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THE ROLE OF GROWTH IN PHOTOTROPISM AND NUTATION OF *ARABIDOPSIS THALIANA* SEEDLINGS

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ABSTRACT

The role of growth in phototropism and nutation of *Arabidopsis thaliana* seedlings

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In response to short- or long-term unilateral blue light-(BL) irradiation, etiolated seedlings of *Arabidopsis thaliana* exhibit first and second positive phototropism, respectively. Pre-irradiation of seedlings with red light-(RL) for one hour decreases the lag phase and increases the magnitude of first positive phototropism. First and second positive phototropism, RL-enhanced first positive phototropism and first positive phototropism of seedlings rotated on a clinostat are all transient responses with different magnitudes. Neither RL pre-irradiation nor rotating the seedlings on the clinostat affects the process of straightening which occurs after the seedlings reach their maximum phototropic curvature.

Phototropism is the result of unequal elongation rates on opposite sides of the seedling. Regardless of the duration of BL photostimulation, the previous history of seedlings (-RL or +RL) or temperature conditions, the shaded side of the seedlings grows faster, while the lighted side of the seedlings grows slower thus producing phototropic curvature. These changes in elongation rates are subsequently reversed such that the shaded side starts growing slower and lighted

side faster. As the elongation rate of the lighted side becomes equal to, and then exceeds the elongation rate of the shaded side, seedlings stop bending toward the light source and start straightening. The kinetics for growth distribution during first and second positive phototropism are similar. However, RL pre-irradiation or a decrease in ambient temperature change kinetics of growth distribution (lag time) during first positive phototropism.

Undirected growth of seedlings is not altered by the RL or short BL pulses from above. However, transfer of seedlings from 25 °C to either a lower or higher temperature resulted in a decrease of growth rate.

Etiolated *Arabidopsis* seedlings exhibit nutation, while about 10% of them circumnutated. Circumnutation dissappears after RL pre-irradiation. Nutation of *Arabidopsis* seedlings appeared to be independent from gravitropism and phototropism.

Independence of the different types of movements points toward complex control of growth in *Arabidopsis thaliana* seedlings.

To The TRUTH

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LIST OF ABBREVIATIONS

ABA: Abscisic Acid
BL: blue light
C: degree Celsius
deg: degree
FW: fresh weight
g: gram
GL: green light
GTP: Guanosine tri-phosphate
h: hour
HPLC: High Performance Liquid Chromatography
IAA: Indole-3-acetic acid
IR: infra-red
m: meter
M: molar
min: minute
ml: mililiter
mm: milimeter
nm: nanometer
PAA: phenyl-acetic acid
RL: red light
s: second
SE: standard error
W: Watt
WL: white light
WT: wild type
w/v: weight per volume
µg: microgram
µm: micrometer
µmol: micromole

CHAPTER I

Introduction

Since plants are sessile organisms, it is of great importance for them to perceive environmental stimuli and respond appropriately by changing certain growth parameters. Light as an environmental stimulus has a double function in the life of plants. First, the energy of photons is used in the process of photosynthesis for the generation of carbohydrates necessary for plant growth. Second, light represents information which is used to trigger such processes as seed germination, control of stem elongation, de-etiolation, phototropism, control of flowering, and others (Mohr, 1972).

All biological responses induced by light exhibit specific requirements regarding the wavelength of actinic light. For example, the quality of light most effective in inducing photosynthesis in green plants is in the blue-green and red region of the visible spectrum, while the light most effective in inducing phototropism is in the blue region of the visible spectrum (Curry,1969; Denffer and Ziegler, 1982). The specific wavelengths utilized in light-mediated processes are determined by the nature of the photoreceptor pigment(s). The photoreceptor pigment responsible for mediation of particular physiological response is excited only by the light of certain wavelength thereby determining the light requirement of the response.

The identity of the photoreceptor pigment mediating blue-light-induced processes, including phototropism, is unknown. However, it is believed that this molecule is most likely a flavoprotein (Song, 1984). Phytochrome is the photoreceptor pigment responsible for mediation of processes induced by red

and far-red light (Smith and Whitelam, 1990). This latter pigment exists in two photoconvertible forms. The first is Pr which is converted to Pfr after absorption of red light. Pr is thought to be the inactive form of phytochrome. The second form of phytochrome is Pfr, which is considered the active form and which is converted to Pr after absorption of far-red light (Smith and Whitelam, 1990).

