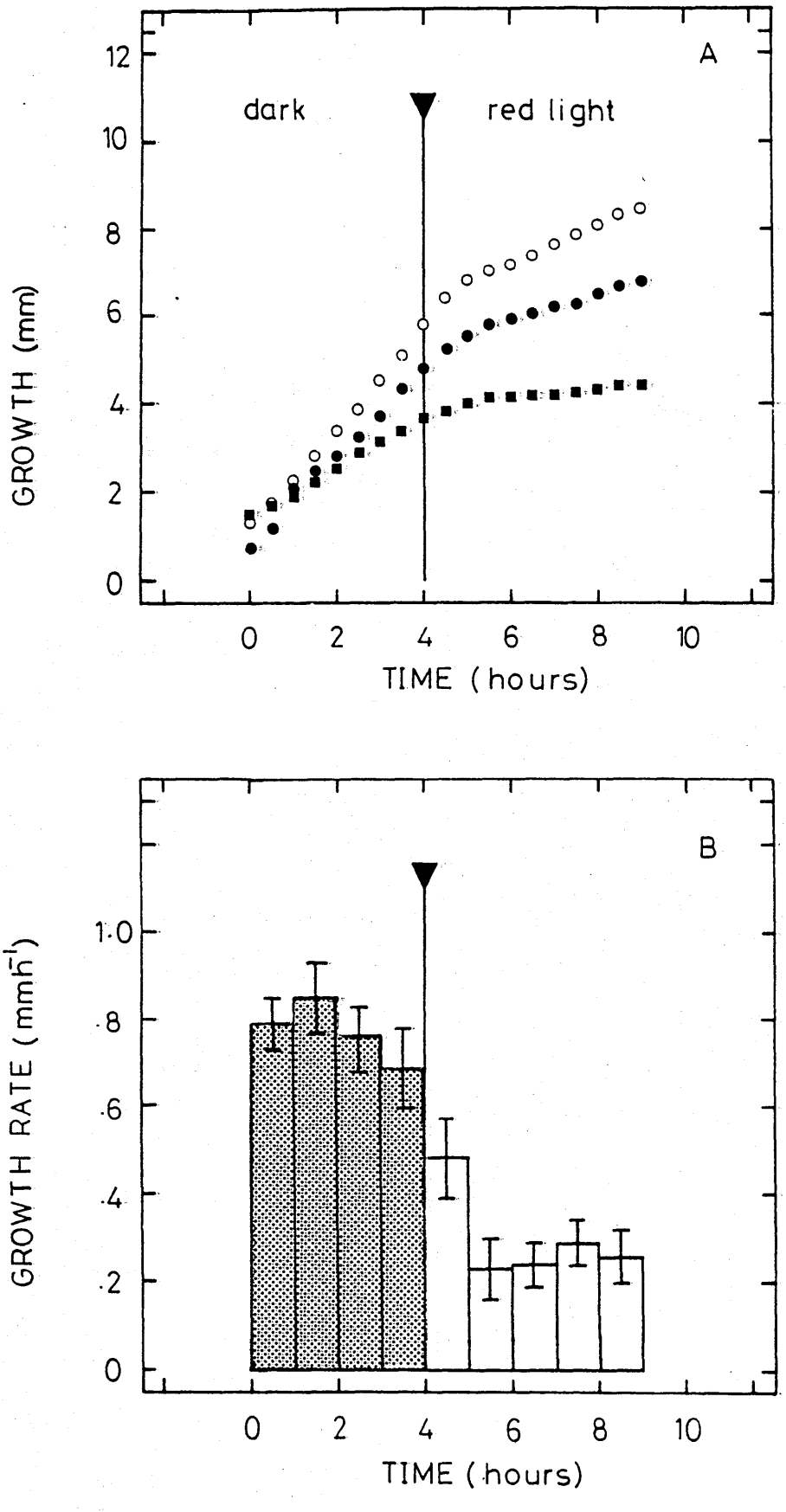


Figure 3.9 Increase in length (A) and mean growth rate (B) of *Z. mays* roots exposed to red light (660nm; 5.0 x 10^{18} quanta $m^{-2}s^{-1}$) after 4 h growth in darkness.

TABLE 3.17 Length of *Z. mays* roots exposed to red light (660nm; 5.0×10^{18} quanta $m^{-2}s^{-1}$) after 4h growth in darkness.

Time (Hrs)	Sample No. RL2	RL4	RL5	5L6	RL7	RL9	RL10	RL11	RL12	RL13
0	1.25	1.35	1.30	1.63	1.33	1.63	1.65	1.55	1.50	1.18
	1.42	1.57	1.47	1.72	1.47	1.85	1.92	1.72	1.58	1.42
	-	1.75	1.70	1.93	1.72	2.07	2.23	1.83	1.75	1.60
	1.68	2.10	2.00	2.22	1.88	2.30	2.50	1.92	1.83	1.07
1	1.97	2.32	2.30	2.48	2.07	2.47	2.75	2.10	1.93	1.87
	2.08	2.57	2.50	2.75	2.28	2.58	3.03	2.25	2.15	2.18
	2.20	2.80	2.82	3.00	2.45	2.70	3.27	2.38	2.33	2.45
	2.38	3.20	3.08	3.25	2.63	2.83	3.48	2.55	2.48	2.77
2	2.50	3.47	3.38	3.55	2.87	2.98	3.63	2.70	2.67	3.05
	2.62	3.73	3.62	3.83	3.13	3.13	3.83	2.82	2.82	3.23
	2.73	3.98	3.83	4.08	3.37	3.33	4.08	2.98	2.95	3.45
	2.83	4.27	4.02	4.33	3.63	3.48	4.20	3.12	2.10	3.70
3	2.92	4.57	4.17	4.55	3.83	3.62	4.43	3.27	3.17	3.92
	3.02	4.88	4.30	4.83	4.10	3.75	4.57	3.38	3.28	4.18
	3.07	5.15	4.47	5.10	4.35	3.88	4.75	3.45	3.43	4.43
	3.13	5.47	4.67	5.30	4.58	4.00	4.97	3.53	3.52	4.63
4	3.17	5.83	4.88	5.37	4.78	4.10	5.08	3.58	3.68	4.87
	3.22	6.15	5.07	5.53	5.03	4.17	5.17	3.63	3.78	5.10
	3.25	6.40	5.22	5.67	5.25	4.27	5.88	3.72	3.88	5.30
	3.27	6.63	5.37	-	5.42	4.28	5.35	3.73	3.98	5.48
5	3.30	6.83	5.43	5.85	5.55	4.32	5.40	3.75	4.02	5.65
	3.30	6.97	5.50	5.92	5.62	4.32	5.40	3.77	4.05	5.78
	3.33	7.07	5.55	5.93	5.73	4.32	5.40	3.78	4.08	5.90
	3.33	7.17	5.62	5.95	5.80	4.32	5.40	3.82	4.13	6.05
6	3.37	7.27	5.67	5.97	5.92	4.32	5.42	3.83	4.15	6.20
	3.40		5.78	5.98	5.97	4.32	5.42	3.87	4.15	6.33
	3.45	7.45	5.83	6.02	6.05	4.32	5.47	3.92	4.17	6.48
	3.50	7.58	5.93	6.07	6.13	4.32	5.50	4.02	4.17	6.57
7	3.52	7.72	6.02	6.16	6.25	4.32	5.58	4.05	4.18	6.75
	3.57	5.83	6.12	6.18	6.28	4.37	5.67	4.10	4.22	6.92
	3.62	7.92	6.18	6.27	6.33	4.38	5.72	-	4.25	7.07
	3.65	8.07	6.23	6.32	6.45	4.40	5.77	-	4.30	7.25
8	3.70	8.17	6.40	6.35	6.58	4.45	5.80	-	4.35	7.40
	3.73	8.25	6.53	6.43	6.67	4.60	5.83	-	4.37	7.48
	3.77	8.35	6.62	6.57	6.73	4.53	5.85	-	4.40	7.73
	3.82	8.45	6.73	6.57	6.78	4.53	5.88	-	4.43	7.88
9	3.85	8.52	6.78	6.60	6.83	4.55	5.92	-	4.47	7.97

TABLE 3.18 Growth rate of intact *Z. mays* roots exposed to red light (660nm; 5.0×10^{18} quanta $m^{-2}s^{-1}$) after 4h growth in darkness.

Time (Hrs)	Sample No. RL2	5L4	RL5	RL6	RL7	RL9	R40	R41	RL12	RL13	n	$\bar{x}$	SD	SD	Overall mean gr.
0-1	0.72	0.97	1.00	0.85	0.74	0.84	1.10	0.55	0.43	0.69	10	0.79	0.196	0.062	
1-2	0.53	1.15	1.08	1.07	0.80	0.57	0.88	0.60	0.74	1.18	10	0.85	0.244	0.077	0.77 ± 0.029
2-3	0.42	1.10	0.79	1.00	0.96	0.64	0.80	0.55	0.50	0.87	10	0.76	0.217	0.069	
3-4	0.25	1.26	0.71	0.82	0.95	0.48	0.65	0.33	0.51	0.95	10	0.69	0.296	0.094	
4-5	0.13	1.00	0.55	0.48	0.77	0.22	0.32	0.17	0.34	0.78	10	0.48	0.279	0.088	
5-6	0.07	0.44	0.24	0.12	0.37	0.00	0.02	0.08	0.13	0.78	10	0.23	0.231	0.073	
6-7	0.15	0.45	0.35	0.19	0.33	0.00	0.16	0.22	0.03	0.55	10	0.24	0.167	0.053	0.30 ± 0.041
7-8	0.18	0.45	0.38	0.19	0.33	0.13	0.22	-	0.17	0.55	9	0.29	0.137	0.046	
8-9	0.15	0.35	0.38	0.25	0.25	0.10	0.12	-	0.12	0.65	9	0.26	0.167	0.056	

light. The mean growth rate histogram, Figure 3.9B clearly shows the decrease in rate upon illumination. The average growth rate was decreased by 64% from 0.77 to 0.30mm h^{-1}. In darkness the growth rate initially rose to reach 0.85mm h^{-1} at 2h, before declining to 0.69mm h^{-1} just prior to illumination. The growth rate fell to 0.21mm h^{-1} 2h after the onset of the light period, after which it stayed between 0.24 and 0.27mm h^{-1} for the final 3h of the observation period. None of these changes in darkness and light were significant ($p = 0.05$) (App.1, Table 9).

3.2.10 Dark to blue light transition (peak 445nm)

Figure 3.10A shows the increase in length of several roots illuminated with blue light following 4h growth in darkness. As with red and white light 2 different rates of increase in length were observed; one in darkness and the other in light. The mean growth rate histogram (Fig. 3.10B) shows that the rate decreases slightly from 0.88 to 0.75mm h^{-1} in darkness. Upon illumination the rate is significantly reduced by 50% (App.1, Table 10) and, 5h after the onset of the light period, has attained a value of 0.34mm h^{-1}. The average growth rate over the 5h in blue light was 0.41mm h^{-1}.

The magnitude of the reduction of the growth rate appears to vary according to the wavelength of light with which the roots are illuminated. When the mean decreases in growth rate for the 3 samples are compared it is found that blue light is significantly more effective than white light ($p = 0.05$) but there is no significant

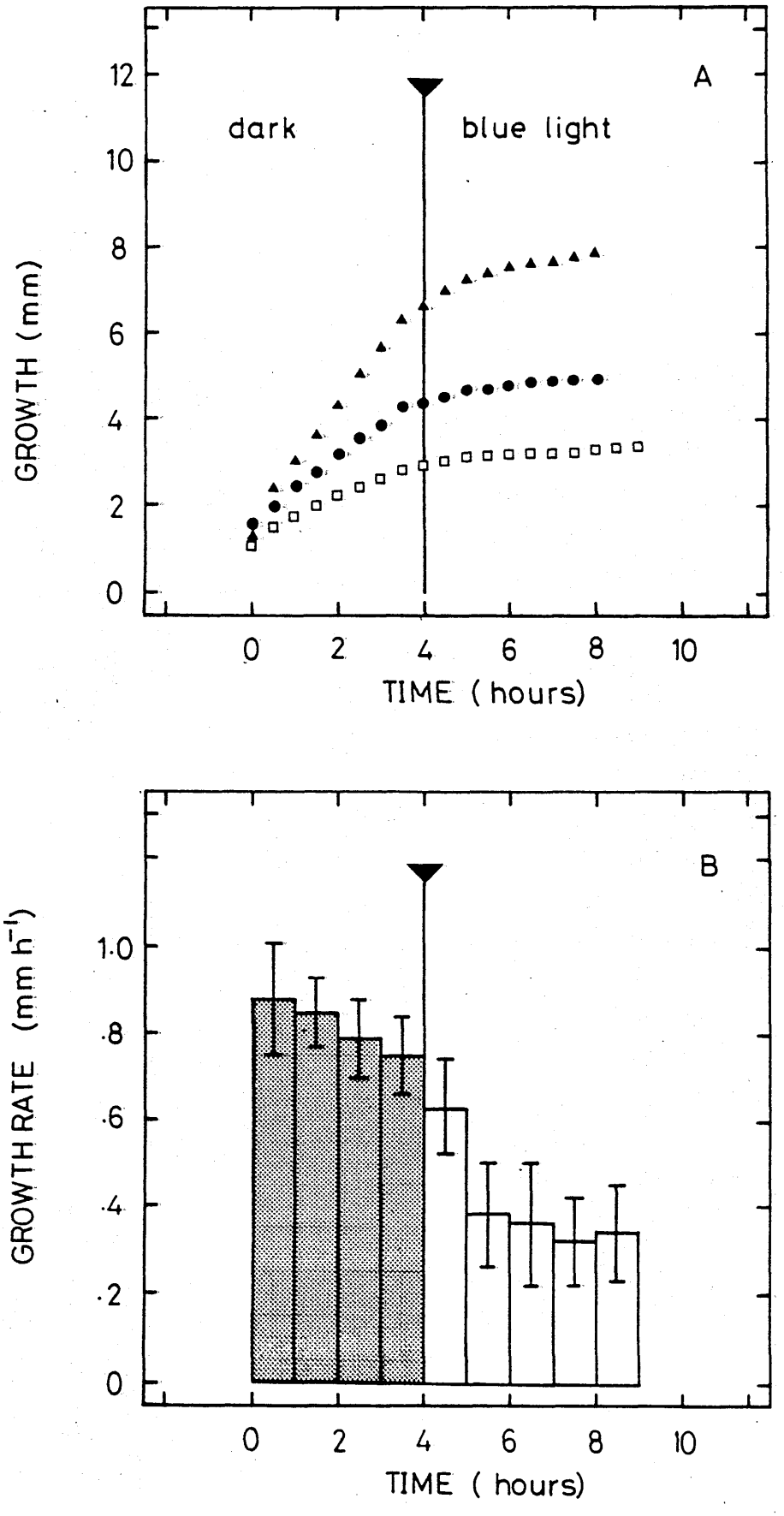


Figure 3.10 Increase in length (A) and mean growth rate (B) of Z. mays roots exposed to blue light (445nm; 4.2 x 10^{18} quanta $m^{-2}s^{-1}$) after 4 h growth in darkness.

TABLE 3.19 Length of intact *Z. mays* roots exposed to blue light (445nm; 4.2×10^{18} quanta $m^{-2}s^{-1}$) after 4h growth in darkness.

Time (Hrs)	Sample No. BL1	BL2	BL3	BL4	BL5	BL6	BL7	BL8	157	BL9	152	BL10	153
0	1.48	1.37	1.60	1.17	1.48	1.50	1.65	1.48	1.32	1.43	0.96	1.82	0.96
	1.50	1.50	1.87	1.33	1.77	1.88	1.98	1.87	2.04	1.75	0.98	-	1.26
	1.53	1.65	2.08	1.50	1.97	2.00	2.17	2.25	2.35	2.02	1.05	-	1.65
	1.67	1.82	2.37	1.62	2.13	2.15	2.40	2.47	2.72	2.30	1.18	-	2.00
1	1.92	2.02	2.62	1.77	2.42	2.42	2.67	-	3.07	2.67	1.32	2.02	2.35
	2.03	2.20	2.83	1.87	2.73	2.62	2.95	3.12	3.33	3.05	1.49	2.30	2.68
	2.12	2.37	3.12	2.02	2.92	2.78	3.17	3.42	3.68	3.32	1.67	2.40	2.96
	-	2.55	3.37	2.15	3.12	2.97	3.42	3.68	4.04	3.67	1.87	2.55	3.21
2	2.33	2.73	3.60	2.23	3.33	3.15	3.58	4.00	4.30	4.00	2.00	2.78	3.46
	2.48	2.95	3.85	2.33	3.53	3.35	3.77	4.32	4.67	4.40	2.14	2.93	3.63
	2.58	3.15	4.17	2.43	3.77	3.55	3.97	4.58	5.05	4.75	2.35	3.10	3.86
	2.78	3.35	4.48	2.55	3.92	3.77	4.25	4.88	5.37	5.05	2.54	3.20	4.02
3	2.87	3.58	4.80	2.60	4.05	3.90	4.45	5.05	5.68	5.33	2.70	3.50	4.21
	3.00	3.77	5.13	2.67	4.20	4.02	4.67	5.45	6.09	5.62	2.89	3.68	4.33
	3.13	4.02	5.45	2.77	4.35	4.25	4.90	5.85	6.25	5.90	3.07	3.87	4.47
	3.20	4.25	5.85	2.82	4.48	-	5.05	6.12	6.42	6.18	3.23	4.00	4.56
4	3.33	4.48	6.18	2.88	4.58	4.33	5.30	6.38	6.56	6.33	3.37	4.10	4.70
	3.40	4.75	6.50	2.95	4.72	4.37	5.47	6.80	6.79	6.62	3.54	4.15	4.79
	3.45	4.98	6.87	3.00	4.78	4.53	5.63	7.10	7.02	-	3.68	4.23	4.89
	3.53	5.22	7.13	3.08	4.85	4.63	5.82	7.42	7.18	7.00	3.87	4.30	4.96
5	3.63	5.42	7.53	3.10	4.92	4.70	5.97	7.72	7.30	7.13	3.95	3.32	5.02
	3.67	5.58	7.90	3.12	4.97	4.72	6.08	7.92	7.39	7.25	4.02	4.32	5.07
	3.68	5.72	8.33	3.13	5.00	4.73	6.15	8.20	7.47	7.35	4.14	4.33	5.09
	3.70	5.87	8.73	3.13	5.02	4.77	6.23	8.42	7.54	7.42	4.25	4.37	5.12
6	3.72	5.98	9.13	3.15	5.05	4.78	6.25	8.62	7.61	7.48	4.33	4.40	5.12
	3.73	6.12	9.52	3.15	5.08	4.82	6.28	8.78	7.61	7.56	4.39	4.42	5.12
	3.77	6.23	9.92	3.17	5.12	4.85	6.28	8.97	7.63	7.62	4.42	4.45	5.12
	3.80	6.38	10.52	3.17	5.15	4.87	6.28	9.10	7.67	7.70	4.46	4.47	5.14
7	3.88	6.55	10.90	3.18	5.22	4.88	6.32	9.43	7.68	7.75	4.51	4.48	-
	3.95	6.73	11.38	3.18	5.25	4.90	6.35	9.72	7.75	7.83	4.58	4.52	-
	4.00	6.90	11.73	3.22	5.30	4.92	6.40	10.03	7.77	7.90	-	4.57	-
	4.05	7.12	-	3.25	5.38	4.95	-	10.22	7.81	7.95	-	4.63	-
8	4.07	7.32	-	3.28	5.43	4.97	-	10.38	7.81	8.02	-	4.68	-
	4.12	7.52	-	3.30	5.48	5.02	-	10.63	-	8.08	-	4.72	-
	4.15	7.72	-	3.32	5.52	-	-	10.85	-	-	-	4.75	-
	4.25	7.90	-	3.32	5.57	-	-	11.00	-	8.20	-	4.78	-
9	4.32	8.03	-	5.62	-	-	-	11.20	-	8.27	-	4.82	-

TABLE 3.20 Growth rate of intact *Z. mays* roots exposed to blue light (445nm; 4.2×10^{18} quanta $m^{-2}s^{-1}$) ater 4h growth in darkness.