Phototropism is the process by which primary plant organs and fungal sporangiophores orient with respect to light. This type of movement is called positive phototropism if it is directed toward the light source and negative phototropism if it is directed away from the light source. Phototropism is the most extensively studied blue-light-induced process in plants. By utilizing the sensory system for phototropism, plants can detect the direction from which the light is incident and orient their growth accordingly. The ecological importance of this response is obvious. Leaves, the organs where most photosynthesis takes place, are brought into a position which provides the highest exposure to the sun. This allows the leaves to capture the maximum number of photons and consequently, to increase the potential yield of photosynthesis.

However, it is important to stress that a change in growth as a final response of plants to environment is always the result of the integration of information perceived through a variety of receptors rather than the response to a single stimulus perceived through a single receptor.

EFFECT OF BLUE LIGHT ON PLANT GROWTH

Light of different wavelengths affects plant growth differently. Blue light elicits two types of responses depending on the fluence applied and the exposure time. The first type of response is the high-irradiance response which requires high fluence and prolonged exposure to the light (Mancinelli and Rabino,1978). The high-irradiance response is exemplified by the inhibition of hypocotyl elongation. The second type of response, the low-fluence response is characterized by the short-term irradiation and low fluence requirements. An example of the low fluence response to the blue light is phototropism.

BLUE LIGHT-INDUCED INHIBITION OF ELONGATION

Blue light and far-red light are the most effective in inhibiting hypocotyl elongation (Koornneef *et al.*, 1980). However, white light consists of all the wavelengths in the visible part of spectrum and, therefore, is more effective than blue or far-red light alone (Koornneef *et al.*, 1980). Blue-light-induced inhibition of hypocotyl elongation has been well described for seedlings of *Arabidopsis* (Liscum and Hangarter, 1991). Etiolated seedlings of *Arabidopsis* were approximately three and four times longer than seedlings irradiated with blue light and far-red light for five days, respectively (Liscum and Hangarter, 1991). Such an irradiation procedure

has been used in the identification of many photomorphogenic mutants such as *blu* and *hy* (Koornneef *et al.*, 1980; Liscum and Hangarter, 1991; Parks and Quail, 1993). Seedlings of *blu* mutants lacked the characteristic inhibition of hypocotyl growth by blue light (Liscum and Hangarter, 1991). The different *hy* mutants lacked the inhibition of hypocotyl growth by white, blue and far-red light (Koornneef *et al.*, 1980; Parks and Quail, 1993). Studies of the *blu* and phototropism mutants of *Arabidopsis* led to the suggestion that blue-light-induced inhibition of elongation and phototropism are genetically separable, and that they may be mediated by different transduction pathways (Liscum *et al.*, 1992).

Blue-light-induced inhibition of stem elongation has been described for several species including pea, mustard and cucumber (Cosgrove, 1981, 1985; Laskowski and Briggs, 1988). In pea epicotyls as well as in mustard seedlings, the kinetics of the inhibition of stem elongation are very rapid, beginning within five minutes after the start of irradiation (Cosgrove, 1981; Laskowski and Briggs, 1988). Etiolated cucumber seedlings also responded quickly to unilateral blue light with decrease in elongation rate. However, 4.5 hours of unilateral photostimulation were required to initiate phototropism in the cucumber seedlings. This report suggests that inhibition of hypocotyl elongation and phototropism may have separate transduction chain (Cosgrove, 1985).

PHOTOTROPISM

The photoreceptor pigment for phototropism

In spite of extensive study in a variety of systems, the blue light absorbing photoreceptor pigment which mediates phototropism has not been identified. Three groups of potential blue-light photoreceptor pigments have been proposed: carotenoids, flavoproteins, and very recently, pterins.

Action spectra. Action spectra for phototropism reported for *Avena*, alfalfa and *Phycomyces* are very similar (Curry, 1969; Galland, 1983; Baskin and Iino, 1987). Wavelengths between 400-500 nm are most effective in inducing phototropism (Curry, 1969). Within this region, the action spectra characteristically have three maxima. These peaks are at approximately 430, 450 and 475 nm. Two smaller peaks are found at 370 nm and 280 nm (Baskin and Iino, 1987).

Carotenoids. Absorption spectra of some carotenoids, especially β-carotene, are very similar to the action spectra reported for phototropism (Britton, 1988). This similarity was taken as a major argument for the hypothesis that carotenoid(s) are the photoreceptor pigments for phototropism. However, many reports presented data contrary to this idea. First, the absorption spectrum of β-carotene lacks the peak at 370 nm present in the action spectra for phototropism (Curry, 1969). A mutant of *Phycomyces* that has $1x10^{-5}$ of the wild-type amount of carotenoids retained wild-type sensitivity to blue light stimulation (Presti *et al.*, 1977). Corn

seedlings in which the amount of carotenoids was decreased by growing the seedlings on medium supplemented with carotenoid synthesis inhibitors were used to study phototropism. Although the magnitude of the phototropic response was reduced, these plants exhibited first and second positive phototropism similar to responses of untreated plants (Vierstra and Poff, 1981; Piening and Poff, 1988). Sensitivity to blue light is still unchanged in mutants plants of *A. thaliana* that have only 2.5-3% of the amount of carotenoids present in the wild-type (Appendix A). Taken together, these reports make carotenoids unlikely candidates for the photoreceptor pigment for phototropism.

Flavins. In the early 1950's, Galston suggested a role for riboflavin in phototropism of *Avena* (Galston, 1950). Flavins were proposed to be photoreceptor pigments for many other biological processes (Schmidt, 1984). However, phototropism research or any other work with organisms having reduced amounts of flavins is very difficult. Flavins play an important role as coenzymes in overall metabolism, and decreasing their level will inevitably lead to non-selective effects in living systems. One of the rare reports of this kind described a mutant of *Phycomyces* affected in the biosynthesis of riboflavin (Otto *et al.*, 1981). It was shown that roseoflavin can replace riboflavin as the blue light photoreceptor pigment for phototropism up to 80%, when the fluence thresholds for phototropism of seedlings grown on riboflavin or roseoflavin were compared. However, quantum efficiency for phototropism in the mutant was estimated to be restored to about 0.1% of the wild-type level (Otto *et al.*, 1981). Song (1987) compared flavins to

carotenoids as possible photoreceptor pigments for blue-light-mediated processes and offered many arguments against carotenoids. Based primarily on photochemistry and reactivity, flavins are favoured over carotenoids as the likely photoreceptor pigment for blue-light-mediated processes.

Pterins. Recently, pterins were detected in *Phycomyces* sporangiophores (Kiewisch and Fukshansky, 1991). The role of pterins in phototropism was suggested based on results which revealed that some photobehavioral mutants of *Phycomyces* have altered pterin patterns when compared to WT fungi (Hohl *et al.*, 1992).

Evidence for multiple pigments. Recent reports for *Phycomyces* (Galland and Lipson, 1987) and *A. thaliana* (Konjević *et al.*, 1989) suggest that multiple pigments control the phototropic response. In *Phycomyces*, analyses of kinetics for phototropism under different experimental conditions, and partial action spectra for the bending rate led Galland and Lipson to propose involvement of multiple pigments. Dark-adapted sporangiophores of *Phycomyces* upon unilateral irradiation with BL at a fluence rate higher than $1x10^{-6}$ $W.m^{-2}$ exhibited a 15 min latency period for the early component of the time-course for phototropism. When dark-adapted sporangiophores were photostimulated with the BL of fluence rate lower than $1x10^{-6}$ $W.m^{-2}$, the latency period for the early component of the time-course for phototropism was 40 min. Partial action spectra for the bending rate mediated by the low- and high-intensity photoreceptor systems had maxima at 350 nm and 400 nm, respectively. On the basis of these results, multiple photoreceptor pigments

mediating phototropism were suggested in *Phycomyces* (Galland and Lipson, 1987).