Time (Hrs)	Sample No. BL1	BL2	BL3	BL4	BL5	BL6	BL7	BL8	157	BL9	n	$\bar{x}$	SD	SE	Overall mean gr.
0-1	0.44	0.65	1.02	0.60	0.94	0.92	1.02	-	1.75	1.24	12	0.88	0.433	0.125	
1-2	0.41	0.71	0.98	0.46	0.91	0.73	0.91	-	1.23	1.33	12	0.85	0.271	0.078	0.82 ± 0.025
2-3	0.54	0.85	0.22	0.37	0.72	0.75	0.87	1.05	1.38	1.33	13	0.79	0.316	0.088	
3-4	0.46	0.90	1.38	0.28	0.53	0.43	0.85	1.33	0.88	1.00	13	0.75	0.328	0.091	
4-5	0.30	0.94	1.35	0.22	0.34	0.37	0.67	1.34	0.74	0.80	13	0.63	0.378	0.105	
5-6	0.09	0.56	1.60	0.06	0.13	0.08	0.28	0.90	0.31	0.35	13	0.38	0.423	0.117	
6-7	0.16	0.57	1.77	0.03	0.17	0.10	0.07	0.87	0.07	0.27	12	0.36	0.481	0.139	0.41 ± 0.050
7-8	0.19	0.77	-	0.10	0.21	0.09	-	0.95	0.13	0.27	9	0.32	0.295	0.098	
8-9	0.25	0.71	-	0.04	0.19	-	-	0.82	-	0.25	7	0.34	0.277	0.105	

difference between the effectiveness of red light compared to white and blue light. The approximate fluence rates of illumination used were 5.0×10^{18}, and 4.2×10^{18}, quanta m^{-2} s^{-1}, for red and blue light respectively. Bearing in mind that broad band filters were used, the similarity of these quantum fluence rates make it possible to state that the effect on the growth rate of roots was similar in both cases, at least at the fluence rates used indicating that both the red and blue spectral bands are capable of eliciting this photobiological response.

3.3.0 DISCUSSION

The results obtained when roots were given a dark to light transition treatment are consistent with the reports in the literature which state that light inhibits root elongation in *Zea mays* (H. Wilkins *et al.*, 1974a,b; H. Wilkins and Wain, 1974), that the perception of light by the root is almost instantaneous, and that the reduction persists for at least 6h (H. Wilkins *et al.*, 1974a). The reduction in root elongation is believed to be brought about by the light-induced production of inhibitor (H. Wilkins and Wain, 1974, 1975; H. Wilkins *et al.*, 1974a,b; Pilet, 1975b, 1976a, 1980) and it would perhaps be expected that, upon returning illuminated roots to darkness, the production of inhibitor would cease and hence, the growth rate would regain its initial value. A certain lag-period of sufficient duration for inhibitor already present in the elongation zone to be metabolised would also be expected. When seedlings in the present study were returned to darkness for 8h, following 4h illumination, their rate of elongation did not increase significantly

and thus did not regain its initial value, at least during the observation period. However, it was established that the growth rate of roots was significantly reduced by illumination (3.2.1). The light-induced inhibition of elongation is reported to be dependent upon the presence of an intact root cap (H. Wilkins and Wain, 1974), and the results in this study confirm this finding with decapped roots showing no significant change in growth rate when illuminated (3.2.3).

On the basis of these facts it would be expected that the growth rate would not change when roots were decapped in darkness. However, this assumption is at variance with the findings in this thesis, where the growth rate of roots in darkness was reduced by decapping (3.2.4). H. Wilkins et al. (1974b) also investigated the effect of decapping roots in darkness and they found that there was no change in their rate of elongation. Although this finding is inconsistent with those of the present study, it is in agreement with the conclusions of Baehler and Pilet (1981), who carried out studies using root segments.

In accordance with the published reports an increase in growth rate of roots decapped in light would have been expected. H. Wilkins et al (1974b) reported such an increase which resulted in an elongation equivalent to that of intact dark-grown roots. An increase in growth rate was also reported by Pilet (1972a, 1977) but only during the first 3h after decapping. These accounts are in disagreement with those of Juniper et al. (1966) and earlier work by Pilet (1971a) the results of which led to the conclusion that decapping in light did not result in an increase in the growth rate. However, in these studies measurements were not begun until 4h after

decapping, so any transient change in rate, during this time, would have been missed. To complete the list of possible growth rate changes, Baehler and Pilet (1981) found that the elongation of decapped horizontal segments was less than that of intact, horizontal, segments. Thus, there is a great deal of disagreement in the published reports as to the effect of decapping on the growth rate of illuminated seedlings. The results obtained in the present study are in agreement with those of Juniper et al. (1966) and Pilet (1971a) with no measurable change in the growth rate upon decapping.

As suggested earlier (3.2.5), the absence of a change in the growth rate on decapping light-grown roots could be due to the fact that during the first 3h in light saturating quantities of inhibitor were produced and these did not decline sufficiently after the removal of the root cap to be reflected as a change in the growth rate. Indeed, H. Wilkins et al. (1974a) found that the reduction in root elongation was related to the duration of the light period. For example, a one second flash of light was sufficient to cause a 33% reduction in root elongaton, and one minute of light a 43% reduction. It is therefore possible that a large amount of inhibitor had accumulated over the 3h prior to decapping.

In this study it was found that 10 min light reduced the growth rate to a lesser extent than 4h light. Despite the shortness of the 10 min light period the growth rate of the roots stayed at its reduced level with no evidence of an increase, for at least 8h following illumination.

It was thought that since the root cap was the source of the light-induced inhibitor (Gibbons and Wilkins, 1970; H. Wilkins et al.,

1974a,b; H. Wilkins and Wain, 1974, 1975; Pilet, 1975a) removal of the root cap after 10 min light should, unless the movement was very rapid, prevent inhibitor moving back to the elongation zone. This removal of the source of inhibitor should be demonstrated by a reduction in the amount of inhibition of the root's growth rates, as compared to that observed when only the 10 min light was given. The result of decapping after the 10 min light was a slightly greater reduction in rate than found when light alone was given, and slightly less than that with 7h light. It thus appears that decapping immediately after a short light period increases, rather than decreases, the inhibition of root elongation.

It is reported (Pilet and Ney, 1978) that the light effects are very rapid, occurring within 5 min of illuminating the root cap. Feldman in his review of 1984 questions, whether or not, such a rapid response can be solely accounted for by movement of chemical inhibitors; the apparently rapid movement of information found in the present study appears to support this criticism, and such a rapid transmission of the message is indicative of an electrical signal. It is known that when a vertical root is placed horizontally an asymmetry in electrical current is established, at the root tip, within 30s of displacement with the flow of current on the upper side being basipetal and on the lower side acropetal. Furthermore, within 3 min the basipetal flow on the distal part of the meristem changes to an acropetal flow, whereas, that on the lower side, remains a basipetal current. This change in the direction of current flow in the root indicates a connection between current-flow and transduction of information from the root cap to the elongation zone (Behrens,

Weisensel and Sievers, 1982a). Thus it is at least possible that the observed inhibition of elongation may be brought about by electrical and chemical signals passing from the root cap to the elongation zone.

Incisions were made in the root cap to ensure that the results obtained in the experiments involving the removal of the root cap were not a combinaton of the growth response and wounding effects. Pilet (1973b) tested the effect of decapitation on the root by removing the cap and then immediately replacing it on the root-tip. The results of these experiments showed that there was no effect on the growth rate. This method was not used in the present study due to the difficulty in ensuring that the root cap was replaced exactly back on the root-tip. H. Wilkins et al. (1974b) made one-millimeter vertical incisions in the tips of Zea roots and found no enhancement of elongation. This method was similar to that used in the present study where the same conclusion was reached.

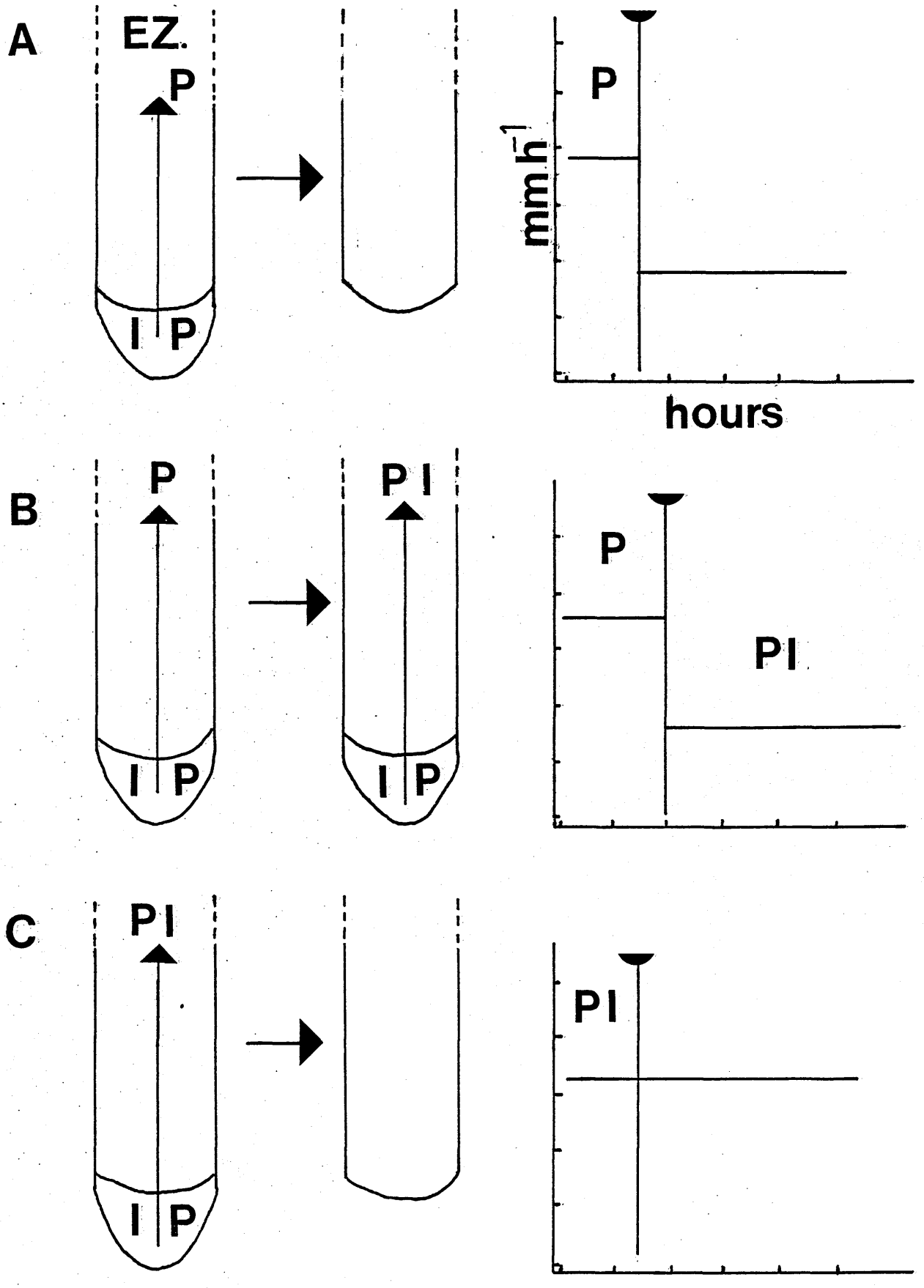
Thus the results of this study confirm those of a number of other studies reported in the literature. It is, however, difficult to explain some of the results with regard to the light-induced production of inhibitor being responsible for the reduction in growth rate. In particular a new explanation has to be sought for the observed inhibition of growth rate upon decapping roots kept in darkness. One possible explanation of the latter response is that at least one growth promoting substance is produced in darkness, and just as the light inhibition of elongation is dependent upon the presence of the root cap, the same may apply to this dark production of promoter. Thus, the removal of the root cap in darkness would remove the source of promoter production/release and hence lead to a reduction in the growth rate.

It is, therefore, possible that a growth promoter may be produced by the root cap, and this hypothesis requires that the observed growth rate changes discussed so far in this chapter are re-examined, and the various conclusions expanded to encompass dark-production of promoter. It is equally feasible that more than one promoter is produced by the root cap, but since the simplest explanation is of only one promoter this latter possibility will be considered in developing the new hypothesis of growth regulator levels involved in the growth rate changes in the root.

The simplest, but by no means only, explanation of the observed growth rate changes reported in this chapter, would be one involving both promoter and inhibitor, production and release, by the root cap. In darkness it is assumed that more promoter is synthesised than inhibitor, and that only promoter, or a net promoting influence, is transported to the elongation zone. Thus, when the root cap is removed, the level of promoter is reduced, and this change in the growth regulator levels would be manifest as a reduction in the growth rate (Fig. 3.11A).

Having proposed this promoter production in darkness it is necessary to ask whether or not this theory can also explain the light induced inhibition of growth, observed when roots were exposed to light after a 4h dark period. In fact, the new hypothesis is applicable, if there was production of promoter in darkness, and if on exposure to light, this promoter production was replaced or accompanied by production of inhibitor, resulting in a particular ratio of these 2 opposing influences such that there was a net inhibiting influence on root growth. The change from just promoter,

Figure 3.11 A diagrammatic representation of the possible growth regulator changes underlying the observed growth rate changes in *Z. mays* roots when (A) decapped in darkness, (B) exposed to darkness then light, and (C) decapped in light. Where EZ is the elongation Zone, P is a net promoting influence and I is a net inhibiting influence.


A
EZ.
P
I P
P
mm h⁻¹
hours
B
P
I P
P I
P
PI
C
PI
I P
PI

to a balance between promoter and inhibitor, would be manifest as a net reduction in the overall growth rate (Fig. 3.11B). This pattern of events can also explain why exposure of a decapped root to light has no influence on the growth rate. Furthermore, it is now possible to offer a further explanation why no change was observed in the growth rate upon decapping illuminated roots. When the root cap is removed from roots in light the site of production of both inhibitor and promoter is removed and therefore the levels of both these regulators would decrease. The fact that no change in the growth rate is observed over the 14h observation period suggests that the decline in the growth regulator levels is very slow (Fig. 3.11C). Objections could arise due to the fact that it has been previously shown that on removal of the root cap, promoter levels rapidly decline seen as a decrease in growth within one hour of decapping (3.2.4). There are, however, numerous explanations of this apparent discrepancy, a few of which are itemised below:-

1) in light, promoter is transformed so that it is no longer rapidly metabolised;

2) promoter/inhibitor interaction stops rapid metabolism;

3) a different promoter is produced in light to that in darkness: In the dark to light transition experiment there is photodestruction of the original promoter and a new promoter is produced;

4) promoter is photodestroyed and only inhibitor is present.

When roots were exposed to light for only 10 min there was no significant difference in the reduction in the growth rate to that when they were exposed for up to 7h. The reduction in growth rate of

roots, decapped immediately after 10 min illumination, was also not significantly different to either that in roots just given the light exposure, or that for roots given 7h light. Thus, a 10 min light period appears to be as effective as 7h illumination, possibly indicating very rapid movement of inhibitor. The rate of decay of inhibitor is again shown to be slow since the growth rate did not rise during the 8h following illumination. This slow decay seems feasible since H. Wilkins et al. (1974a) have reported that it takes between 9 and 12h, for the inhibition caused by a one second flash of light to decay. Furthermore, the level of inhibitor produced must have been saturating since it has to be assumed that once the roots are returned to darkness the promoter is still synthesised, and released, by the root cap. When the roots are decapped following 10 min illumination, not only is the source of inhibitor removed, but also that of promoter, thus promoter breakdown must also be slow.

It, therefore, appears that the hypothesis of dark-production of promoter can account for the observed growth rate changes. The changes in growth regulator levels may be far more complex than assumed, but in this thesis it has only been possible to describe and discuss the observed growth rate changes caused by altering certain environmental conditions, and it was not possible to obtain any direct information as to the underlying changes in growth regulator levels.

Since it has been outside the scope of this thesis to locate and identify the growth regulators involved in the growth rate changes in roots, the published literature has been the source of such information. Results presented in this chapter show that roots have the capacity to grow and regulate their growth rate without the

presence of the root cap (3.2.3). This independence could be accounted for by the slow decay of regulators which had accumulated in the elongation zone prior to decapping. Alternatively, it is reasonable to assume that the decapped roots continue to grow at a steady rate under the control of regulators that are acropetally transported in the root. An acropetal flow of a number of regulators such as IAA has been demonstrated (Pilet, 1964). These regulators (ABA, IAA, Gibberellins) come from either the caryopsis (Rivier and Pilet, 1974; Pilet, 1975; Pilet et al., 1979), the differentiated regions of the root (Reinhold, 1978) or the shoot (Iino and Carr, 1982). One or a combination of these regulators could control the growth of decapitated roots. If such acropetally transported regulators can control the growth of roots it must follow that in intact roots the growth rate is regulated by a balance between acropetally and basipetally transported regulators (Pilet and Senn, 1980; Beffa and Pilet, 1982). It thus appears that the role of the cap could be one of 'finely-tuning' the growth rate of the root.

Having discussed the movement of regulators in the root and proposed a hypothesis involving promoters and inhibitors consideration must now be given to which regulators have been identified in the root and root cap, and whether any of these compounds can fulfil the role of either the proposed promoter or inhibitor. In the introduction to this thesis the presence of gibberellins, cytokinins, Ca^{2+}, K^{+}, ^{32}P, IAA, ABA and the unidentified compounds of Suzuki et al. (1979) and Feldman (1982) in the root was mentioned. As discussed in the introduction, most of these ions and compounds are inhibitory in their action on root elongation. There is, however, evidence that these,

and other substances in the root, can promote root growth. The best known of these promoting compounds is IAA. IAA is, however, acropetally transported in the stele (Scott and Wilkins, 1968; Bowen et al., 1972) and although it is found in the root cap (Rivier and Pilet, 1974) it appears that the direction of transport is inconsistent with the theory of a promoter produced in the cap. Despite this obvious objection, IAA could still be the promoter involved in the dark-growth of roots if there were to be a sensitiser, rather than IAA itself, which travelled back to initiate IAA's growth promoting properties.

Mertens and Weiler (1983) used the very sensitive technique of radio-immunoassay to examine the distribution of endogenous regulators in a variety of plant organs. Following their observation that there was only a transient asymmetrical distribution of ABA in Zea roots, they examined the effect of exogenous ABA on the endogenous ABA levels and root growth. They applied ABA unilaterally to vertical root-tips and found that ABA concentrations between 10^{-8} and 10^{-3} M, slightly enhanced elongation compared with the controls. Mertens and Weiler concluded that it was this stimulation, rather than an inhibition of growth, which induced root curvature. Wareing et al. (1968) have also shown that ABA is stimulatory in its action in circumstances in which it antagonises the action of other inhibitory growth regulators. Thus ABA, at certain concentrations, could be the growth rate promoter; this conclusion is, however, inconsistent with the fact that H. Wilkins and Wain (1974) could not detect any ABA in extracts from dark-grown roots.

There are in addition to IAA and ABA, a number of as yet

unidentified compounds in the root which promote root growth. Examination of assay data in various reports in the literature indicate that in root extracts there are a number of compounds which are promoters of root elongation. For example, the chromatograms of extracts from light-grown seedlings, presented by H. Wilkins et al. (1974a) show up to 20% promotion of growth by compounds at a variety of R_F values. These promoters could possibly be found to be in much greater amounts in extracts of dark-grown seedlings.

Feldman (1982) found a promotory influence in the extract of a 2mm portion of root, taken from 1mm behind the apex. Using the stomatal closure test for ABA, he observed larger apertures, than in controls, for roots given 60 and 120 min illumination. These extracts were from what would normally be the acid-inhibitor zones of the chromatogram. The stimulation of stomatal opening observed, Feldman suggests, may be caused by the 'acid' inhibitor which has reached low enough levels in these segments to be stimulatory to growth. Thus, it again appears, that a compound identified as being inhibitory in its action can, at certain concentrations, promote root growth.