In *Arabidopsis*, the wavelength dependence of the shape of the fluence-response curve led Konjević and coworkers to suggest that at least two pigments are mediating phototropism (Konjević *et al.*, 1989). The fluence-response curve for phototropism in the range of light doses inducing first positive phototropism has two maxima if the fluence rate of actinic BL (λ=450 nm) is higher than 0.3 μmol.m^{-2}s^{-1} and one maximum when the fluence rate is lower. Pre-irradiation of seedlings with BL or GL made maxima in the fluence-response curve disappear selectively. The kinetics of recovery to subsequent unilateral BL pulse, after the pre-irradiation with the BL was slower than after the GL pre-irradiation (Konjević *et al.*, 1989). Furthermore, it was shown by the shift of one of the maxima in the fluence-response curve that JK224, a mutant strain of *A. thaliana*, has one of the pigments responsible for phototropism altered while the other appears unaffected (Konjević *et al.*, 1992).

Biochemical events possibly associated with photoreception. Over the last few years, Briggs and his group have published several papers describing a 120 kDa plasma-membrane protein which is phosphorylated upon exposure to blue light (Short and Briggs, 1990). This protein was found to be phosphorylated in several plant species including *Arabidopsis*. In the *Arabidopsis* strain JK224, a putative photoreceptor mutant, phosphorylation of this protein reaches levels only 5% of that found in the wild-type plants (Reymond *et al.*, 1992). Based on these

data, it appears that phosphorylation of the 120 kDa protein after the blue light irradiation is associated with a blue-light photoreceptor pigment that is responsible for phototropism in *A. thaliana* (Reymond *et al.*, 1992).

G-proteins are known to participate in signal transduction for many physiological processes in animals (Linder and Gillman, 1992). Recently, a G-protein that is activated by the blue light irradiation was isolated from the plasma-membranes of etiolated pea buds (Warpeha *et al.*, 1992). The blue light activation of this protein results in binding of GTPγS. Binding of GTP to G-proteins is considered the initial step in signal transduction mediated by the G-proteins. Binding of GTPγS to the G-protein isolated from the plasma membranes of pea buds is inhibited by the PAA and KI, compounds which interact with and quench the excited states of flavins that are suggested to be photoreceptor pigments for phototropism (Warpeha *et al.*, 1992). However, additional studies will be necessary to determine the relationship of G-proteins to signal transduction for blue-light-induced phototropism.

Fluence-response relationship for phototropism

The fluence-response relationship describes the dependence of the phototropic response on the fluence of unilaterally administered actinic light, and has been measured for many plant species (reviewed in Pohl and Russo, 1984; Steinitz and Poff, 1986; Iino, 1987,1988; Janoudi *et al.*, 1992).

If phototropism was induced by a single photochemical reaction mediated by a single photoreceptor pigment, the phototropic response would be expected to increase with the log of the number of quanta of applied light once the threshold dose of quanta is exceeded. As the available photoreceptor pigment becomes saturated, the response would be expected to reach a plateau and stay unchanged with the further increase of fluence (Poff *et al.*, 1992).

The observed fluence-response relationships for phototropism do not follow these predictions (Poff *et al.*, 1993). The phototropic response does increase to a maximum with increasing fluence of applied light. However, after reaching a maximum, the response starts decreasing with increasing fluence (Poff *et al.*,1993). This results in a bell-shaped portion of the fluence-response curve that is known as the first positive response. With a further increase of dose of light, the response reaches zero and in some species even becomes negative (Blaauw and Blaauw-Jansen, 1970). With even further increase of fluence of administered light, plants start responding again. This response at higher fluences is called second positive phototropism (Poff *et al.*,1993).

Only a few investigators have tried to explain the apparent complexity of the fluence-response curve for phototropism (Iino, 1987; Poff *et al.*,1993). It has been suggested that the major factor affecting the shape of fluence-response curve for phototropism for corn and *A. thaliana* is adaptation (Iino, 1987; Poff *et al.*,1993).

In a series of papers from Poff's laboratory, the shape of the fluence-response curve for phototropism in *Arabidopsis* has been examined in detail.