In summary, it appears that the growth rate changes observed in the experiments reported in this chapter confirm the reports of earlier researchers. An expansion of ideas as to the underlying changes in growth regulators has been necessary to encompass all the observed changes. In the published literature it has been possible to find evidence of a number of growth regulators which could possibly have a role as the growth promoter which is thought to be involved in regulation of root growth.

CHAPTER FOUR

GRAVITROPIC CURVATURE STUDIES (I)

4.0.0 INTRODUCTION

Gravitropic curvature has been studied over many years, the most commonly used method of estimating the angle of bending being that of exposing a sample of seedlings to a particular treatment for several hours and then calculating the average curvature of the sample. However, just as the rate of straight growth varies from one organ to the next (Chapter 3) the curvature of an individual root is different to that of another root, and it may be that the mean curvature quoted is not representative of the behaviour of the individuals in the sample.

Measuring the angle of curvature after a fixed period of time using destructive sampling gives no information about the way in which individual roots respond to the gravitational stimulus over time. The final angle measured could have developed in a number of ways:-

1) a steady increase in curvature over the whole time period;

2) a significant lag phase followed by rapid bending;

3) rapid bending to the final angle and then no further curvature; or

4) rapid bending to an angle greater than the final angle, followed by straightening out; - an "overshooting" mechanism.

Previous studies have been restricted by the technology at the time they were performed and advances in the field of I.R. (infra-red)-sensitive camera equipment have justified reinvestigating

some of the basic features of gravitropic curvature using radiation of a non-physiologically active wavelength to manipulate the seedlings and record curvature.

The curvature studies reported in this chapter have been carried out firstly, to compare the results obtained using samples of roots with those of individual roots and, secondly, to elucidate how the curvature develops over time using continuous recording and ultimately to relate these to changes in growth regulator levels in the organ.

4.1.0 METHODS

4.1.1 Samples of ten roots at low magnification

Seedlings of Zea were grown and selected as described in chapter 2. A sample of 10 seedlings was studied using a magnification of x1 to x1.5 lifesize. The seedlings were contained in a perspex box 21 x 3.5 x 6.5cm with a ten-hole holder, and this was placed in front of the recording camera with the roots orientated horizontally. The seedlings were continuously illuminated with white light from the start of the recording period, which was of between 6 and 12h duration, and video pictures were taken every 30 min. The experiment was repeated 4 times and data were obtained for 39 roots since 1 root out of a total of 40 failed to grow.

4.1.2 Samples of one to three roots at higher magnification

The magnification used when recording the gravitropic curvature of the roots was increased to between x8 and x14 lifesize; this enabled measurement of curvature to be more precise than in the

previous experiments using a lower magnification. The number of seedlings in a sample varied from 1 to 3 depending upon the magnification used. Initially the roots were orientated vertically and straight growth recorded. After 2h the box containing the seedlings was rotated so that the radicles were suspended horizontally, and recordings were made over a further 4 to 6h. The curvature was studied in both darkness and continuous white light.

4.2.0 RESULTS

4.2.1 Low magnification: continuous light

Figure 4.1A, B and C each show 3 roots taken from 3 different samples of roots each being examined on one of 3 separate occasions. The data for all 39 roots examined are presented in Table 4.1. The data show that the roots complete a period of rapid curvature within approximately 2 to 3h, during which time they have almost reached their maximum angle. In the majority of roots there appears to be a lag phase of 30 min, but in a number the curvature began between the first reading at 0h and the second reading at 30 min. After 2 to 3h the rate at which the roots bend decreases and the angle of curvature fluctuates about the final 'average' angle of response which varies from root to root.

The maximum angle of curvature is also found to be different in different roots, for example, in Graph 4.1C the maximum angles shown are 71°, 81°, and 105°, under the same experimental conditions.

The mean curvature of each of the 3 samples of roots was calculated and the data are plotted in Figures 4.1D, E and F. The curves obtained are in each case much smoother than those plotted

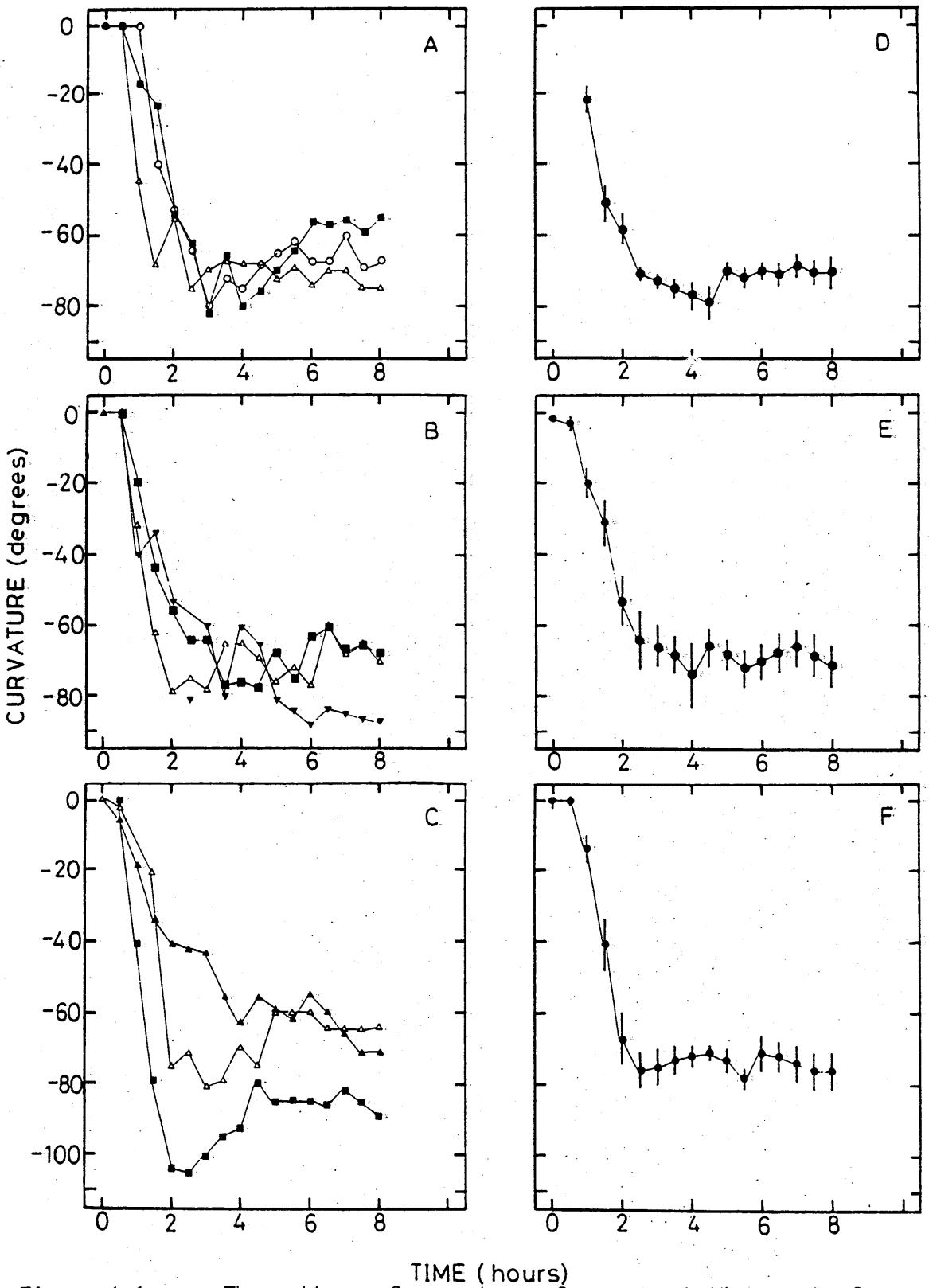


Figure 4.1 The pattern of curvature of representative roots from 3 samples (A, B and C) and the respective mean curvature of the samples (D, E and F) of Z. mays roots exposed to white light (3.67 $Jm^{-2}s^{-1}$).

TABLE 4.1 Curvature of samples of up to 10 *Z. mays* roots growing in continuous white light.

Time (Hrs)											$\bar{x}$	SE
0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
1	-17	-16	-25	-23	-19	0	-20	-45	-45	-10	-22	24
	-24	-41	-67	-68	-65	-40	-55	-60	-68	-60	-52	5
2	-55	-35	-67	-77	-67	-53	-61	-62	-55	-50	-58	4
	-62	-67	-71	-72	-78	-64	-76	-82	-75	-70	-72	2
3	-82	-58	-64	-76	-82	-80	-75	-75	-70	-75	-74	2
	-66	-73	-68	-75	-92	-72	-82	-75	-67	-70	-74	3
4	-80	-71	-78	-61	-100	-75	-92	-74	-68	-70	-77	4
	-76	-78	-66	-76	-116	-68	-86	-89	-68	-67	-79	5
5	-70	-73	-65	-67	-85	-65	-80	-65	-72	-60	-70	2
	-64	-73	-71	-72	-80	-62	-90	-74	-69	-60	-72	3
6	-56	-73	-65	-75	-74	-67	-83	-70	-74	-66	-70	2
	-57	-72	-72	-81	-75	-67	-89	-65	-70	-65	-71	3
7	-56	-67	-72	-75	-77	-60	-88	-66	-70	-61	-69	3
	-59	-78	-71	-73	-77	-69	-93	-64	-75	-55	-71	3
8	-55	-76	-74	-76	-70	-67	-97	-63	-75	-53	-71	4

											$\bar{x}$	SE
0	+ 3	- 3	0	-21	0	0	0	-12	0		- 4	3
	+ 5	- 4	0	-20	-10	- 6	0	-24	0		- 7	3
1	+ 5	- 5	-10	-25	-20	-15	-35	-39	- 7		-17	5
	+ 2	-17	-15	-26	-22	-47	-45	-31	-12		-24	5
2	0	-33	-29	-30	-49	-60	-63	-55	-27		-38	7
	-41	-33	-43	-26	-48	-67	-65	-42	-52		-46	5
3	-17	-45	-52	-32	-52	-82	-57	-56	-62		-51	6
	-29	-62	-65	-41	-35	-81	-67	-55	-70		-56	6
4	-55	-55	-68	-45	-58	-83	-62	-62	-74		-62	4
	-65	-60	-67	-52	-47	-86	-68	-47	-82		-64	5
5	-82	-58	-75	-60	-55	-92	-68	-63	-89		-71	5
	-80	-65	-72	-40	-52	-90	-60	-52	-102		-68	7
6	-85	-57	-73	-44	-50	-95	-66	-51	-104		-69	7
	-85	-50	-50	-45	-50	-93	-68	-55	-104		-67	8
7	-85	-54	-60	-35	-55	-93	-67	-64	-114		-70	8
	-72	-60	-63	-31	-44	-93	-65	-48	-116		-66	9
8	-65	-57	-69	-38	-50	-96	-65	-53	-122		-68	9

											$\bar{x}$	SE
0	-11	0	0	0	0	0	0	- 4	0	- 6	- 2	
	-10	0	0	0	0	0	0	-11	0	-12	- 3	
1	-26	-32	- 9	-22	- 5	-40	0	-13	-20	-33	-20	
	-60	-62	-42	-32	-26	-34	0	-34	-44	-45	-31	
2	-76	-79	-45	-75	-41	-53	0	-51	-55	-55	-53	
	-75	-75	065	-80	-76	-81	0	-60	-65	-66	-64	
3	-81	-78	-76	-72	-81	-60	-11	-67	-64	-62	-65	
	-74	-65	-72	-82	-67	-80	-22	-78	-77	-66	-68	
4	-83.	-65	-70	-91	-83	-60	-72	-71	-76	-71	-74	
	-70	-69	-56	-83	-80	-65	-29	-72	-77	-65	-67	
5	-66	-76	-68	-83	-75	-81	-35	-70	-67	-60	-68	
	-75	-72	-68	-86	-77	-87	-31	-81	-75	-65	-72	
6	-81	-77	-70	-85	-63	-88	-39	-72	-63	-62	-70	
	-76	-60	-62	-91	-68	-84	-32	-74	-61	-60	-67	
7	-75	-68	-66	-80	-65	-85	-23	-71	-68	-62	-66	
	-77	-65	-75	-82	-72	-86	-16	-77	-65	-70	-69	
8	-85	-70	-68	-86	-69	-87	-23	-75	-68	-75	-71	

											$\bar{x}$	SE
0	0	0	0	-1	0	0	0	+10	-12	0	0	2
	-6	0	-2	0	0	0	0	+10	- 4	0	0	1
1	-19	-20	-19	-14	-24	-41	-12	+ 7	- 5	0	-15	4
	-34	-54	-21	-23	-55	-79	-35	-65	-10	-42	-42	7
2	-41	-80	-75	-53	-81	-104	-69	-82	-20	-67	-67	8
	-42	-81	-72	-81	-76	-105	-83	-73	-70	-76	-76	5
3	-43	-73	-81	-80	-73	-101	-66	-73	-67	-92	-75	4
	-56	-75	-79	-66	-73	-95	-55	-76	-74	-82	-73	4
4	-63	-71	-70	-68	-64	-93	-62	-74	-68	-83	-72	3
	-56	-72	-75	-70	-80	-80	-61	-66	-72	-73	-71	2
5	-59	-80	-60	-65	-76	-85	-64	-90	-83	-70	-73	4
	-62	-72	-60	-67	-76	-85	-52	-68	-76	-65	-68	3
6	-55	-72	-60	-65	-81	-85	-52	-64	-105	-66	-71	5
	-60	-73	-65	-65	-77	-86	-55	-70	-103	-68	-72	4
7	-66	-74	-65	-65	-78	-82	-65	-74	-112	-63	-74	5
	-71	-73	-65	-70	-78	-85	-65	-72	-113	-63	-76	5
8	-71	-70	-64	-80	-77	-90	-63	-65	-112	-68	-76	5

Overall mean $\bar{x}$	SE
- 1	± 0.80
- 2	0.99
-18	2.20
-40	3.30
-55	3.5
-65	3.1
-66	3.0
-68	2.5
-72	1.8
-70	2.3
-70	1.8
-70	2.2
-70	2.4
-69	2.5
-69	2.6
-70	2.9
-71	2.9

TABLE 4.2 Curvature of *Z. mays* roots sampled one, two or three at a time whilst growing in darkness.

Sample No.	46	50		52	55		036c	038c	039c		045c		$\bar{x}$	SE
Magnification	x14	x14		x14	x14		x8	x8	x8		x8.5			
Time after turning horizontal (Hrs)														
0		+22	+20	0	-13	0							+7.25	8.38
	0	+10	+12	0	-10	-10	- 8	0	0	-25	- 9	+18	1.83	3.37
	-11	0	0	0	-16	-11	-10	0	0	-30	-14	+10	6.00	3.33
1	-17	-18	-28	-11	-22	-24	- 8	-15	-30	-28	-16	+15	16.33	3.52
	-17	-30	-32	-12	-34	-31	-15	-32	-37	-41	+21	+18	23.67	4.62
	-22	-38	-37	-20	-39	-37	-16	-35	-39	-42	-22	+14	27.75	4.60
	-24	-45	-35	-18	-45	-40	-24	-35	-38	-38	-25	+11	29.67	4.48
2	-22	-52	-35	-19	-49	-42	-20	-38	-41	-37	-26	+10	30.92	4.88
	-22	-52	-25	-23	-48	-44	-24	-39	-47	-37	-29	0	32.50	4.28
	-20	-47	-29	-28	-46	-44	-16	-48	-43	-37	-29	0	32.25	4.28
	-20	-48	-22	-30	-51	-48	-20	-38	-41	-38	-30	0	32.17	4.30
3	-20	-44	-15	-36	-50	-42	-13	-43	-40	-34	-30	0	30.58	4.41
	-20	-46	-15	-30	-47	-41	-12	-40	-45	-30	-29	0	29.58	4.39
		-43	-17	-30	-47	-42	-19	-42	-43	-35	-33	0	31.91	4.37
		-46	-15	-30	-47	-43	-15	-43	-44	-34	-35	0	29.00	5.68
4		-49	-15	-30	-50	-40	-15	-40	-40	-33	-33	0	31.36	4.68

Max. av. angle 37 ± 4.16°

using the data for single roots. Angles approximately equal to the maximum angle of curvature for the sample are achieved by the end of the period of rapid curvature, which again lasts between 2 and 3h. After this time there is little variation in the curvature. Thus, a slightly different pattern of curvature is obtained from the mean data which reveal little of the fluctuations in angle that occur as the individual roots hunt around their final 'average' angle of response.

4.2.2 Higher magnification. Samples of one to three roots: continuous darkness

The curvatures exhibited by 5 individual roots, which are a representative sample of a total of 12 roots examined on a number of separate occasions, are shown in Figure 4.2A and B. The roots rapidly curve to their maximum angle during the first 2 to 2.5h of gravistimulation after which time their angle of curvature fluctuates about an angle, which is generally slightly less than the maximum for each particular root. In addition to the maximum angle and the angle about which the roots' curvature fluctuates, having a different value for different roots, the amplitude of the oscillations observed also varies from root to root. In the 12 roots examined in the present study the minimum amplitude of the oscillations was 4° and the maximum 20° (Table 4.3). Furthermore the frequency of the oscillations varies from between 15 min to 45 min.

The mean curvature of the 12 roots was calculated and is shown in figure 4.2C. The rapid curvature during the first 2h is clear, as it is in the individual curves, but after this time the curve is very smooth with only a 1° or 2° change in the average angle

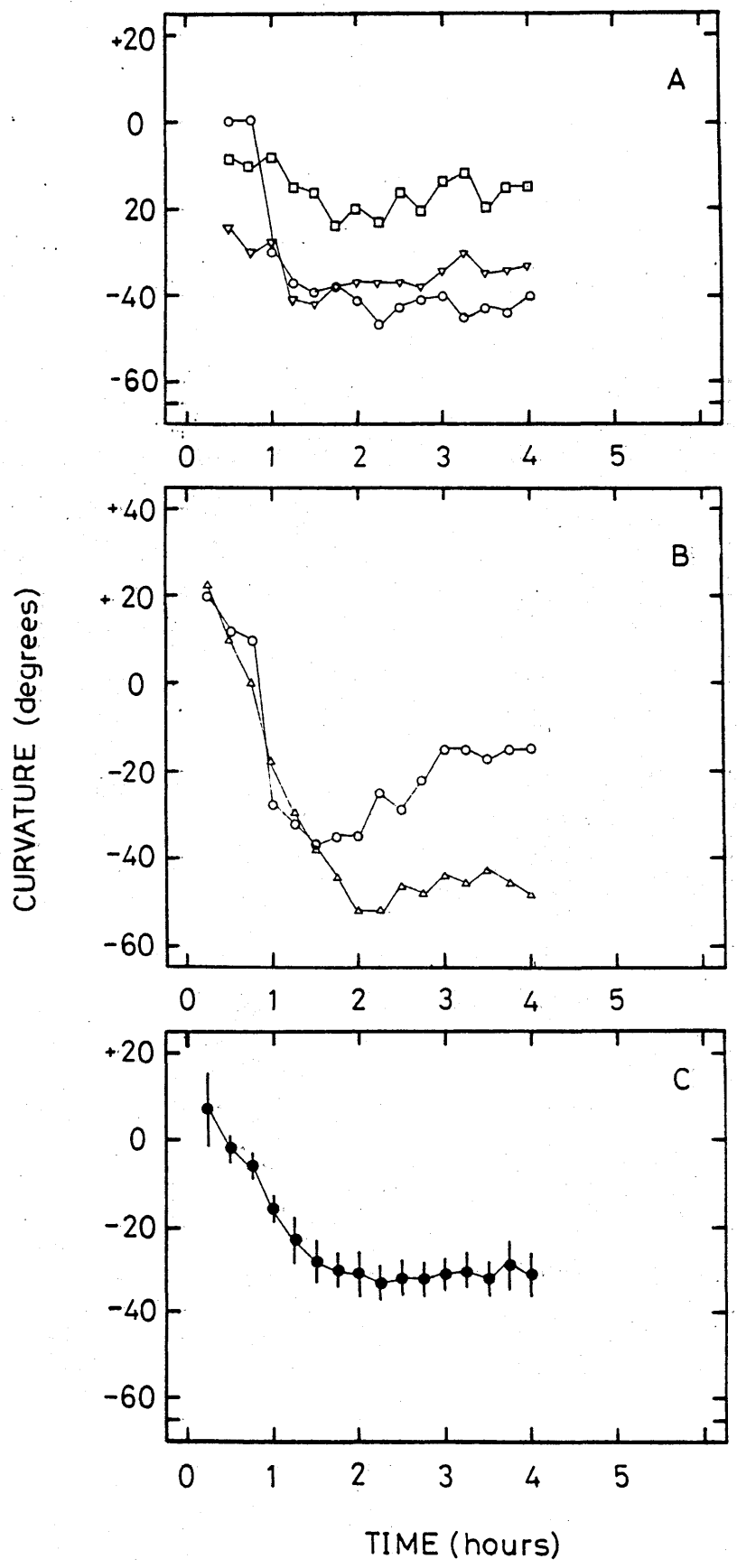


Figure 4.2 The curvature of representative roots (A, B) and the mean curvature (C) of Z. mays roots kept in darkness.

TABLE 4.3 Maximum angle and fluctuation in angle of roots in darkness.

Sample No.	Max. angle of curvature	Range of Oscillations		°oscillation
46	-24	-20	-24	4
50a	-37	-10	-30	20
b	-52	-43	-52	9
52	-36	-30	-30	0
55a	-57	-47	-50	3
b	-48	-36	-50	8
036c	-24	-12	-24	12
038c	-48	-38	-43	5
039c	-47	-40	-45	5
	-42	-32	-38	6
046c	-35	-33	-35	2
	0	0		0

of 37° over the whole time period. This lack of change in angle is in contrast to the fluctuation in curvature that occurs in individuals and thus, the mean curve presented masks the actual behaviour found in individual roots.

4.2.3 Higher magnification. Samples of one to three roots: continuous light

It is possible to divide the curvature exhibited by the 31 roots studied in continous light (Table 4.4) into the two distinct groups shown in figures 4.3A and B. Figure 4.3A shows 3 representative roots from a total of 19 individuals which exhibited a pattern of curvature similar to that displayed by roots kept in continous darkness (Fig. 4.2); however the roots did curve to a greater extent when illuminated. During the first 2 to 3h the roots bent rapidly to their maximum angle and after this initial period the rate of curvature decreases and oscillates about the final angle. Once again, as in continuous darkness, the amplitude of these fluctuations is different for different roots. The magnitude of fluctuation found in the 19 roots in the present study was, in most cases, between 5° and 25° , although one root was observed to oscillate over as large a range as 37° (Table 4.5).

This pattern of curvature was designated as type 1 response in light.

A different pattern of curvature was exhibited by the other 12 roots examined, 3 examples of which are shown in Figure 4.3B. In these roots the final angle of curvature was achieved by curvature increasing continuously at an approximately constant rate over virtually the whole of the observation period. The average maximum

TABLE 4.4 Curvature of *Z. mays* roots in continuous light.

Sample No.	026c		027c		028c	031c		033c		47		49		53		56		003		007			008		010	009	021	
Magnification	x8.5		x8.5		x8.5	x8.5		x8.5		x14		x14		x14		x14		x10		x10			x10		x10	x10	x10	
Time (Hrs)																												
0	+30	-27	+16	-11	-	0	-17	0	0	0	0	0	-11	+ 5	-12	0	+13	-12	0	0	-12	+10	-10	-	+27	-25	-10	
	+27	-31	0	-15	0	-21	-20	-11	-16	-17	-23	-10	-16	+10	-18	-12	+12	- 7	-22	-19	-18	-20	-16	- 7	+19	-39	-19	
	0	-28	-15	-24	-21	-30	-22	-20	-27	-31	-35	-17	-20	0	-28	-29	0	-20	-37	-30	-30	-36	-27	- 9	- 9	-39	-31	
1	-23	-33	-23	-30	-34	-42	-40	-22	-37	-50	-45	-30	-24	-12	-38	-37	-18	-36	-25	-40	-41	-42	-32	-25	-22	-47	-43	
	-35	-42	-29	-37	-39	-45	-46	-25	-37	-60	-55	-46	-34	-25	-42	-53	-19	-36	-19	-58	-47	-56	-34	-50	-27	-50	-50	
	-39	-47	-34	-41	-50	-55	-59	-36	-38	-69	-63	-	-38	-31	-56	-70	-12	-33	-25	-60	-65	-62	-33	-61	-	-66	-61	
	-46	-47	-39	-46	-66	-67	-62	-39	-35	-50	-66	-63	-47	-40	-63	-82	-25	-27	-29	-54	-70	-70	-40	-64	-49	-69	-69	
2	-39	-62	-45	-55	-73	-78	-70	-48	-30	-101	-68	-72	-58	-45	-78	-85	-35	-25	-35	-45	-80	-82	-41	-62	-56	-76	-80	
	-37	-63	-62	-62	-87	-83	-70	-52	-28	-103	-68	-85	-55	-50	-72	-89	-20	-19	-31	-45	-80	-85	-40	-50	-65	-73	-80	
	-37	-65	-70	-66	-84	-89	-85	-60	-32	-104	-62	-89	-57	-58	-73	-86	-25	-16	-32	-39	-87	-87	-35	-47	-68	-70	-74	
	-37	-75	-76	-70	-87	-95	-91	-64	-33	-100	-52	-92	-62	-62	-73	-72	-	-	-30	-31	-90	-89	-32	-30	-79	-70	-74	
3	-42	-60	-82	-72	-75	-95	-91	-73	-36	-88	-53	-92	-72	-67	-56	-70	-14	-13	-25	-29	-80	-93	-38	-30	-78	-77	-71	
	-	-	-84	-78	-80	-97	-96	-65	-45	-83	-53	-95	-73	-72	-43	-63	-12	-14	-25	-31	-72	-95	-33	-34	-77	-77	-69	
	-	-	-87	-87	-79	-116	-	-70	-43	-79	-60	-92	-75	-70	-38	-64	-10	-15	-23	-35	-68	-89	-34	-38	-85	-73	-69	
	-	-	-92	-85	-76	-116	-	-69	-50	-104	-60	-99	-83	-	-48	-65	-13	-18	-22	-44	-58	-82	-30	-44	-84	-63	-59	
4	-	-	-90	-84	-77	-113	-	-75	-53	-	-	-	-94	-	-64	-60	-14	-17	-31	-52	-60	-84	-29	-47	-89	-58	-72	
Type of curvature	1	1	2	2	1	2	2	2	2	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	1	

TABLE 4.4 (continued)

022c		024c			
x10		x8.5		$\bar{x}$	SE
0	0	0	0	+ 1.59	2.31
-11	- 7	0	- 8	-10.81	2.50
-22	-25	-23	-19	-22.71	1.88
-33	-40	-27	-37	-33.16	1.66
-40	-51	-26	-49	-40.84	2.09
-48	-60	-36	-53	-48.31	2.77
-55	-65	-37	-54	-50.74	2.99
-58	-74	-37	-69	-60.06	3.41
-72	-95	-61	-72	-61.13	4.30
-90	-93	-45	-70	-64.35	4.13
-74	-92	-46	-65	-67.22	4.09
-80	-95	-49	-68	-63.35	4.38
-83	-94	-57	-73	-64.59	4.64
-93	-101	-52	-79	-64.93	5.04
-77	-104	-56	-82	-66.37	5.11
-70	-108	-55	-86	-65.92	5.36
1	2	1	2		

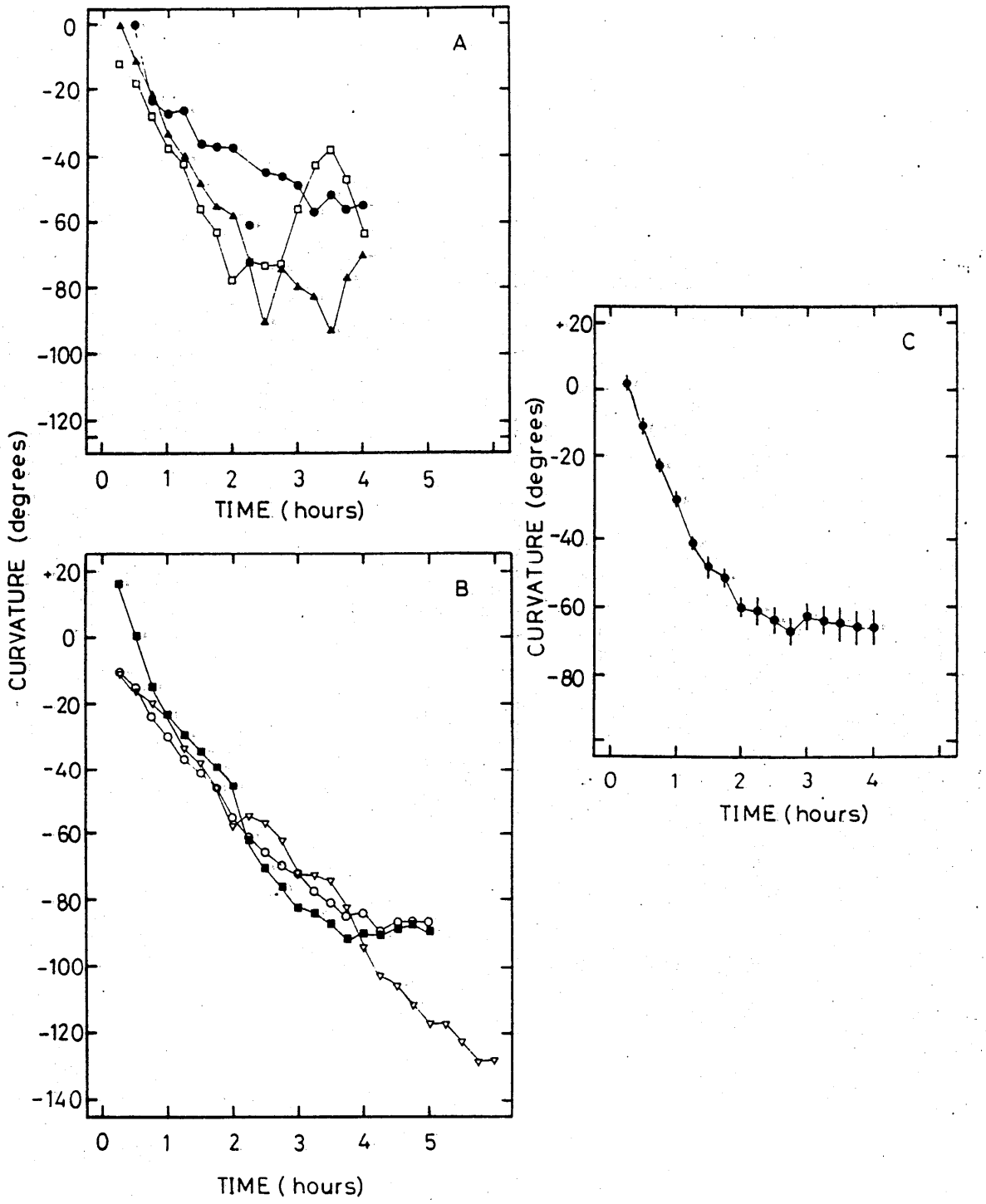


Figure 4.3 The type 1 curvature (A) type 2 curvature (B) and the mean curvature (C) of Z. mays roots illuminated with white light (3.67 $Jm^{-2}s^{-1}$).

TABLE 4.5 Fluctuation in angle of curvature in roots showing type-1 curvature in light.

Sample No.	Max angle of curvature (°)	Range of oscillation		oscillation
026c a	-46	-37	-42	5
b	-75	-60	-75	15
028c	-87	-75	-87	12
47 a	-104	79	-104	25
b	-68	-52	-60	8
56	-78	-38	-75	37
	-89	-51	-59	8
003 a	-35	-10	-29	19
b	-36	-12	-19	7
007 a	-37	-22	-32	10
b	-60	-29	-52	23
c	-90	-58	-80	22
008 a	-95	-82	-89	7
b	-41	-29	-38	9
010	-64	-35	-49	14
012c a	-77	-58	-77	19
b	-80	-69	-74	5
022c a	-93	-70	-93	23
b	-61	-45	-57	12

angle of curvature was 93° ± 6.1, which is significantly greater than the 70° ± 5.1 reached by the roots showing the fluctuating pattern of curvature after 2 to 3h of gravistimilation. This response will be referred to as type 2 response.

The overall mean curvature of 31 roots was calculated and is shown in Fig. 4.3C. The curve shows fewer fluctuations than those for individual roots. The mean curve shows a period of rapid curvature during the first 3h followed by a period where there is little change in the angle of curvature. The average maximum curvature in light is 77° ± 3.90 which is approximately twice as large as the 37° ± 4.16 curvature executed by the non-illuminated roots. The mean data, however, conceal the 2 distinct patterns of curvature exhibited.

4.3.0 DISCUSSION

One of the aims of the experiments reported in this chapter was to establish whether or not mean gravitropic curves are truly representative of the curvatures executed by individual roots. The phenomenon of gravitropic curvature has been studied fairly comprehensively over the past 50 years but the data presented are usually mean data, and although some of these studies have involved monitoring the responses of a number of individual roots, these individual results are rarely presented. Recently Hillman and Wilkins (1982) studying the return of gravitropic responsiveness following decapping, commented that the mean response masked the behaviour of individuals, and they therefore placed little emphasis on mean data in their study. In the present study it is very evident from the graphs in Figures 4.1, 4.2 and 4.3 that when the mean data are plotted a

different pattern of curvature emerges to that obtained by plotting the curvature executed by each individual root separately. In the individual curves there is a considerable amount of variation in angle especially after the first 2 to 3h of gravistimulation, a fact not evident from the mean curve. In addition to this fluctuation in the angle of curvature in a single root, the magnitude of the curvature varies from root to root. It is this inherent variability in roots that makes the use of mean data a not wholly accurate or acceptable way of representing the gravitropic curvature of Zea roots.

A few of the reports in the literature have included responses of individual roots (Ney and Pilet, 1981; [illegible] Hillman and Wilkins, 1982). Ney and Pilet (1981), used a continuous filming method to follow the gravicurvature of Zea mays cv. LG 11 roots in white light. The curvature observed is remarkably similar to the curvature exhibited in the present study by roots in darkness and by those roots showing a type I response in light; a period of rapid curvature to approximately 70° during the first 3h followed by oscillation over the rest of the time period. The amplitude of oscillation found by Ney and Pilet was between 5°and 20°, which is similar to the 5°to 25° variation reported here. The roots showing curvature designated as type 2 response in light did not conform to the pattern of curvature described by Ney and Pilet, since these roots showed no oscillation in angle after 2 to 3h of gravistimulation.

Ney and Pilet (1981) described the curve they found as biphasic; the first phase, up to 3h being gravicurvature and the second phase, after 3h, nutation. These two phases could be assigned

to most of the curves described in this chapter.

There are two schools of thought as to the mechanism of nutation, which is defined as the spiral course pursued by the apex of a plant organ during growth (Dictionary of Biology, Penguin). The first, and earliest theory, is that nutation is an autonomous oscillator system, and this theory was first proposed by Dutrochet in 1843. The second theory (Gradmann, 1926) ascribes the movement to a gravitropic feedback mechanism. Although the autonomous oscillator system and the gravireaction system are separate, both will act via modulation of growth rate within the growing organ, and will therefore interact in their expression, the simplest way that this can occur being additively. The feedback system will involve discrete perception and response times that will create oscillations between limits on either side of the preferred orientation. A delay between the change in orientation and the corrective growth change in the elongation zone, will result in the curvature overshooting one way and then the other. This system is analagous to thermostatic regulation of a mean temperature in a room or a water-bath.

The responses observed in Figures 4.1, 4.2 and 4.3, could therefore be showing one of two possible sequences of events; firstly a period of gravireaction up to 3h and then nutation for the remainder of the time period, or secondly, the combined effect of nutation and gravireaction during the first 3h and then nutation alone after this time. Heathcote (1982) reanalysed Ney and Pilet's (1981) data and apparently showed that during the first 3h the nutational oscillation is merely masked by its additive affect with the gravitropic curvature.

The data presented in this chapter cannot resolve which mechanism is involved in nutational movements or whether the response after 3h is gravity-related or not, an autonomous oscillator system being independent of gravity; only future work in space or artificial low gravity environments can solve these problems. It can be noted, however, that the oscillations observed were in the vertical plane only and not spiral in nature, a finding in accordance with that of Ney and Pilet (1981). Any spiral movement would have resulted in a distortion of the image on the monitor screen and all of the video pictures were sharp indicating that no movement out of the plane of focus of the camera had occurred. If nutation is occurring over the whole of the time period it could account for variation in the gravicurvature of individual roots. It is possible that all roots react equally to gravity and it is the magnitude of nutational oscillations, and the point in the oscillation at which the curvature is measured, that causes the variation observed in the curvatures exhibited by the individual roots.

Another problem in classifying the type of curvature exhibited arises since not all of the roots curving in light show the same patterns of curvature. Almost 50% of the roots studied in light have no oscillatory period of growth. This variation does not arise because of the different numbers of roots in the samples used in these experiments, since in one case 3 roots were examined together and two showed a type I response and the other a type 2 response. There must, therefore, be some other explanation as to why roots in light show these two types of response under identical experimental conditions.

The other feature of the results presented is confirmation

that light enhances the gravitropic response (Scott and Wilkins, 1969; Gibbons and M.B. Wilkins, 1970; H. Wilkins and Wain, 1974, 1975; Pilet, 1971; Beffa and Pilet, 1982). The effect of light on gravicurvature was reinvestigated since all of the previous studies had involved the use of dim green light (510-550nm) for selection and manipulation of the seedlings, whereas in the present study I.R. radiation was used. Using seedlings of Zea mays cv. LG II., Beffa and Pilet (1983) found a mean curvature of approximately 30° in darkness and 60° in light. These curvatures correspond closely with the 30° and 70° found in the present study. Initially, therefore, it appears that there is little difference between the curvatures in seedlings which were exposed to green safelights and those exposed to I.R. radiation. However, Beffa and Pilet (1983) kept their seedlings vertical for 4h prior to gravistimulation, whereas the roots in this study were turned horizontally either immediately or after only 2h vertical growth. It may be that the 4h dark period is of sufficient duration for any effect of green light to be nullified. Also, it must be remembered that 2 different maize cultivars, LG II and Fronica, were used in these studies, and a difference in the magnitude of the graviresponse in light may just coincidentally result in the 2 sets of results coinciding. Further work with these 2 maize cultivars under identical conditions could confirm whether or not there is a difference in their reaction to gravistimulation.

A small amount of curvature (30°) is found in darkness. This curvature may arise from the fact that the roots are mechanically stimulated in being mounted in the plant holder before being suspended horizontally in humid air while the gravicurvature is studied since

roots kept on the agar slabs in the germination boxes show little evidence of gravicurvature when left in the experimental box and exposed to I.R. radiation during recordings.

CHAPTER FIVE

GRAVITROPIC CURVATURE STUDIES (II)

5.0.0 INTRODUCTION

Gravitropic curvature of a primary root or shoot is the result of differential growth of the upper and lower surfaces of the organ (Larsen, 1953; Audus and Brownbridge, 1957a; Bennet-Clark et al., 1959; Konings, 1964; Pilet and Nougarede, 1974; Bejaoui and Pilet, 1977). Such differential growth could be achieved in a number of ways:-

1) an increase in growth rate of the upper surface (Iversen, 1973; Pilet and Nougarede, 1974; Jotterand-Dolivo and Pilet, 1976);

2) a decrease in growth of the lower surface (Gibbons and Wilkins, 1970; Pilet, 1971a, 1977; Audus, 1975; Wilkins, 1977);

3) an unequal decrease in the growth rate of both surfaces (Audus and Brownbridge, 1957a; Konings, 1964; Bejaoui and Pilet, 1977);

4) an unequal increase in the growth rate of both surfaces; and

5) an increase in the growth rate of the upper surface and a simultaneous decrease of that of the lower surface. The nature of the growth rate changes is of importance since it provides an insight into the possible regulatory mechanisms initiated by gravistimulation.