Desensitization is described to be the first phase in the process of adaptation. It was found that fluences of blue light inducing phototropism overlap with fluences inducing desensitization, the first phase in the process of adaptation. Thus, the blue light signal that induces phototropism simultaneously desensitizes the seedlings (Janoudi and Poff, 1993). As a result, seedlings that receive a fluence of unilateral blue light in excess of that needed to induce maximal first positive phototropism are also subject to desensitization which results in a reduced phototropic response. Therefore, Poff *et al.* (1993) claimed that the descending arm of first positive phototropism is due to the process of desensitization.

The indifferent region of the fluence-response curve represents a range of fluences of unilateral light to which seedlings do not exhibit a curvature response. According to Janoudi and Poff (1993), seedlings receiving these fluences have undergone complete desensitization and lost responsiveness to the unilateral stimulation. The period of time before the seedlings regain their responsiveness was called the refractory period and represents the second phase of adaptation (Janoudi and Poff, 1993).

In order for the second positive phototropism to be induced, two requirements must be met: 1) the amount of unilateral light administered to the seedlings must be in excess of the fluence threshold and; 2) this amount of light must be applied for a period of time in excess of a time threshold (Janoudi and Poff, 1990). The time threshold for second positive phototropism can be decreased by pre-irradiating seedlings with red light (Janoudi *et al.*, 1992). In *Arabidopsis* the

time threshold for second positive phototropism is decreased from 20 min to about 4 min (Janoudi *et al.*, 1992). This decrease in the time threshold for second positive phototropism also decreases the range over which the indifferent region of the fluence-response curve spans pointing to a direct relationship of the two parameters.

During a prolonged irradiation, seedlings have time to recover their sensitivity and respond with second positive phototropism. Recovery is the third phase of adaptation as described by Janoudi and Poff (1993). Although it appears that the second positive response is exclusively dependent on the time required for recovery, the time threshold for second positive phototropism is always longer than the time for recovery from desensitization. This suggests that, although the time threshold sets a time during which recovery occurs, the time threshold is controlled by some element other than recovery time.

The magnitude of second positive phototropism is greater than the magnitude of first positive phototropism. This difference may be a consequence of the fourth and final phase of adaptation, enhancement (Janoudi and Poff, 1991). In contrast to desensitization which is a rapid process requiring only a few seconds, enhancement is a slow process which can take up to two hours (Janoudi and Poff, 1991). Since the magnitude of enhancement is time dependent, so is the magnitude of curvature. Enhancement of curvature in *Arabidopsis* can be induced by pre-irradiation with either blue and red light (Janoudi and Poff, 1991).

Thus, the complexity of the fluence-response curve for phototropism in *A.*

thaliana can be explained by considering the involvement of adaptation which modulates the sensitivity and the responsiveness of the seedlings to blue light.

Growth distribution and kinetics during phototropism

Phototropism in seedlings of higher plants is the result of different elongation rates on the two sides of the shoot. Measurements of growth distribution during both first and second positive phototropism have been reported for monocotyledonous and dicotyledonous species (reviewed in Trewavas, 1992). The diversity of results obtained in the studies of growth distribution and a lack of knowledge about the mechanisms mediating the development of curvature led to conflicting hypotheses.

Blaauw Theory. In the beginning of this century, Blaauw suggested that light mediates phototropism by inhibiting the growth of cells. As a gradient of light is established across the seedlings during unilateral irradiation, different elongation rates on the two sides of the seedlings result (Went and Thimman, 1937). This theory, based on localized effect of light was soon disputed by Boysen-Jensen. Boysen-Jensen proposed that there is transmission of the stimulus down the coleoptile during phototropism, based on the movement of curvature from the top to the base of coleoptiles (Went and Thimman, 1937). A recent report about phototropism in oat, refuted Blaauw's theory based on the fact that changes in growth rates during phototropism took place within the segments of coleoptiles that

were not irradiated by actinic light (Macleod *et al.*, 1985).

Paal Theory. Paal suggested that unilateral light induces inhibition of growth on the lighted side more than on the shaded side of shoot. He ascribed this general inhibition of growth to a detrimental effect of light on either transport or synthesis of the growth factor (Pohl and Russo, 1984). Although both Blaauw and Paal hypothesized that a result of unilateral irradiation would be greater growth inhibition on the lighted side of the seedlings, their initial premise was different. Blaauw claimed that light affects the growth of individual cells while Paal suggested the inhibition resulted from an effect of light on the transmissible growth regulator.