A number of studies have been made of the growth rate changes in gravitropically responding organs. Sachs (1837) marked roots of Vicia faba with Indian ink dots and reported that the growth of the

convex (upper) side surface was greater than the mean rate of growth of the whole organ, whereas that of the concave (lower) surface was less. More precise measurement of the upper and lower surfaces of the roots and hypocotyls of Zea were made by Erickson and Sax (1956.) and Silk- and Erickson (1978) by applying carbon particles to the surfaces of the organ to act as reference points. Other procedures have involved the use of resin beads to examine the growth of Chara rhizoids (Hejenowicz et al., 1977) and Sephadex resin beads to monitor the growth of Zea roots (Pilet et al., 1983).

The variation in the results of previous publications needs to be clarified. The infra-red videoequipment has therefore been used to investigate the growth rate changes in graviresponding Zea roots following the application of Sephadex resin beads to the upper and lower surfaces of the organ to act as markers.

5.1.0 METHODS

A root between 10 and 15mm in length was selected and, using a glass micropipette, soaked Sephadex G50, ion-exchange, resin beads (approx. 0.20mm diameter) (hereafter referred to simply as beads) were placed at intervals of between 0.5 and 3mm along the terminal 1-6mm of 2 opposite surfaces of the root so as to divide them into recognisable regions. Beads soaked in distilled water were used since preliminary experiments had revealed that unsoaked beads absorbed moisture from the surface of the root and thus caused cessation of growth. However, other experiments showed that over 7h of vertical growth there was no significant difference between the increase in length of roots marked with soaked beads and that of unmarked roots

(Table 5.1A and B).

After bead application the root was placed inside a perspex box and allowed to grow vertically for 2h; the box was then rotated, so that the root was orientated horizontally, and left for a further 4h. At the end of the recording period the distances between adjacent beads were measured for every 15 min time interval (Table 5.2) and the growth rate calculated (mm h^{-1}) for both surfaces (Table 5.3). This procedure was repeated for individual roots on 25 separate occasions.

5.1.1 Effect of G50 beads on curvature

To determine whether or not curvature was induced by placing beads on the root-tip, beads were placed along only one surface of 20 vertically orientated roots. After 8h growth the roots were examined for any sign of curvature.

In all of the roots there was no evidence of curvature either towards or away from the side of the root with the beads.

5.2.0 RESULTS

The mean growth rate of 25 roots kept in the vertical position, and the growth rates of the upper and lower surfaces after horizontal displacement are shown in Fig. 5.1A. When orientated vertically, the roots grew at a rate of approximately 0.53 ± 0.06mm h^{-1}.

Within 15 min of the roots being placed horizontally, the growth rate of the upper surface had increased, and continued to do so until it reached a maximum value of 0.95mm h^{-1} after 1h. The growth rate then gradually declined to reach the original value of approximately 0.53mm h^{-1} after 4h. The growth rate of the lower surface of the

TABLE 5.1A Length of *Z. mays* roots with G50 Sephadex resin beads attached.

	No beads					Soaked beads						Unsoaked beads				
Sample No.	G16	G17	G18	G22	G23		G11	G12	G13	G14	G15		712	G7	G8	G9	G10
Time (H)						(H)						(H)					
0	1.67	2.58	2.50	2.85	2.55	0	-	2.69	2.52	2.69	2.43	0	2.25	2.65	2.35	2.35	2.40
	1.77	2.65	2.67	3.02	2.87		-	2.87	2.54	2.79	2.67		2.33	3.23	2.60	2.31	2.31
	2.25	2.70	2.80	2.10	3.03		-	3.08	2.56	2.94	2.88		2.44	3.37	2.79	2.38	2.17
	2.52	2.77	3.00	3.27	3.23		-	3.21	2.62	3.12	3.00		-	3.92	2.88	2.40	2.27
1	2.72	2.80	3.22	3.45	3.42	1	2.79	3.44	2.69	3.21	3.17	1	2.52	4.00	2.98	2.44	2.44
	2.95	2.83	3.33	3.50	3.48		2.83	3.65	2.77	3.38	3.33		2.54	4.08	3.06	2.48	2.62
	3.12	2.85	3.43	3.58	3.62		2.88	3.90	2.87	3.48	3.48		2.56	4.13	3.12	2.50	2.69
	3.28	2.95	3.50	3.65	3.77		2.88	4.13	2.98	3.56	3.63		2.60	4.15	3.17	2.52	2.75
2	3.43	3.03	3.58	3.82	3.92	2	2.88	4.22	3.08	3.73	3.80	2	2.69	4.19	3.23	2.56	2.87
	3.62	3.17	3.63	4.00	4.00		2.98	4.67	3.17	3.87	3.93		2.79	4.23	2.39	2.60	2.90
	3.77	3.25		4.10	4.08		3.04	5.08	3.29	4.04	4.10		2.88	4.25	3.35	2.63	2.96
	3.90	3.38		4.35	4.23		3.08	5.38	3.37	4.23	4.27		2.94	4.27	3.44	2.69	3.08
3	4.03	3.42	3.88	4.58	4.45	3	3.10	5.65	3.50	4.37	4.42	3	3.00	4.33	3.48	2.77	3.17
	4.15	3.43	4.12	4.73	4.67		3.15	5.87	3.58	4.48	4.50		3.06	4.37	3.54	2.83	-
	4.28	3.48	4.30	4.97	4.83		3.21	6.17	3.57	4.63	4.62		3.13	4.42	3.58	2.87	3.31
	4.37	3.53	4.42	5.25	5.02		3.25	6.46	3.88	4.57	4.77		3.19	4.52	3.63	2.90	3.40
4	4.45	3.65	4.50	5.45	5.25	4	3.31	6.75	4.02	4.90	4.92	4	3.25	4.54	3.69	3.00	3.50
	4.53	3.67	4.62	5.73	5.40		3.33	7.10	4.19	5.04	5.08		3.31	4.54	3.73	3.06	3.58
	4.62	3.73	4.75	6.00	5.55		3.37	7.27	4.38	5.15	5.25		3.35	4.56	3.75	3.12	3.65
	4.73	3.82	4.80	6.37	5.72		3.40	7.42	4.58	5.29	5.42		3.40	4.58	3.79	3.23	3.85
5	4.82	3.85	4.92	6.72	5.87	5	3.42	7.62	4.75	5.46	5.58	5	3.46	4.60	3.83	3.27	3.94
							3.46	7.96	4.87	5.60	5.67		3.48	4.62	3.88	3.35	4.00
							3.48	8.23	5.04	5.73	5.75		3.54	4.65	3.92	3.42	4.08
							3.52	8.46	5.15	5.96	5.87		3.58	4.69	3.96	3.52	4.15
						6	3.54	8.58	5.33	6.12	6.02	6	3.62	4.71	4.00	3.58	4.25
							3.58	8.73	5.50	6.25	6.17		3.65	4.73	4.04	3.65	4.35
							3.62	8.92	5.62	6.38	6.25		3.69	4.79	4.08	3.73	4.44
							3.69	9.17	5.73	6.56	6.33		3.75	4.79	4.12	3.79	-
						7	3.71	9.44	5.83	6.73	6.43	7	3.79	4.83	4.17	3.90	-
													3.85	4.87	4.17	3.90	-
													3.87	4.88	4.29	4.04	-
													3.92	4.98	4.33	4.08	-
												8	3.96	4.98	4.38	4.19	-

TABLE 5.1B Growth rate of *Z. mays* with either soaked, unsoaked or no resin beads attached.

	No Beads					$\bar{x}$	+ Soaked Beads					$\bar{x}$	+ Unsoaked Beads					$\bar{x}$
0-1	1.05	0.22	0.72	0.60	0.87	0.69		0.75	0.17	0.52	0.74	0.55	0.27	1.35	0.63	0.09	0.04	0.48
1-2	0.71	0.23	0.36	0.37	0.50	0.43	0.09	10.98	0.39	0.52	0.35	0.47	0.17	0.19	0.25	0.12	0.43	0.23
2-3	0.60	0.39	0.30	0.76	0.53	0.52	0.22	0.23	0.42	0.64	0.60	0.62	0.31	0.14	0.25	0.21	0.30	0.24
3-4	0.42	0.23	0.62	0.87	0.80	0.59	0.21	1.10	0.52	0.53	0.50	0.57	0.25	0.21	0.21	0.23	0.33	0.25
4-5	0.37	0.20	0.42	1.27	0.62	0.58	0.11	0.87	0.73	0.56	0.66	0.59	0.21	0.06	0.14	0.27	0.44	0.22
5-6	-	-	-	-	-	-	0.12	0.96	0.58	0.66	0.44	0.55	0.16	0.11	0.17	0.31	0.31	0.21
6-7	-	-	-	-	-	-	0.17	0.86	0.50	0.61	0.41	0.51	0.17	0.12	0.17	0.32	-	0.20
7-8	-	-	-	-	-	-					-	-	0.17	0.15	0.21	0.29	0.21	-

t-values for no beads vs. soaked beads t_s = 0.07 NS

t-values for no beads vs. unsoaked beads t_{us} = 3.28 ***

TABLE 5.2A Length (distance between adjacent resin beads) of the upper surface of horizontal *Z. mays* roots.

Sample No.		G29				G30			G31				G32				G33				G34				
Distance of bead from root tip (mm)		5.55	3.88	3.18	1.97	6.10	4.37	1.77	5.67	4.43	2.86	1.33	5.75	4.15	2.80	1.35	5.08	3.48	2.33	1.20	5.62	3.40	2.92	1.77	1.13
Time	(H)																								
	0	1.38	0.45	0.92	1.08	1.52	2.35	1.45	1.02	1.30	1.32	1.03	1.35	1.12	1.12	0.83	1.40	0.93	0.85	0.98	1.40	0.53	0.95	0.35	0.85
		1.42	0.50	1.03	1.08	1.58	2.50	1.45	1.08	1.38	1.38	1.05	1.35	1.25	1.25	0.83	1.57	0.95	0.97	0.98	1.53	0.57	0.95	0.35	0.97
		1.55	0.53	1.03	1.12	1.58	2.93	1.47	1.10	1.63	1.48	1.07	1.37	1.33	1.32	0.83	1.65	1.08	0.97	1.02	1.58	0.62	1.03	0.43	1.00
		1.60	0.58	1.09	1.12	1.58	3.13	1.53	1.20	1.72	1.53	1.07	1.42	1.45	1.42	0.83	1.75	1.15	1.00	1.02	1.58	0.70	1.07	0.43	1.02
	1	1.67	0.63	1.15	1.13	1.58	3.50	1.53	1.25	1.82	1.57	1.07	1.42	1.70	1.45	0.83	1.88	1.28	1.07	1.02	1.58	0.73	1.07	0.48	1.02
		1.80	0.75	1.17	1.23	1.60	3.75	1.55	1.25	1.90	1.62	1.13	1.42	1.73	1.50	0.83	2.08	1.40	1.15	1.07	1.62	0.77	1.12	0.48	1.02
		1.82	0.78	1.30	1.23	1.60	4.20	1.60	1.27	2.02	1.72	1.15	1.43	1.73	1.53	0.83	2.12	1.43	1.20	1.12	1.62	0.85	1.20	0.53	1.02
		1.95	0.78	1.30	1.23	1.60	4.20	1.60	1.37	2.17	1.72	1.15	1.53	1.83	1.53	0.83	2.32	1.50	1.20	1.12	1.62	0.95	0.53	1.02	
	2	2.03	0.85	1.32	1.23	1.60	4.38	1.63	1.40	2.28	1.82	1.15	1.53	1.83	1.53	0.83	2.48	1.72	1.23	1.12	1.62	1.00	1.30	0.57	1.02
		2.08	0.93	1.38	1.25	1.60	4.62	1.70	1.40	2.30	1.82	1.15	1.53	1.83	1.53	0.83	2.48	1.75	1.27	1.13	1.63	1.13	1.43	0.63	1.02
		2.12	0.97	1.40	1.27	1.60	4.80	1.70	1.40	2.47	1.82	1.25	1.53	1.83	1.63	0.83	2.52	1.75	1.27	1.15	1.66	1.22	1.50	0.63	1.02
		2.12	0.97	1.43	1.30	1.60	4.97	1.70	1.40	2.55	1.82	1.25	1.53	1.83	1.53	0.83	2.55	1.98	1.32	1.15	1.70	1.38	1.50	0.63	1.02
	3	2.18	1.02	1.53	1.30	1.62	5.25	1.70	1.40	2.63	1.82	1.25	1.53	2.03	1.53	0.83	2.62	2.07	1.32	1.20	1.75	1.48	1.62	0.63	1.02
		2.18	1.10	1.58	1.35	1.62	5.40	1.70	1.40	2.65	1.82	1.28	1.53	2.12	1.60	0.83	2.70	2.20	1.40	1.20	1.75	1.60	1.62	0.63	1.05
		2.20	1.12	1.62	1.35	1.62	5.40	1.70	1.40	2.72	1.83	1.28	1.53	2.25	1.67	0.86	2.72	2.27	1.40	1.20	1.75	1.65	1.62	0.63	1.10
		2.25	1.23	1.72	1.35	1.62	5.65	1.73	1.40	2.87	1.83	1.28									1.75	1.75	1.62	0.63	1.10
	4	2.25	1.27	1.83	1.50	1.62	5.68	1.73	1.40	2.95	1.88	1.32									1.75	1.83	1.67	0.67	1.10
		2.25	1.33	1.88	1.55	1.62	5.83	1.73	1.50	2.95	1.97	1.37									1.75	1.93	1.73	0.67	1.10
		2.25	1.42	1.98	1.55	1.62	5.98	1.73													1.75	2.00	1.73	0.67	1.10
		2.25	1.42	2.07	1.55																1.75	2.02	1.85	0.67	1.10
	5	2.25	1.45	2.12	1.55																1.75	2.07	1.98	0.70	1.10
		2.26	1.45	2.15	1.55																1.75	2.10	2.07	0.70	1.10
		2.26	1.45	2.20	1.55																1.75	2.10	2.22	0.70	1.10
																					1.75	2.12	2.35	0.70	1.10
	6																				1.75	1.13	2.47	0.75	1.10
																					1.75	2.17	2.65	0.75	1.10
																					1.75	2.17	2.83	0.78	1.10
																					1.75	2.17	3.00	0.82	1.12
	7																								

TABLE 5.2A (continued)

	G36				G37			G42					G43				G47'					G48			
	5.35	4.08	2.90	1.48	3.90	2.68	1.18	5.42	4.30	3.05	1.87	0.83	5.63	4.72	3.75	2.48	4.23	3.38	2.53	1.87	1.03	5.12	4.17	2.57	1.54
0	1.20	0.97	1.13	1.20	1.02	0.92	0.86	0.75	0.97	0.87	0.68	0.57	0.67	0.68	0.95	2.12	0.58	0.62	0.45	0.65	0.77	0.73	1.33	0.75	1.37
	1.20	1.05	1.17	1.20	1.02	0.92	0.86	0.75	1.02	0.92	0.77	0.57	0.67	0.68	1.02	2.33	0.58	0.62	0.45	0.67	0.80	0.75	1.42	0.75	1.42
	1.35	1.18	1.32	1.30	1.02	0.93	0.88	0.75	1.03	1.00	0.83	0.58	0.68	0.72	1.10	2.38	0.58	0.62	0.45	0.72	0.82	0.75	1.67	0.78	1.42
	1.50	1.30	1.32	1.33	1.10	1.03	0.92	0.75	1.08	1.03	0.83	0.58	0.72	0.87	1.35	2.47	0.58	0.62	0.45	0.72	0.82	0.77	1.98	0.92	1.45
1	1.50	1.37	1.37	1.33	1.18	1.12	0.92	0.75	1.12	1.08	0.83	0.58	0.72	0.87	1.35	2.52	0.58	0.62	0.48	0.72	0.82	0.77	2.15	1.02	1.45
	1.67	1.53	1.42	1.33	1.22	1.12	0.92	0.75	1.13	1.12	0.83	0.58	0.72	0.93	1.43	2.57	0.58	0.62	0.48	0.75	0.83	0.78	2.27	1.08	1.45
	1.75	1.60	1.45	1.38	1.33	1.25	0.93	0.75	1.17	1.20	0.83	0.58	0.72	1.00	.63	2.57	0.58	0.62	0.48	0.78	0.87	0.78	2.52	1.17	1.45
	1.76	1.72	1.45	1.38	1.55	1.37	0.93	0.78	1.20	1.27	0.83	0.58	0.72	1.10	1.77	2.83	0.60	0.63	0.53	0.83	0.87	0.78	2.63	1.17	1.45
2	1.76	1.72	1.47	1.38	1.58	1.47	0.95	0.78	1.28	1.32	0.88	0.58	0.72	1.13	1.83	2.88	0.60	0.65	0.58	0.87	0.87	0.78	2.73	1.27	1.45
	1.76	1.78	1.53	1.38	1.80	1.57	0.95	0.78	1.28	1.42	0.90	0.58	0.72	1.13	1.95	2.97	0.60	0.65	0.63	0.87	0.87	0.78	2.90	1.17	1.45
	1.88	1.83	1.53	1.38	2.00	1.63	0.95	0.78	1.33	1.47	0.90	0.60	0.72	1.18	2.02	2.97	0.60	0.65	0.68	0.90	0.88	0.78	3.09	1.22	1.45
	1.92	1.83	1.57	1.38	2.00	1.72	1.00	0.78	1.38	1.53	0.90	0.60	0.72	1.18	2.18	2.98	0.62	0.67	0.75	1.00	0.88	0.78	3.12	1.25	1.45
3	1.93	1.92	1.57	1.43	2.00	1.72	1.00	0.78	1.40	1.60	0.90	0.62	0.72	1.20	2.30	3.03	0.62	0.68	0.83	1.00	0.88				
	1.93	1.98	1.62	1.43	2.05	1.78	1.00	0.82	1.40	1.70	0.92	0.62	0.75	1.22	2.43	3.03	0.62	0.70	0.92	1.13	0.88				
	1.95	2.01	1.63	1.47	2.15	1.83	1.08	0.82	0.43	1.80	0.92	0.62					0.62	0.75	0.97	1.22	0.88				
	1.95	2.12	1.67	1.47	2.23	1.83	1.08	0.82	1.47	1.92	0.92	0.62					0.62	0.78	1.12	1.22	0.92				
4	2.00	2.12	1.68	1.47	2.25	1.85	1.08	0.82	1.48	1.98	0.95	0.62					0.62	0.80	1.18	1.23	0.92				
								0.82	1.50	2.05	0.97	0.62					0.62	0.83	1.28	1.30	0.92				
								0.82	1.52	2.13	0.97	0.62					0.62	0.88	1.42	1.35	0.92				
								0.82	1.52	2.22	0.97	0.62					0.62	0.95	1.48	1.38	0.92				
5								0.82	1.53	2.30	0.97	0.62					0.62	0.97	1.58	1.38	0.92				
								0.82	1.53	2.35	0.98	0.62					0.62	1.00	1.65	1.42	0.92				
								0.82	1.55	2.48	0.98	0.62					0.62	1.00	1.72	1.42	0.95				
								0.82	1.57	2.48	0.98	0.62													
																	0.62	1.05	1.82	1.42	0.95				
6								0.82	1.58	2.58	1.02	0.62													
7																									

TABLE 5.2A (continued)