Boysen-Jensen Theory. A third theory which describes the growth distribution during phototropism is the Boysen-Jensen theory. Boysen-Jensen proposed that, as a result of unilateral stimulation, the growth rate of lighted side of oat coleoptiles does not change while the growth rate on the shaded side increases (Pohl and Russo, 1984). This theory was tested only for second positive phototropism. Additional evidence in support of this theory has not been reported.

Cholodny-Went Theory. One of the most cited theories describing the distribution of growth during phototropism is the Cholodny-Went (C-W) theory. This theory was proposed independently at approximately the same time by N. Cholodny and F. Went (Went and Thimman, 1937). The major premise of this theory was that phototropism is regulated by auxins. As a result of unilateral irradiation, auxin transport is affected in such a way that there is a higher accumulation of

auxin on the shaded side of the shoot. The change in distribution of auxin across the stem results in different growth rates on the two sides. Thus, growth on the lighted side would be slower while, at the same time, the growth on the shaded side would be enhanced as a consequence of photostimulation (Went and Thimman, 1937).

Measurements of growth during phototropism of maize coleoptiles fit the predictions of the C-W theory (Iino and Briggs, 1984; Baskin *et al.*, 1985). In addition, the redistribution of IAA studied in phototropically-stimulated corn coleoptiles was consistent with the predictions of C-W theory (Iino, 1991). Thus, in corn coleoptiles stimulated by a unilateral blue light pulse sufficient to induce first positive phototropism, detectable redistribution of extractable IAA occurs such that after about 10 min there is more auxin on the shaded than on the lighted side. Approximately 20 min after the blue light pulse, curvature becomes evident which is consistent with the simultaneous increase of the growth rate on the shaded side and the decrease of growth rate on the lighted side of coleoptile (Iino, 1991). Moreover, when the orientation of microtubules in the cell walls on opposite sides of phototropically stimulated maize coleoptile was monitored, changes in accord with C-W theory were exhibited. Since it is thought that auxin regulates the orientation of microtubules in the cell walls, a pattern of microtubule milieu resembling that of a fast growing cell on the shaded side and the slow growing cell on the lighted side was expected and observed (Nick *et al.*, 1992).

A redistribution of growth during phototropism that is consistent with the

predictions of C-W theory was also reported for seedlings of mustard and pea (Rich *et al.*, 1985; Baskin, 1986). In both of these species, seedlings developed curvature due to a simultaneous increase of elongation rate on the shaded side and a decrease of the elongation rate on the lighted side (Rich *et al.*, 1985; Baskin, 1986). However, it was recently reported that in pea epicotyls exhibiting both first and second positive phototropism, the amount of IAA was equal on two opposite sides (Hasegawa and Yamada, 1992).

In contrast to reports confirming the validity of the C-W theory for maize coleoptiles, Togo and Hasegawa (1991) have reported that blue light stimulation does not induce unequal distribution of IAA for either first or second positive phototropism. Reports regarding phototropism in oat coleoptiles also argued against the C-W theory and in favour of the Blaauw/Paal (B/P) theory.

After the blue light photostimulation, the growth rate on the lighted side of the oat coleoptiles substantially decreased while the growth rate of shaded side remained unchanged (Macleod *et al.*, 1986). The response of oat coleoptiles to the blue light stimulation was shown to depend on where along the coleoptile the light stimulation was administered (Macleod *et al.*, 1984). Phototropism in oat to white light was reported to occur although the amount of IAA on the opposite sides of coleoptiles was equal. Based on this finding, unequal distribution of an acidic growth inhibitor different from ABA was suggested to mediate phototropic curvature of oat coleoptiles (Hasegawa and Sakoda, 1988).

A specific case regarding the control of phototropism which may be partially

explained by the B/P theory was reported in cress hypocotyls (Hart *et al.*, 1982). The development of curvature in dark adapted seedlings of cress occurred primarily as a consequence of stronger inhibition of growth on the lighted than on the shaded side of the hypocotyl. Ninety minutes after the onset of photostimulation, the growth rate on the shaded side became even greater than the growth rate of control seedlings irradiated from above. This increase of the growth rate on the shaded side exceeding the growth rate of control seedlings further contributed to development of curvature (Hart *et al.*, 1982).