	G38				G49				G51				G52				G53				060				062			
	5.72	3.78	2.58	1.60	5.85	4.65	3.20	1.92	5.62	4.33	2.87	1.83	3.92	3.68	2.60	1.67	6.28	5.27	4.02	2.42	4.87	3.67	2.55	1.45	4.28	3.43	2.34	1.3
0	1.55	0.90	0.65	1.23	0.98	1.22	0.98	1.57	0.80	1.07	0.67	1.35	0.73	0.72	0.50	1.30	0.62	0.95	1.28	2.17	0.74	0.61	0.59	0.86	0.62	0.80	0.74	0.9
	1.72	1.02	0.78	1.25	1.00	1.23	1.08	1.57	0.92	1.10	0.67	1.38	0.87	0.78	0.53	1.37	0.63	0.98	1.37	2.17	0.74	0.70	0.61	0.86	0.74	0.82	0.74	1.0
	2.02	1.20	0.95	1.25	1.00	1.38	1.23	1.57	0.97	1.17	0.73	1.45	0.92	0.78	0.55	1.38	0.63	0.98	1.38	2.17	0.77	0.73	0.61	0.87	0.74	0.85	0.77	1.0
	2.30	1.42	1.95	1.28	1.00	1.57	1.33	1.57	1.05	1.17	0.73	1.47	0.93	0.80	0.58	1.38	0.63	1.00	1.38	2.18	0.81	0.73	0.63	0.91	0.74	0.88	0.77	1.0
1	2.42	1.45	1.00	1.30	1.00	1.72	1.45	1.58	1.07	1.18	0.73	1.00	1.00	0.87	0.63	1.40	0.65	1.00	1.47	2.18	0.81	0.74	0.64	0.93	0.74	0.88	0.77	1.0
	2.43	1.55	1.02	1.30	1.03	1.87	1.58	1.58	1.07	1.18	0.77	1.50	1.13	0.97	0.63	1.43	0.65	1.00	1.52	2.20	0.86	0.74	0.64	0.93	0.74	0.88	0.77	1.0
	2.55	1.62	1.03	1.32	1.12	1.95	1.75	1.50	1.12	1.30	0.77	1.50	1.22	1.05	0.63	1.43	0.65	1.00	1.58	2.22	0.89	0.77	0.67	0.93	0.74	0.89	0.77	1.0
	2.55	1.80	1.08	1.33	1.12	2.05	1.75	1.58	1.12	1.42	0.87	1.50	1.33	1.20	0.67	1.45	0.65	1.00	1.68	2.30	0.90	0.81	0.70	0.93	0.77	0.91	0.77	1.0
2	2.73	2.03	1.12	1.33	1.12	2.12	1.75	1.58	1.15	1.50	0.87	1.58	1.42	1.25	0.78	1.57	0.65	1.00	1.73	2.30	0.93	0.83	0.71	0.94	0.83	0.91	0.78	1.0
	2.77	2.20	1.17	1.33	1.12	2.15	1.83	1.08	1.20	1.57	0.87	1.58	1.50	1.45	0.78	1.57	0.65	1.02	1.83	2.30	0.83	0.94	0.82	1.08	1.25	1.23	1.13	1.1
	2.83	2.32	1.18	1.33	1.12	2.17	1.85	1.58	1.20	1.68	0.90	1.60	1.58	1.50	0.83	1.57	0.67	1.02	1.83	2.30	0.93	0.87	0.71	0.94	0.83	0.94	0.83	1.0
	2.87	2.45	1.23	1.40	1.12	2.22	1.88	1.58	1.25	1.78	0.92	1.67	1.58	1.80	0.83	1.68	0.67	1.03	1.83	2.30	0.96	0.91	0.71	0.94	0.83	0.94	0.85	1.0
3	2.95	2.58	1.32	1.42	1.12	2.22	1.95	1.58	1.25	1.80	0.95	1.67	1.62	1.92	0.92	1.75	0.67	1.03	1.83	2.42	0.97	0.91	0.73	0.94	0.83	0.97	0.88	1.1
	3.02	2.75	1.33	1.42	1.12	2.22	2.00	1.58	1.25	1.90	1.05	1.70	1.67	2.13	1.00	1.75	0.67	1.03	1.92	2.43	0.97	0.97	0.79	0.97	0.83	1.02	0.89	1.1
	3.03	2.92	1.38	1.42	1.12	2.22	2.05	1.58									0.67	1.03	1.92	2.52	0.97	1.00	0.89	0.99	0.83	1.02	0.95	1.1
	3.03	3.07	1.42	1.42	1.12	2.45	2.17	1.58									0.67	1.03	1.92	2.62	0.99	1.11	0.93	1.00	0.83	1.05	1.05	1.1
4																	0.67	1.03	2.00	2.75	0.99	1.21	0.93	1.10	0.83	1.05	1.05	1.1
																	0.67	1.05	2.00	2.85	1.00	1.26	0.93	1.07	0.83	1.05	1.05	1.1
																					1.00	1.33	0.96	1.01	0.83	1.05	1.08	1.1
																					1.01	1.43	0.97	1.03	0.85	1.05	1.12	1.1
																					1.01	1.44	1.07	1.03	0.85	1.08	1.18	1.1
																					1.01	1.54	1.01	1.03				
																					1.01	1.60	1.10	1.03				

TABLE 5.2A (continued)

	G55				G56				G60				G61				G62				G47					
	5.75	4.20	2.83	1.60	4.48	2.98	1.73	0.70	4.72	3.32	2.13	0.98	4.28	3.20	2.23	1.45	3.88	2.72	1.83	1.00	5.55	4.17	2.95	1.67	$\bar{x}$	SE
0	1.05	0.88	0.92	1.15	1.13	0.72	0.67	0.40	1.13	0.92	0.83	0.67	0.82	0.67	0.47	1.17	0.90	0.63	0.53	0.70	0.90	0.97	1.00	1.32		
	1.10	1.05	0.97	1.17	1.27	0.73	0.70	0.50	1.23	0.98	0.85	0.67	0.82	0.75	0.52	1.17	0.90	0.63	0.53	0.70	1.00	0.98	1.00	1.32		
	1.10	1.05	0.97	1.17	1.30	0.75	0.70	0.50	1.23	1.00	0.88	0.78	0.87	0.78	0.55	1.17	0.90	0.63	0.53	0.73	1.05	1.07	1.00	1.32		
	1.17	1.05	0.97	1.17	1.30	0.75	0.70	0.50	1.23	1.00	0.90	0.78	0.88	0.78	0.58	1.18	0.90	0.63	0.57	0.75	1.05	1.07	1.00	1.32		
1	1.17	1.05	0.97	1.17	1.30	0.75	0.72	0.50	1.33	1.00	0.90	0.80	0.88	0.83	0.58	1.18	0.92	0.63	0.57	0.80	1.13	1.18	1.02	1.33	0.37	0.03
	1.20	1.13	0.98	1.17	1.30	0.75	0.73	0.50	1.35	1.00	0.90	0.80	0.90	0.85	0.58	1.18	0.92	0.63	0.58	0.80	1.13	1.22	1.03	1.33		
	1.23	1.17	1.05	1.17	1.33	0.75	0.77	0.50	1.35	1.05	0.97	0.80	0.90	0.85	0.58	1.18	0.92	0.63	0.58	0.80	1.17	1.38	1.13	1.42		
	1.23	1.18	1.07	1.17	1.35	0.82	0.83	0.50	1.38	1.07	0.97	0.80	0.95	0.93	0.63	1.30	0.97	0.65	0.58	0.80	1.22	1.47	1.18	1.43		
2	1.25	1.22	1.08	1.17	1.42	0.92	0.83	0.50	1.43	1.23	1.10	0.82	0.95	0.98	0.65	1.32	0.97	0.65	0.58	0.80	1.22	1.65	1.33	1.45	0.42	0.05
					1.55	1.05	0.90	0.52	1.43	1.42	1.10	0.82	0.98	1.05	0.68	1.37	0.97	0.67	0.58	0.80	1.28	1.85	1.40	1.53		
	1.25	1.30	1.17	1.17	1.58	1.18	0.92	0.52	1.50	1.52	1.10	0.83	0.93	1.13	0.72	1.40	0.97	0.68	0.58	0.80	1.28	1.92	1.45	1.53		
	1.28	1.37	1.23	1.17	1.60	1.25	0.92	0.55	1.53	1.60	-	0.83	0.98	1.18	0.72	1.40	0.98	0.68	0.58	0.80	1.28	2.13	1.65	1.53		
3	1.28	1.42	1.27	1.17					1.55	1.63	1.13	0.83	1.02	1.27	0.77	1.40	0.98	0.68	0.58	0.80	1.37	2.25	1.77	1.58	0.48	0.07
	1.28	1.43	1.35	1.17									1.03	1.42	0.83	1.40	0.98	0.68	0.58	0.80						
	1.30	1.52	1.48	1.17									1.03	1.53	0.85	1.43	0.98	0.72	0.58	0.80						
	1.30	1.52	1.53	1.17									1.05	1.68	0.88	1.43	0.98	0.72	0.62	0.80						
4	1.30	1.58	1.60	1.18									1.05	1.80	0.92	1.45	1.00	0.72	0.63	0.80					0.50	0.06
	1.30	1.58	1.72	1.23									1.05	1.97	0.92	1.47	1.00	0.75	0.63	0.80						
	1.30	1.58	1.77	1.23									1.05	2.10	0.92	1.48										
	1.30	1.58	1.93	1.23									1.05	2.22	0.97	1.50										
5	1.30	1.58	2.02	1.23																						
	1.32	1.58	2.07	1.23																						
	1.32	1.58	2.20	1.26																						
	1.33	1.58	2.33	1.32																						
	1.37	1.62	2.47	1.32																						
6																										
7																										

TABLE 5.2B Length (distance between adjacent resin beads) of the lower surface of horizontal *Z. mays* roots.

Sample No.		G29				G30			G31					G32				G33				G34			
Distance of bead from root tip.		5.33	4.03	2.88	2.40	5.72	3.93	1.67	4.97	4.05	3.23	2.43	1.40	5.67	4.45	2.77	1.42	5.18	4.33	2.72	1.32	4.80	3.75	2.80	1.48
Time	(H)																								
	0	1.13	0.83	0.25	2.07	1.73	2.08	1.45	0.67	0.67	0.63	0.80	1.02	0.98	1.33	0.97	0.90	0.62	1.17	1.17	0.90	0.80	0.73	0.95	1.13
		1.20	0.87	0.27	2.13	1.78	2.17	1.45	0.73	0.70	0.63	0.83	1.18	0.98	1.38	0.98	0.93	0.68	1.22	1.17	0.92	0.82	0.73	0.95	1.28
		1.22	0.87	0.38	2.15	1.82	2.17	1.47	0.73	0.70	0.63	0.83	1.18	1.02	1.42	0.98	0.93	0.72	1.25	1.18	0.92	0.82	0.73	0.98	1.28
		1.22	0.87	0.42	2.17	1.83	2.20	1.53	0.73	0.70	0.63	0.85	1.18	1.02	1.45	0.98	0.93	0.75	1.28	1.18	0.92	0.82	0.73	1.00	1.28
	1	1.30	0.87	0.42	2.17	1.87	2.22	1.60	0.75	0.70	0.63	0.85	0.18	1.02	1.57	0.98	0.93	0.75	1.32	1.23	0.92	0.83	0.75	1.00	1.28
		1.32	0.87	0.42	2.17	1.87	2.25	1.62	0.75	0.70	0.63	0.85	1.25	1.02	1.57	1.02	0.93	0.85	1.33	1.23	0.93	0.87	0.78	1.00	1.28
		1.32	0.92	0.42	2.17	1.87	2.30	1.63	0.75	0.70	0.67	0.85	1.25	1.02	1.60	1.07	0.95	0.87	1.42	1.27	0.93	0.88	0.80	1.05	1.25
		1.32	0.98	0.42	2.17	1.87	2.42	1.65	0.75	0.70	0.70	0.85	1.25	1.03	1.75	1.17	0.97	0.87	1.53	1.27	1.00	0.88	0.80	1.05	1.32
	2	1.32	1.05	0.42	2.17	1.87	2.50	1.72	0.75	0.70	0.70	0.85	1.25	1.03	1.90	1.20	0.97	0.88	1.58	1.33	1.00	0.92	0.80	1.05	1.35
		1.32	1.12	0.42	2.17	1.87	2.50	1.72	0.82	0.75	0.75	0.90	1.25	1.05	1.92	1.22	0.97	0.88	1.70	1.37	1.00	0.92	0.80	1.10	1.35
		1.33	1.17	0.45	2.20	1.87	2.63	1.73	0.82	0.75	0.82	0.90	1.32	1.05	1.95	1.22	0.97	0.88	1.77	1.40	1.00	0.92	0.83	1.13	1.37
		1.33	1.22	0.45	2.20	1.92	2.72	1.77	0.83	0.75	0.90	0.90	1.32	1.05	2.00	1.22	0.97	0.90	1.93	1.48	1.00	0.92	0.83	1.17	1.37
	3	1.33	1.23	0.50	2.22	1.93	2.80	1.80	0.83	0.83	0.95	0.95	1.32	1.05	2.07	1.22	0.97	0.90	1.97	1.55	1.00	0.92	0.83	1.27	1.37
		1.35	1.33	0.50	2.25	1.93	2.97	1.80	0.83	0.85	1.07	1.05	1.32	1.05	2.12	1.22	0.97	0.90	2.07	1.60	1.00	0.92	0.90	1.27	1.40
		1.37	1.48	0.50	2.32	1.97	3.07	1.80	0.83	0.85	1.07	1.07	1.32	1.05	2.13	1.33	0.97	0.90	2.12	1.78	1.00	0.92	0.92	1.37	1.40
		1.37	1.53	0.50	2.32	1.97	3.22	1.88	0.83	0.95	1.13	1.07	1.32									0.92	0.92	1.42	1.43
	4	1.38	1.67	0.57	2.40	1.97	3.37	1.88	0.83	0.97	1.20	1.08	1.32									0.93	1.03	1.45	1.48
		1.38	1.72	0.57	2.43	1.97	3.42	1.98	0.83	0.97	1.20	1.08	1.32									0.93	1.07	1.57	1.48
		1.38	1.75	0.57	2.43	1.97	3.58	1.98														0.93	1.08	1.73	1.52
		1.38	1.75	0.62	2.43																	0.93	1.12	1.73	1.52
	5	1.38	1.83	0.62	2.47																	0.93	1.25	1.92	1.53
		1.40	1.88	0.65	2.55																	0.93	1.25	1.95	1.55
		1.40	1.92	0.75	2.58																	0.93	1.30	2.08	1.55
																						0.95	1.33	2.22	1.60
	6																					0.95	1.33	2.32	1.60
																						0.95	1.40	2.42	1.60
																						0.97	1.42	2.55	1.60
																						0.97	1.43	2.73	1.60
	7																								

TABLE 5.2B (continued)

	G36				G37					G42					G43					G47'					G48			
	5.83	4.62	2.90	1.57	5.05	4.08	2.86	1.86	1.13	5.32	4.15	2.87	1.92	0.83	4.93	4.17	3.20	2.27	0.63	4.58	3.98	3.12	1.98	0.90	5.50	3.85	2.72	1.35
0	0.88	1.47	1.12	1.42	0.58	0.83	0.73	0.52	0.85	0.90	1.07	0.67	0.80	0.55	0.50	0.70	0.62	0.70	0.95	0.35	0.67	0.87	0.82	0.67	1.33	0.97	1.03	1.03
	0.97	1.67	1.18	1.42	0.67	0.86	0.73	0.52	0.85	0.90	1.07	0.68	0.80	0.55	0.52	0.72	0.65	0.72	0.95	0.37	0.70	0.87	0.83	0.67	1.33	1.05	1.03	1.12
	0.97	1.67	1.18	1.50	0.67	0.88	0.75	0.53	0.88	0.90	1.08	0.68	0.80	0.57	0.52	0.77	0.65	0.72	1.07	0.40	0.70	0.87	0.55	0.67	1.33	1.08	1.08	1.15
	0.97	1.70	1.22	1.53	0.67	0.90	0.80	0.53	0.92	0.92	1.08	0.72	0.80	0.62	0.53	0.78	0.67	0.72	1.07	0.40	0.70	0.57	0.87	0.67	1.33	1.12	1.08	1.15
1	0.97	1.70	1.22	1.53	0.67	0.95	0.88	0.58	0.92	0.92	1.08	0.72	0.80	0.63	0.58	0.83	0.67	0.72	1.07	0.40	0.70	0.87	0.87	0.67	1.33	1.23	1.08	1.18
	1.00	1.70	1.22	1.58	0.67	0.95	0.85	0.58	0.92	0.92	1.13	0.75	0.80	0.63	0.63	0.85	0.68	0.78	1.07	0.40	0.70	0.87	0.90	0.67	1.33	1.33	1.13	1.18
	1.00	1.73	1.22	1.58	0.67	0.95	0.88	0.58	0.92	0.92	1.13	0.75	0.80	0.63	0.63	0.87	0.72	0.78	1.07	0.40	0.73	0.88	0.90	0.67	1.33	1.37	1.13	1.18
	1.00	1.76	1.28	1.58	0.67	0.97	0.88	0.67	0.92	0.92	1.13	0.75	0.80	0.63	0.67	0.93	0.72	0.78	1.07	0.40	0.75	0.90	0.92	0.72	1.33	1.40	1.13	1.18
2	1.00	1.83	1.32	1.58	0.67	0.97	0.88	0.72	0.92	0.92	1.17	0.75	0.80	0.63	0.68	1.00	0.73	0.82	1.07	0.40	0.75	0.90	0.93	0.75	1.33	1.48	1.20	1.18
	1.05	1.95	1.38	1.58	0.67	1.10	0.88	0.72	0.92	0.92	1.17	0.75	0.80	0.63	0.68	1.07	0.78	0.83	1.07	0.40	0.75	0.90	0.93	0.75	1.33	1.60	1.23	1.18
	1.05	2.03	1.43	1.58	0.67	1.15	1.00	0.72	0.92	0.92	1.18	0.75	0.80	0.67	0.70	1.15	0.82	0.83	1.07	0.40	0.75	0.90	0.93	0.75	1.33	1.62	1.23	1.18
	1.05	2.10	1.43	1.58	0.67	1.15	1.03	0.82	0.92	0.92	1.18	0.77	0.80	0.67	0.70	1.18	0.85	0.83	1.08	0.40	0.75	0.90	0.95	0.75	1.33	1.73	1.30	1.18
3	1.05	2.12	1.43	1.58	0.67	1.17	1.15	0.82	0.92	0.92	1.18	0.83	0.80	0.67	0.70	1.30	1.00	0.88	1.08	0.40	0.75	0.90	0.95	0.75	1.33	1.73	1.37	1.18
	1.05	2.18	1.45	1.62	0.67	1.25	1.18	0.82	0.97	0.92	1.18	0.83	0.80	0.67	0.70	1.38	1.10	0.90	1.13	0.40	0.75	0.92	0.95	0.75				
	1.07	2.28	1.50	1.62	0.70	1.25	1.18	0.82	1.02	0.92	1.18	0.83	0.82	0.67						0.40	0.75	0.92	0.98	0.75				
	1.07	2.33	1.50	1.63	0.73	1.26	1.23	0.85	1.02	0.92	1.18	0.92	0.83	0.67						0.40	0.75	0.97	0.98	0.75				
4	1.07	2.40	1.65	1.63	0.73	1.32	1.40	0.85	1.02	0.92	1.20	0.97	0.83	0.67						0.40	0.75	0.97	0.98	0.75				
										0.92	1.23	1.02	0.83	0.67						0.40	0.75	0.98	0.98	0.75				
										0.92	1.23	1.07	0.87	0.67						0.40	0.75	1.00	1.08	0.75				
										0.92	1.23	1.12	0.90	0.67						0.40	0.75	1.03	1.08	0.80				
5										0.92	1.23	1.20	0.90	0.67						0.40	0.75	1.05	1.13	0.80				
										0.92	1.23	1.27	0.92	0.67						0.40	0.75	1.12	1.23	0.80				
										0.92	1.23	1.35	0.92	0.67						0.40	0.75	1.17	1.28	0.80				
										0.92	1.23	1.42	0.95	0.67						0.40	0.75	1.23	1.33	0.80				
6										0.92	1.27	1.50	0.98	0.67						0.40	0.75	1.30	1.42	0.80				

TABLE 5.2B (continued)

	G38				G49				G51			G52				G53				O60				O62			
	5.12	3.35	2.42	1.60	5.10	3.62	2.55	1.50	5.58	4.30	3.05	4.83	3.63	2.30	1.27	5.15	4.15	2.48	1.40	5.85	4.27	2.82	1.50	5.40	3.92	2.52	1.3
0	1.47	0.60	0.42	1.12	1.18	0.75	0.78	1.20	0.82	0.93	2.70	0.93	1.07	0.77	0.85	0.65	1.33	0.62	1.10	1.14	0.93	0.89	0.71	0.66	0.95	0.94	0.9
	1.53	0.60	0.48	1.23	1.18	0.75	0.80	1.20	0.92	1.00	2.75	0.97	1.18	0.83	0.95	0.68	1.45	0.65	1.12	1.14	1.00	0.93	0.71	0.68	1.00	0.94	0.9
	1.53	0.62	0.48	1.27	1.22	0.75	0.80	1.20	1.05	1.13	2.82	1.18	1.33	0.92	0.97	0.70	1.67	0.75	1.12	1.14	1.10	1.04	0.80	0.68	1.08	1.15	1.0
	1.63	0.62	0.48	1.27	1.22	0.78	0.80	1.23	1.08	1.22	2.87	1.42	1.43	0.97	0.97	0.70	1.67	0.75	1.12	1.20	1.14	1.11	0.80	0.69	1.17	1.26	1.0
1	1.65	0.62	0.50	1.27	1.22	0.80	0.85	1.23	1.17	1.37	3.00	1.57	1.67	0.98	1.02	0.70	1.80	0.87	1.12	1.23	1.23	1.16	0.87	0.69	1.20	1.45	1.0
	1.80	0.62	0.50	1.28	1.22	0.83	0.87	1.23	1.33	1.50	3.08	1.67	1.72	1.07	1.02	0.70	1.97	0.95	1.12	1.24	1.31	1.20	0.83	0.72	1.28	1.54	1.0
	1.92	0.70	0.53	1.32	1.22	0.85	0.90	1.23	1.45	1.60	3.20	1.80	1.90	1.13	1.07	0.70	2.07	1.00	1.12	1.29	1.47	1.33	0.83	0.75	1.32	1.63	1.0
	2.10	0.77	0.57	1.35	1.22	0.88	0.90	1.25	1.51	1.70	3.28	1.87	2.10	1.17	1.07	0.72	2.17	1.08	1.12	1.30	1.59	1.40	0.83	0.75	1.34	1.72	1.1
2	2.25	0.82	0.63	1.37	1.22	0.95	0.97	1.25	1.53	1.75	3.28	2.10	2.28	1.25	1.08	0.72	2.35	1.10	1.12	1.30	1.59	1.40	0.83	0.75	1.34	1.72	1.1
	2.35	0.85	0.63	1.37	1.25	1.00	1.00	1.28	1.67	1.75	3.30	2.10	2.43	1.32	1.08	0.72	2.38	1.17	1.12	1.30	1.66	1.40	0.83	0.75	1.38	1.82	1.1
	2.47	0.92	0.63	1.37	1.25	1.00	1.02	1.28	1.67	1.78	3.37	2.18	2.70	1.32	1.08	0.72	2.55	1.28	1.12	1.30	1.79	1.40	0.83	0.77	1.40	1.89	1.1
	2.55	1.12	0.65	1.43	1.25	1.03	1.08	1.28	1.70	1.93	3.40	2.47	2.87	1.32	1.08	0.72	2.77	1.50	1.17	1.30	1.84	1.40	0.83	0.77	1.42	2.00	1.1
3	2.62	1.27	0.68	1.43	1.25	1.15	1.20	1.28	1.78	2.12	3.55	2.47	3.00	1.45	1.08	0.72	2.77	1.50	1.17	1.33	1.96	1.40	0.83	0.77	1.52	2.11	1.1
	2.70	1.38	0.72	1.43	1.25	1.23	1.27	1.28	1.75	2.27	3.68	2.48	3.25	1.50	1.08	0.72	2.90	1.58	1.17	1.33	2.06	1.50	0.83	0.77	1.52	2.15	1.1
	2.75	1.52	0.75	1.43	1.25	1.30	1.32	1.28								0.72	2.92	1.60	1.25	1.33	2.13	1.53	0.91	0.77	1.55	2.20	1.1
	2.80	1.63	0.82	1.43	1.25	1.30	1.37	1.28								0.72	2.93	1.63	1.25	1.33	2.21	1.54	0.91	0.77	1.62	2.31	1.1
4	2.83	1.73	0.83	1.43												0.72	2.97	1.67	1.30	1.33	2.29	1.54	0.91	0.77	1.62	2.32	1.1
																0.72	3.00	1.67	1.30	1.33	2.36	1.60	0.91	0.77	1.63	2.40	1.2
																				1.33	2.43	1.60	0.91	0.78	1.65	2.42	1.2
																				1.33	2.46	1.60	0.97	0.80	1.69	2.45	1.2
5																				1.33	2.51	1.61	1.03	0.85	1.69	2.46	1.2
																				1.33	2.61	1.71	1.04	0.88	1.69	2.52	1.2
																				1.33	2.66	1.79	1.04				
																				1.33	2.69	1.84	1.04				
6																											
7																											

TABLE 5.2B (continued)

	G55				G56				G60				G61				G62				G47			$\bar{x}$	SE
	5.63	4.42	3.60	1.92	5.63	4.42	3.60	1.92	5.07	3.58	2.38	1.08	3.88	2.72	1.92	0.93	4.00	2.90	1.98	1.12	4.90	3.35	2.13		
0	1.25	1.07	0.82	0.97	0.87	1.02	0.80	1.55	1.13	0.83	0.95	0.76	0.95	0.53	0.63	0.60	0.77	0.68	0.50	0.78	1.17	1.07	1.58		
	1.25	1.17	0.88	0.97	0.87	1.05	0.80	1.55	1.22	0.85	1.00	0.78	1.07	0.68	0.78	0.68	0.77	0.68	0.50	0.78	1.32	1.20	1.72		
	1.28	1.23	0.88	1.00	0.87	1.15	0.90	1.62	1.30	0.97	1.07	0.83	1.15	0.70	0.78	0.68	0.83	0.73	0.58	0.78	1.43	1.27	1.75		
	1.33	1.32	0.98	1.02	0.93	1.22	1.07	1.63	1.45	1.08	1.13	0.83	1.30	0.75	0.80	0.68	0.92	0.78	0.63	0.78	1.67	1.43	1.83		
1	1.33	1.37	1.03	1.05	0.93	1.45	1.15	1.73	1.58	1.25	1.17	0.83	1.43	0.78	0.87	0.70	0.95	0.80	0.65	0.78	1.82	1.53	1.93	0.95	0.07
	1.38	1.52	1.08	1.05	0.93	1.50	1.28	1.82	1.72	1.43	1.32	0.83	1.52	0.82	0.87	0.70	0.98	0.87	0.77	0.78	1.93	1.70	1.93		
	1.43	1.57	1.15	1.07	0.93	1.60	1.40	1.83	1.92	1.58	1.32	0.87	1.68	0.92	0.90	0.72	1.17	0.90	0.78	0.78	2.08	1.85	2.00		
	1.43	1.70	1.17	1.17	0.93	1.75	1.50	1.98	2.00	1.72	1.35	0.87	1.88	0.97	0.90	0.72	1.13	0.95	0.78	0.78	2.27	1.95	2.08		
2	1.43	1.70	1.17	1.17	0.93	1.82	1.55	2.03	2.12	1.78	1.38	0.87	2.03	1.05	0.90	0.73	1.15	0.97	0.80	0.78	2.38	2.05	2.10	0.86	0.06
	1.43	1.72	1.20	1.20	0.93	1.88	1.67	2.05	2.13	1.88	1.43	0.87	2.22	1.05	0.90	0.77	1.23	1.00	0.80	0.80	2.38	2.27	2.13		
	1.47	1.80	1.20	1.20	0.95	1.93	1.78	2.13	2.15	1.88	1.43	0.87	2.32	1.23	1.02	0.77	1.23	1.15	0.83	0.80	2.45	2.42	2.15		
	1.47	1.90	1.27	1.23	0.93	2.02	1.93	2.17	2.15	1.95	-	0.87	2.47	1.27	1.02	0.77	1.23	1.15	0.83	0.80	2.65	2.58	2.15		
3	1.47	2.03	1.30	1.27	0.93	2.05	2.02	2.22	2.20	2.02	1.50	0.87	2.63	1.30	1.03	0.78	1.30	1.17	0.83	0.80	2.78	2.80	2.15	0.69	0.06
	1.47	2.20	1.42	1.27									2.63	1.30	1.05	0.78	1.32	1.25	0.85	0.80					
	1.47	2.25	1.53	1.33									2.68	1.37	1.10	0.78	1.32	1.33	0.92	0.83					
	1.47	2.38	1.62	1.40									2.73	1.55	1.10	0.78	1.40	1.43	0.92	0.83					
4	1.47	2.47	1.63	1.40									2.78	1.70	1.12	0.78	1.43	1.47	0.92	0.83				0.59	0.05
	1.47	2.47	1.77	1.40									2.80	1.78	1.13	0.80	1.47	1.60	1.00	0.85					
	1.47	2.53	1.85	1.42									2.83	1.95	1.13	0.83									
	1.53	2.70	1.92	1.42									2.83	2.08	1.15	0.83									
5	1.53	2.70	2.09	1.45																					
	1.53	2.77	2.12	1.50																					
	1.53	2.83	2.23	1.50																					
	1.53	2.83	2.33	1.00																					
6	1.53	2.87	2.57	1.50																					
7																									

TABLE 5.3 Growth rate of the upper (A) and lower (B) surface of horizontal and vertical (C) *Z. mays* roots in continuous white light (3.67 $Jm^{-2}s^{-1}$).

Time (Hrs)																									$\bar{x}$	SE
A.																										
0-1	0.75	1.29	1.04	0.98	1.09	0.80	1.07	0.42	0.52	1.04	0.12	0.94	1.62	1.00	1.09	1.62	0.79	0.76	0.93	0.67	1.02	1.14	1.07	0.45	1.46	0.95	0.07
1-2	0.85	1.00	0.94	0.32	1.30	0.63	0.76	0.78	0.48	1.10	0.27	0.91	0.81	0.82	1.02	1.47	0.80	0.76	0.70	0.77	1.07	1.32	0.93	0.52	1.25	0.86	0.06
2-3	0.60	0.96	0.45	0.20	0.66	0.99	0.52	0.72	0.46	0.69	0.46	0.57	1.19	0.30	0.89	1.29	0.87	0.53	0.49	0.52	0.89	0.44	1.03	0.40	1.20	0.69	0.06
3-4	0.82	0.46	0.45	-	-	0.52	0.42	0.46	0.55	-	0.74	-	0.99	-	-	-	0.50	0.48	0.39	0.90	-	-	0.64	0.55	-	0.59	0.05
B.																										
0-1	0.48	0.43	0.32	0.32	0.36	0.25	0.53	0.44	0.16	0.40	0.13	0.46	0.43	0.19	0.59	0.65	0.28	0.32	0.28	0.36	0.35	0.48	0.34	0.16	0.47	0.37	0.03
1-2	0.20	0.40	0.14	0.60	0.57	0.26	0.31	0.21	0.12	0.43	0.22	0.37	1.03	0.29	0.62	1.12	0.38	0.29	0.19	0.36	0.40	0.55	0.43	0.08	0.99	0.42	0.05
2-3	0.32	0.44	0.63	0.21	0.63	0.27	0.45	0.60	0.13	0.66	0.02	0.42	0.93	0.49	0.57	1.19	0.27	0.14	0.21	0.42	-	0.56	0.56	0.04	1.32	0.48	0.07
3-4	0.74	0.69	0.52	-	-	0.50	0.57	0.56	0.19	0.10	-	-	0.82	-	-	-	0.50	0.59	0.31	0.52	-	-	0.76	0.11	-	0.50	0.06
C.																										
-2--1	0.76	1.17	0.59	0.71	0.66	0.12	0.62	0.11	0.03	0.28	0.20	0.58	0.83	0.68	0.73	0.89	-	0.43	0.20	0.55	0.68	0.75	0.17	0.31	1.10	0.55	0.06
-1-0	0.45	1.16	0.51	0.34	0.63	0.26	0.58	0.19	0.12	0.20	0.11	0.59	1.00	0.67	0.48	1.11	-	0.28	-	0.41	0.64	0.74	0.61	0.14	0.82	0.52	0.06

t values 1-4 hours upper vs initial

t = 4.25***

t = 3.71***

t = 2.36*

t = 1.32

t values 1-4 hours lower vs initial growth rate

t = 1.66

t = 1.50

t = 1.00

t = 1.14

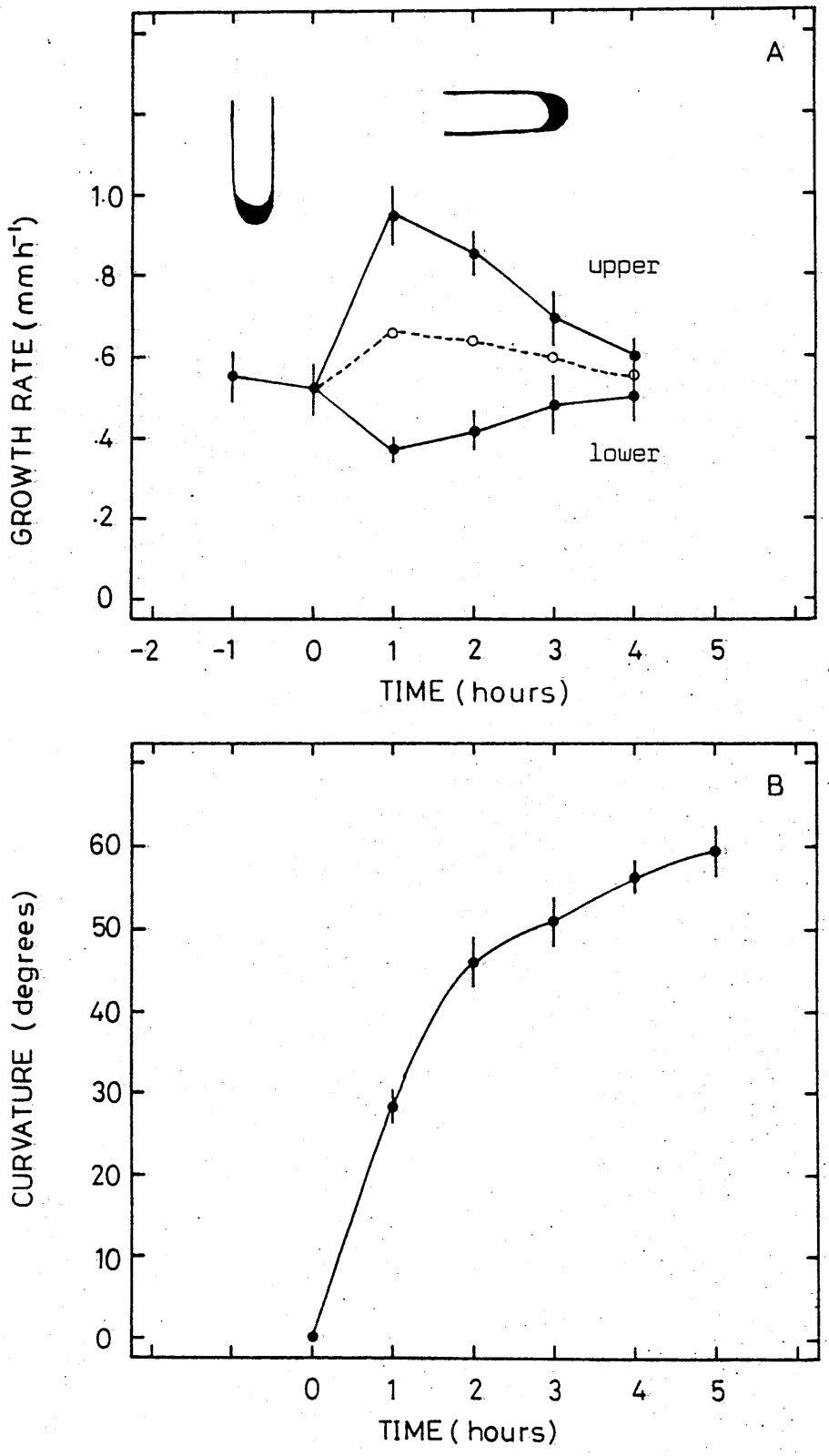


Figure 5.1 The mean growth rate of the upper and lower surface of roots displaced horizontally after 2 h vertical growth (A) and the mean curvature (B) of Z. mays roots growing in white light (3.67 $Jm^{-2}s^{-1}$) o --- o indicates the average mean growth rate whilst horizontal.

horizontal roots decreased to 0.37mm h^{-1} after 1h and then gradually increased over the next 3h to regain approximately its original value. The mean decrease in the growth rate of the lower surface of the organs did not attain significance at the 0.05 probability level at any time during the 4h following horizontal placement of the root (Table 5.3).

The upper surface of a gravistimulated root shows an 80% increase in its growth rate after 1h whereas the lower surface shows a decrease of 30%. The increase on the upper surface is, therefore, over twice as great as the decrease on the lower surface. The average of the growth rates on the upper and lower surfaces, at any particular time after the root is placed horizontally, is found to be greater than the original growth rate of the root when vertical. Gravitropic stimulation thus appears to lead to an overall increase in the growth rate of the root, at least for the two hours or so following horizontal placement.

During the two hours after being placed in the horizontal position the growth rates of the upper and lower surfaces of the roots are highly, significantly, different but by the third and fourth hour the difference has decreased to a value which is no longer significant at the 0.05 level of probability. The differences in the growth rate of the two surfaces of the root are clearly correlated with the downward gravitropic curvature of the root (Fig. 5.1B). During the first hour the roots bend downward to 28° and in the second to 45° . After this the rate of curvature declines to about 5° per hour so that after 5h the mean angle attained is 58°. The lower rate of curvature between the second and fifth hour after horizontal placement agrees

TABLE 5.4 Curvature of *Z. mays* roots in continuous white light (3.67 $Jm^{-2}s^{-1}$).

Time (Hrs)																								$\bar{x}$	SE
0-1	0	-8	0	0	0	0	1	0	0	0	0	0	-8	-5	-3	0	-6	0	0	0	0	0	0	-0.8	0.76
1-2	22	33	42	30	39	32	44	24	12	24	0	20	36	33	35	50	19	26	35	32	22	17	42	28.65	2.35
2-3	34	68	81	19	65	42	57	60	29	45	10	40	40	55	50	55	25	39	58	60	50	30	49	46.13	3.43
3-4	33	86	68	23	78	62	48	65	46	42	29	40	45	41	56	54	73	47	52	44	68	42	46	51.65	2.25
4-5	40	75	60	-	-	64	48	58	57	-	56	-	48	-	-	-	61	54	-	-	58	56	-	56.54	2.25
5-6	36	-	-	-	-	59	-	-	65	-	77	-	-	-	-	-	-	61	-	-	-	-	-	59.60	5.97

clearly with the rather small difference between the growth rates of the two surfaces of the root during this time.

5.3.0 DISCUSSION

The observed response is very similar to the 2-phase model of gravicurvature as described by Bennet-Clark et al. (1959). They characterised the first phase by rapid curvature and reduced growth rate, and the second phase by a very slow change in curvature and normal growth rate. In the present study the rapid curvature to approximately 50° during the first 3h of the response could be assigned to phase 1, and the slower curvature after 3h to phase 2. The pattern of growth rate change does not completely conform to Bennet-Clark et al.'s model since there was an increase rather than a reduction in the growth rate during the first phase.

Pilet and Ney (1981) also reported a decreased growth rate during the first hours of gravicurvature. However, when their data for the growth rates of the two surfaces of the root are examined it is found that the growth rate of the upper surface is not altered significantly whereas that of the lower surface does decrease significantly in the first 2h after turning horizontal. This is in direct contrast with the data reported in this thesis where the growth rate of the upper surface was found to increase significantly whilst that of the lower surface was not significantly decreased at any time during the observation period. However, despite this disagreement in the growth rate data, the pattern of gravicurvature found by Pilet and Ney (1981) is identical to that in this paper; that is, an increase in angle during the period of differential growth followed by a more

gradual increase in angle after 5h have elapsed. Pilet and Ney (1981) also present data for a single root and here an oscillating pattern of curvature similar to that found in present study after 3h is clearly seen.