A role of growth regulators other than auxin was suggested in phototropism of sunflower and radish seedlings (Bruinsma and Hasegawa, 1990). The levels of IAA, ABA and xanthoxin, a potent inhibitor of plant growth, were equal on opposite sides of sunflower seedlings exhibiting second positive phototropism. These findings led the authors to hypothesize that some other growth regulator mediates phototropism in sunflower (Feyerabend and Weiler, 1988). Second positive phototropism of radish seedlings is a result of differential growth inhibition on the lighted and shaded sides (Hasegawa and Togo, 1989). Chemically neutral growth inhibitors called raphanusanins were isolated from radish seedlings; the distribution of these inhibitors across the seedlings agrees with the recorded growth rates during phototropism (Hasegawa *et al.*, 1986; Hasegawa and Togo, 1989). When raphanusanins were applied differentially to seedlings, they induced changes in growth similar to those observed during phototropism (Hasegawa and Togo, 1989).

The kinetics of phototropism. In general, time-course curves for phototropism in higher plants show some similarity. Phototropism usually starts after a lag phase of at least 10 min (Cosgrove, 1985; Rich *et al.*, 1985; Baskin, 1986; Orbović and Poff, 1991; Hasegawa and Yamada, 1992). Once the curvature is initiated, it proceeds in a linear fashion. In several species, after reaching the maximum of curvature at some time after the photostimulation, the seedlings start straightening or bending away from the light source (Orbović and Poff, 1991; Hasegawa and Yamada, 1992). Differences between the kinetics of phototropism in different species are expressed as differences in duration of the lag phase and in the magnitude of the response.

An examination of the kinetics of phototropism is very important for obtaining a more complete understanding of the responses to blue light. The kinetics of phototropism was shown to be different from the kinetics for inhibition of elongation of cucumber hypocotyls, and this has been proposed as an argument against Blaauw's theory (Cosgrove, 1985). Furthermore, Cosgrove has compared the actual time-course for phototropism with the theoretical time-course derived on the basis of light gradients across cucumber seedlings. He concluded that phototropism in this species is controlled by factors other than the light gradient (Cosgrove, 1985). Thus, if the kinetics of two processes differ, a complex system of mediation might be postulated.

Different kinetics of phototropism under different regimes of irradiation led two groups to conclude that more than one pigment is involved in mediation of

phototropism (Galland and Lipson, 1987; Konjević *et al.*, 1989). Also, the shape of the time-course curve for phototropism of stationary seedlings and seedlings rotated on the clinostat points toward possible interaction between phototropism and gravitropism (Nick and Schafer, 1988). Interaction between phytochrome and the blue-light-regulated process was observed by comparing time-course curves for phototropism of etiolated and red light pre-irradiated seedlings (Orbović and Poff, 1991; Janoudi *et al.*, 1992).

EFFECT OF TEMPERATURE ON PLANT GROWTH

The effects of temperature on plants are more difficult to study than the effects of some other environmental factors. Heat energy is permanently present in all living systems and affects every molecule comprising these systems. That one can not provide conditions without heat energy and that temperature is sensed non-specifically make research in this field challenging.

To test for the involvement of heat energy in the control of growth in microorganisms, experiments were designed in which the stimulus was either a spatial temperature gradient or the rate of cooling or heating of the incubation media (Poff *et al.*, 1984). It was suggested that microorganisms may sense the changes in the level of heat energy in their environment by sensing structural changes in their membranes. Since membranes are composed mostly of lipids that

undergo phase transition at particular temperatures, the phase transition would be a recognizable signal in microorganisms (Poff *et al.*, 1984).

A similar approach to investigating temperature effects was undertaken for plants. Plants were grown at particular temperatures and the physiological process of interest was monitored following transfer of the plants to a different temperature conditions (Minorsky, 1989).

The most extensively studied effect of temperature on plant processes is the effect of temperature on photosynthesis. In most cases, it was shown that there is an optimum temperature at which the photosynthetic rate as estimated by consumption of CO_2 was highest, and other temperatures at which the rate was lower (Sams and Flore, 1982).