There are however, reports in the literature which support the data in the present study. Veen (1964) observing the increase in length of marked roots, and Pilet and Nougarede (1974) measuring the increase in length of cortical cells, provide evidence that Vicia faba and Zea mays achieve a curvature by stimulation of the growth rate of the upper surface accompanied by no alteration of the growth rate of the lower surface. Barlow and Hofer (Jackson and Barlow, 1981) have made similar observations with Z. mays LG 11, their results indicating a substantial promotion of cell elongation in the cortex of the upper half of gravicurving roots but little change in the lower half. These researchers have also noted a correlation between cuticular cracking and the presence of fast growing cells in the convex surface of curving roots.

Iversen (1973) and Jotterand-Dolivo and Pilet (1970) also report that the upper surface of a gravicurving root grows faster than the lower surface but they attribute this to a greater amount of inhibition on the lower surface, rather than an acceleration on the upper, a finding that is clearly inconsistent with the data presented here.

A striking feature of the data presented here is that the promotion of growth on the upper surface is not directly equivalent to the inhibition on the lower surface. This pattern of growth rate changes has been quoted as an objection to the Cholodny-Went hypothesis

of gravitropism (Digby and Firn, 1979; Franssen et al., 1981, 1982). The argument used in opposition to this hypothesis is that the predicted co-ordinated change in the growth rates on the upper and lower surfaces is not observed (Digby et al., 1982). However, this absence of a co-ordinated change in rate can be explained in a number of ways, without the Cholodny-Went hypothesis loosing its validity. Two of the ways in which the observed growth rate changes can be accommodated are by the non-linearity of the response of growth rate to inhibitor concentration and by metabolism of the growth regulator.

The first of these explanations is based on the fact that under certain circumstances addition of inhibitor can cause an amount of inhibition quite different to the amount of promotion caused by removal of the same quantity of the inhibitor. Since the circumstances under which these un-coordinated changes can occur, in relation to the dosage-response curve for auxin action on root growth, were detailed in the introduction to this thesis they shall not be re-discussed here.

The second way to explain the responses involves the metabolism of inhibitor and two of the possible ways in which this could have an effect are outlined here. Firstly, the inhibitor could be metabolised as it is transported down through the root tissues, resulting in less reaching the lower surface than leaves the upper surface. This theory could be substantiated if the inhibitor in the gravitropic response was identified and shown to be metabolised in the root tissues. The explanation appears to have some circumstantial support since Feldman (1980a) has presented evidence showing that all root tissues are efficient at metabolising IAA. Although IAA is not

the favourite contender for the role of root cap inhibitor due to its acropetal transport in the root (Pilet, 1964; Wilkins and Scott, 1968a; Scott and Wilkins, 1968) it seems feasible that the growth regulator involved in the gravitropic response would also be metabolised by the root tissues.

Secondly, the metabolism of growth regulator could be involved in the way outlined in Figure 5.2. When a root is kept vertical it is assumed that equal amounts of inhibitor pass back along both surfaces of the root to the elongation zone: for argument's sake, it will be assumed that 10 molecules of inhibitor pass back along both surfaces (Fig. 5.2A). When placed horizontally, downward, lateral, transport of the inhibitor occurs (Shaw and Wilkins, 1973) with inhibitor moving from the upper to the lower surface; let it be assumed that 5 molecules of inhibitor are laterally transported (Fig. 5.2B). If there is the metabolism of 5 molecules of inhibitor on both the upper and the lower surface of the root, there will be no inhibitor left to pass back on the upper surface, that is, 10 molecules less than in the vertical root, being manifest as an increase in the growth rate, but still 10 molecules on the lower surface, resulting in very little change in the growth rate as compared to the initial vertical rate (Fig. 5.2C). The net effect of these changes would be an increase in the overall growth rate of the roots, and this was in fact what was observed in the experiments reported in this chapter.

The explantions outlined above are 3 of the simplest of how the disproportionate increase and decrease in growth rate could arise in gravireacting roots: these simple models do, however, illustrate

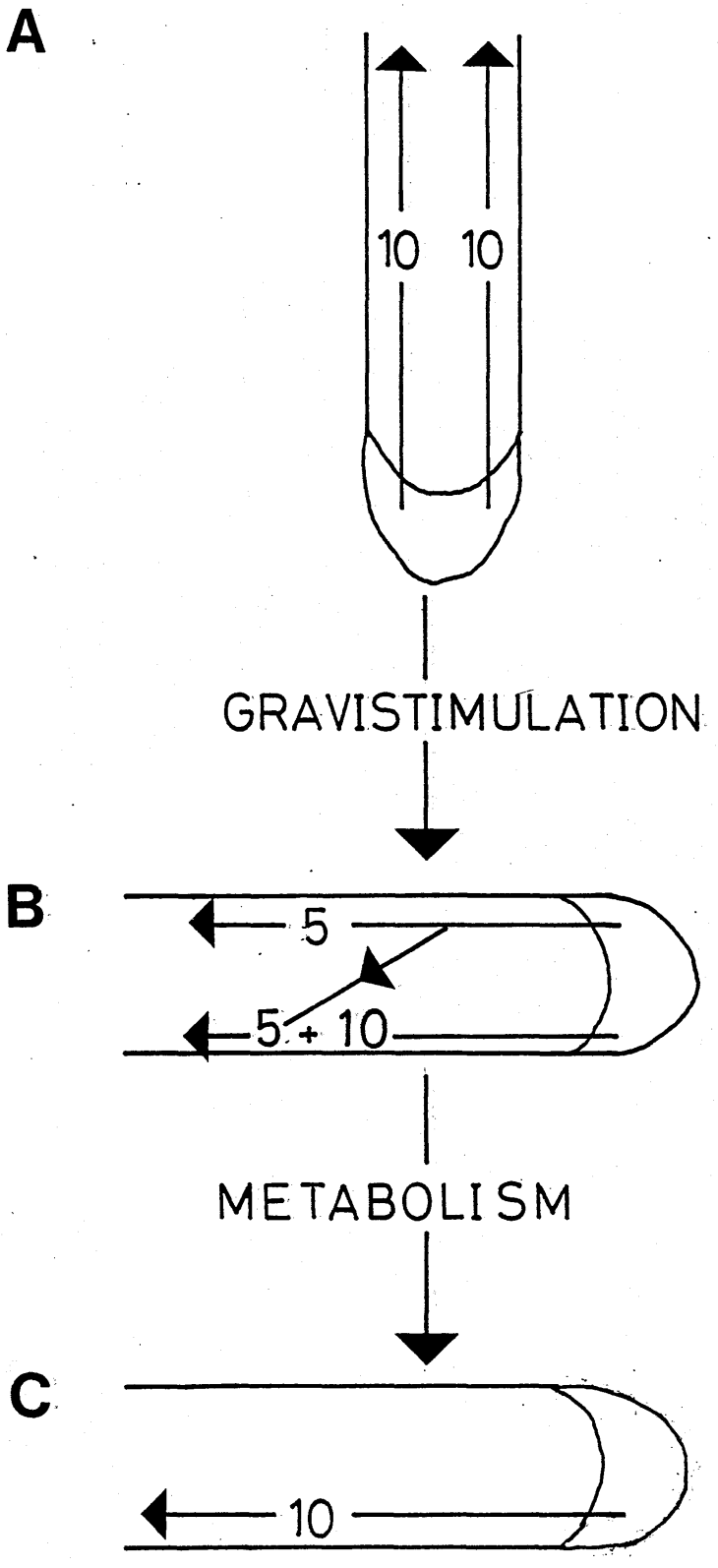


Figure 5.2 A diagrammatic representation of the possible metabolism of growth regulator leading to the observed disproportionate growth rate changes on the opposite surfaces of a horizontal Z. mays root. (A) the transport of inhibitor in a vertical root (B) downward, lateral transport of 5 molecules of inhibitor (C) the levels of inhibitor resulting on both surfaces of the root.

that the unequal changes in the rate observed can be accounted for without invalidating the Cholodny-Went theory, contrary to what $\frac{1}{h}$ was suggested by Digby et al. (1982).

CHAPTER SIX

GENERAL CONCLUSIONS

In undertaking physiological studies of the growth of plant organs it is necessary to ensure that the experimental conditions are as near to those which the plant would encounter in its natural environment. Whilst this is relatively easy to achieve when studying the aerial parts of the plant, difficulties arise in simulating the conditions of the soil environment in root studies. Of particular difficulty is the fact that roots are generally in darkness, but in order to measure and record continuously, without the use of destructive sampling, the behaviour of roots, light is required. In order to overcome this difficulty in the studies reported in this thesis infra-red radiation, which has been shown to have no measurable effect on the growth of seedlings (Iino and Carr, 1981), [illegible] was used to manipulate and monitor the growth and curvature of the roots.

Using this infra-red methodology it was possible to rationalise the conflicting reports in the literature. The data in this thesis confirm that light inhibits the growth of roots (Torrey, 1952; Pilet and Went, 1956; Burström, 1960; Masuda, 1962; H. Wilkins et al., 1973; Pilet and Ney, 1978) enhances gravitropic curvature (Scott and Wilkins, 1969; Gibbons and Wilkins, 1970; Pilet, 1971; H. Wilkins and Wain, 1974, 1975; Beffa and Pilet, 1982) and that the presence of the root cap is a prerequisite for the light induced growth inhibition (H. Wilkins and Wain, 1974, 1975). Of particular

interest were the observations in Chapter 3 which indicated that a promoter may be produced by the root cap in darkness. As discussed earlier (Chapter 3) the presence of this promoter required that the previous mechanisms for explaining the observed growth rate changes were revised and expanded to involve both a promoter and an inhibitor.

One surprising feature of the data in this thesis is that the average growth rate observed for roots, in both darkness and light, was found to vary throughout the study. This variability could be related to a number of factors, for example, a) the age of the seed; b) a seasonal variation in the seed; or c) a variable genotype of the seed. All three of these possibilities seem unlikely: the first two possibilities seem unlikely since no variation was observed in the data from other experiments carried out over the three years of study, and the seeds were stored at a low temperature which should have slowed their metabolic activities. The third possibility was that of variation in the genotype of the seed, that is, that there are fast growing and slow growing individuals and by chance the majority of fast growing seeds have been picked for some experiments and slow growing seedlings for others. This explanation seems unlikely since in all experiments the roots were selected for a root length of 10-15mm and in all cases there were a number of smaller and larger roots in the sample of seedlings germinated for the experiments. Pilet and Saugy (1984) have recently published data which they believe show a bimodal distribution in growth rate of a population of approximately 600 Zea seedlings. Many fewer roots were examined in the present study and it is not possible to state whether or not a bimodal distribution of growth rate occurs.

Although the straight growth data cannot indicate two types of growth rate in the seedlings, the gravicurvature of illuminated roots was clearly divisible into two distinct populations; firstly those which showed a fluctuating pattern of curvature after 2 hours horizontal displacement and, secondly, those which continued to curve to a maximum angle over the whole of the recorded time period (Chapter 4). Whether or not these 2 patterns of curvature are related to the fast and slow growth apparently shown by Pilet and Saugy (1984) cannot be determined from the data in this thesis; results of future work where the vertical growth rate of the individuals is determined before horizontal displacement should demonstrate if these 2 phenomena are related. The most favoured mechanism which results in the downward gravitropic curvature in roots is the Cholodny-Went hypothesis which states that the downward, lateral, transport of IAA leads to a greater inhibition of growth on the lower side of the root and hence curvature. The asymmetric distribution of growth inhibitor should be reflected in the growth rate changes on the opposite sides of the root. The data in Chapter 5 clearly indicate that the curvature develops as a result of a significant increase in the rate on the upper surface and a simultaneous, although insignificant reduction on the lower surface. Thus, promotion of the growth rate on the upper surface is the critical factor in the development of gravicurvature.

The belief that the critical growth regulator was inhibitory in its action in gravicurvature arose from experiments which demonstrated that removal of the root cap from illuminated roots led to an increase in the growth rate (Cholodny, 1926) and that during gravicurvature the overall growth rate of the root was decreased.

(Sachs, 1882; Larsen, 1953; Bennet-Clark et al., 1959). These findings are, however, inconsistent with the results of experiments by Juniper et al. (1966) Pilet (1971a,b) and those of the present study. Pilet (1972) explained the lack of a response in his earlier experiments and those of Juniper et al. by the fact that the initial readings were taken 4h after decapping and that a transient decrease, revealed in his later studies (1972b) had been missed. However, the data of the present study, with readings taken every 15 min from decapping, do not reveal any such decrease in growth rate upon decapping, and during gravicurvature an increase in the overall growth rate was observed (Fig. 5.1).

The absence of a decrease in growth rate upon decapping can be explained without affecting the validity of the Cholodny-Went hypothesis as has been explained in Chapter 3.

Simple analyses of growth rate changes in vertically-orientated and gravitropically curving roots, such as those reported in this thesis, are of considerable importance when trying to establish that a particular physiological factor, such as a growth regulator, is responsible for causing a particular response. However, in order to prove conclusively the validity of any of the models proposed in this thesis, and moreover that of the Cholodny-Went hypothesis, it is imperative that future studies involve the identification of growth regulators inducing gravitropic curvature.

Furthermore, until the growth regulators are identified and their transport and metabolism are established there is little prospect of elucidating the conflicting data in the published literature or to prove unequivocally, or disprove, the validity of the Cholodny-Went hypothesis.

App. 1, TABLE 1. Analysis of variance data of intact Z. mays roots kept in darkness for 4h prior to illumination with white light (3.67 $Jm^{-2}s^{-1}$).

Sum of Sq. = Sum of Squares; D.F. = Degrees of Freedom; Mean Sq. = Mean of Squares.

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	83452.39	14	5960.885	95.22	***
Times	37755.93	7	5393.7029	86.16	***
a v b	32720.42	1	32720.40	522.69	***
in a	244.9665	3	81.6555	1.07	NS
in b	4790.54	3	1596.85	20.88	***
Interactions					
Roots x (a vs b)	870.84	14	62.60	0.82	NS
Remainder	6424.87	84	76.49		

App. 1, TABLE 2. Analysis of variance data of intact Z. mays roots exposed to 4h darkness (A), 4h light (B) and then 8h darkness (C).

	Sum of Sq.	D.F.	Mean Sq.	F	P
A vs B					
Roots	37001.47	8	4625.31	11.26	**
Time	21143.53	7	3020.50	7.35	**
a vs b	13781.64	1	13781.64	33.55	***
in a	4991.66	3	1663.89	25.99	***
in b	2370.24	3	790.08	12.35	***
Roots x (a vs b)	3285.93	8	410.74	6.41	***
Remainder	3073.95	48	64.04		
B vs C					
Roots	18102.13	8	2262.77	4.34	*
Times	3816.35	7	545.19	1.05	NS
b vs c	1112.25	1	1112.25	2.14	NS
in b	2370.24	3	790.08	7.11	***
in c	333.86	3	111.29	1.00	NS
Root x (b vs c)	4167.20	8	520.90	4.69	***
Remainder	5335.32	48	111.15		
A vs C					
Roots	23074.30	8	2884.29	2.88	NS
Time	12609.34	7	1801.33	1.80	NS
a vs c	11706.09	1	11706.09	11.68	**
in a	4991.66	3	1663.89	20.63	***
in c	333.86	3	111.25	1.38	***
Roots x (a vs c)	8019.52	8	1002.44	12.43	***
Remainder	3869.65	48	80.62		

App. 1, TABLE 3. Analysis of variance data of decapped *Z. mays* roots exposed to 4h darkness followed by 4h white light (3.67 $Jm^{-2}s^{-1}$).

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	108817.83	14	7772.70	15.75	***
Times	5016.70	7	716.67	1.45	NS
a vs b	516.57	1	516.57	1.05	NS
within a	4784.08	3	1594.69	15.45	***
within b	2396.07	3	798.69	7.74	***
Interactions					
Roots x (a vs b)	6909.49	14	493.50	4.78	***
Remainder	8672.21	84	103.24		

App. 1, TABLE 4. Analysis of variance data of *Z. mays* roots kept in darkness with the root cap removed at 3h.

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	25876.09	10	2587.61	35.29	*
Times	26495.72	7	2927.96	23.99	**
a vs b	18480.09	1	18480.09	25.207	***
in a	872.87	2	436.48	4.052	*
in b	1142.65	4	285.66	2.652	*
Interactions					
Roots x (a vs b)	7331.41	10	733.14	6.807	***
Remainder	6462.50	60	107.71		

App. 1, TABLE 5. Analysis of variance data of *Z. mays* roots kept in white light (3.67 $Jm^{-2}s^{-1}$) with the root cap removed at 3h.

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	27122.54	10	2712.25	0.20	NS
Times	17047.26	7	2435.33	0.19	NS
a vs b	3186.42	1	3186.42	0.24	NS
within a	1003110.28	2	501555.14	54.70	***
within b	8678.66	4	2169.67	2.46	NS
Interactions					
Roots x (a vs b)	130623.15	10	13062.32	14.82	***
Remainder	45845.42	52	881.64		

App. 1, TABLE 6. Analysis of variance data of intact *Z. mays* roots growing in darkness with a 10 min pulse of white light (3.67 $Jm^{-2}s^{-1}$) at 3h.

	Sum of sq.	D.F.	Mean sq.	F	P
Roots	81897.14	19	4310.37	6.10	***
Time	39462.13	8	4932.77	6.98	***
a vs b	31906.25	1	31906.25	45.14	***
in a	226.90	2	113.45	0.51	NS
in b	7328.98	5	1465.80	6.62	***
Interactions					
Roots x (a vs b)	13429.07	19	706.79	3.19	**
Remainder	28782.72	130	221.41		

App. 1, TABLE 7. Analysis of variance data of *Z. mays* roots growing in darkness with the root cap immediately removed after a 10 min pulse of white light (3.67 $Jm^{-2}s^{-1}$) at 3h.

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	13314.62	11	1210.47	0.26	NS
Time	57696.42	7	8242.35	1.75	NS
a vs b	45925.97	1	42925.97	9.74	**
in a	374.22	2	187.11	2.20	NS
in b	1396.28	4	349.07	4.12	**
Interactions					
Roots x (a vs b)	51854.27	11	4714.02	55.60	***
Remainder	5425.84	64	84.78		

App. 1, TABLE 8. Analysis of variance data of *Z. mays* roots growing in darkness with incisions made in the root cap at 4h.

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	80761.26	9	8973.47	8.96	***
Times	4149	7	592.71	0.59	NS
a vs b	2729.96	1	2729.96	2.72	NS
within a	191.27	2	95.64	0.39	NS
within b	123313.78	4	30828.44	125.64	***
Interactions					
Roots x (a vs b)	9010.78	9	1001.20	4.08	**
Remainder	13250.18	54	245.37		

App. 1, TABLE 9. Analysis of variance data of intact *Z. mays* roots exposed to red light (660nm; 5.0×10^{18} quanta $m^{-2}s^{-1}$) after 4h growth in darkness.

	Sum of Sq.	D.F.	Mean Sq	F	P
Roots	27945.94	9	3105.10	4.81	**
Times	54509.40	8	6813.68	10.54	**
a vs b	49028.83	1	49028.83	75.87	***
in a	1363.48	3	454.49	2.87	**
in b	4117.09	4	1029.27	6.50	***
Interactions					
Root x (a vs b)	5815.80	9	646.20	4.08	***
Remainder	9660.45	61	158.37		

App. 1, TABLE 10. Analysis of variance data of intact *Z. mays* roots exposed to blue light (445nm; 4.2×10^{18} quanta $m^{-2}s^{-1}$) after 4h growth in darkness.

	Sum of Sq.	D.F.	Mean Sq.	F	P
Roots	51611.42	12	4300.95	16.03	***
Times	49585.67	8	6198.21	23.10	***
a vs b	40673.78	1	40673.78	151.61	***
in a	1017615	3	339205	279.44	***
in b	103044.59	4	25761.15	21.22	***
Interactions					
Roots x (a vs b)	3219.35	12	268.28	0.22	NS
Remainder	86184.32	71	1213.86		

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