Extension growth of plants was also examined under different temperature conditions. Extension growth was shown to increase at temperatures between 10°C to 30 °C in mungbean and between 10 °C and 36 °C in both morning glory and bean (Raison and Chapman, 1976; Bagnal and Wolfe, 1978). Due to the limited range of temperatures employed in these two reports it is difficult to estimate whether the growth rate of tested plants would be higher or lower with further increases in ambient temperature.

For the roots of maize that were grown at two different temperatures (16 °C and 26 °C), followed by transfer to new conditions, similar optimal temperatures for elongation were recorded at about 28 °C and 30 °C, respectively (Fortin and Poff, 1991). In the same paper, Fortin and Poff described thermotropism of maize roots.

They showed that growing maize roots in a field of thermal gradients of variable strength bend toward the warmer side. The magnitude of thermotropism was shown to be dependent on the strength of the thermal gradient, the temperature at which the seedlings were grown previously, and the orientation of the roots with respect to the gravity vector (Fortin and Poff, 1991).

In trying to explain how plants sense difference in temperature, researchers borrowed ideas from researchers studying microorganisms. Raison and Chapman used an Arrhenius plot to describe their data regarding the growth rate of mungbeans at different temperatures. Points at which the slope of the plot changed were proposed as the temperatures at which phase transition occurred in the membranes of the cells within the growing stem (Raison and Chapman, 1976). However, Bagnal and Wolfe argued strongly against the simplified way in which Raison and Chapman explained their results. Bagnal and Wolfe suggested that the extension growth of plants is complex process that encompasses several simultaneous processes all of which have their distinct rate constants. Since an Arrhenius plot is used to describe single process, these authors suggested that the Arrhenius plot cannot be used to define plant growth (Bagnal and Wolfe, 1978).

The field of thermal responses and thermosensing in plants is still undeveloped. Isolation and characterization of mutant plants with alterations in their responses to temperature stimulation should provide more knowledge about sensory physiology and growth responses in plants.

NUTATION

Primary organs of plants rarely elongate in a strictly directional manner for a long period of time. As they grow, these organs nutate around their plumblines. The nature of nutations is either oscillatory or irregular, and they are spatially limited to one or many planes. The term circumnutation is used to denote an oscillatory, elliptical type of nutation. The first comprehensive study of nutation was done by Charles Darwin in 1880. He examined over 100 species for the occurrence of nutation and, on the basis of his observations, suggested that nutations were universal to all plants and internally induced (Darwin, 1896).

In more recent years, nutation has been extensively studied in sunflower and to a lesser extent, in peas, beans and cress (reviewed in Brown, 1992). As a result, two modern theories that describe nutational movements have been proposed: the theory of gravitropic overshoot and the theory of internal oscillations.

The theory of gravitropic overshoots states that nutating seedlings are exhibiting constant responses to the opposing gravity stimulation. This theory is based on following assumptions. First, nutation is induced by the gravitropic stimulation and so in its absence, nutation should cease. Second, the rate of nutational movements should be similar to the rate of gravitropism. Third, gravitropic stimulation should have a phase shifting and entrainment effect on nutation; when gravity stimulation is imposed on seedlings that are nutating they should change the period of nutation in such way that as a result all seedlings

nutate in phase. Fourth, the relationship between the period of nutation and the response time of gravitropism should be constant. All these predictions were confirmed for seedlings of sunflower (Israelson and Johnsson, 1967; Johnsson and Israelson, 1969). Furthermore, results obtained for sunflower seedlings also fit well to a mathematical model put forward as numerical definition of the gravitropic overshoot theory (Israelson and Johnsson, 1969).

One of the major challenges to the validity of the gravitropic overshoot theory arose with experiments using seedlings which were kept for a limited time under conditions of micro gravity. In these conditions, hypocotyls of sunflower seedlings and roots of cress continued to exhibit circumnutation (Volkmann *et al.*, 1986; Brown *et al.*, 1990). These results contradict the first prediction of the theory that gravity is necessary for induction of nutation. Another disagreement with this theory came from experiments which showed that gravitropism of bean seedlings was much slower than the nutation rate, and that gravitropic stimulation did not have a phase shifting effect on nutation (Heathcote and Aston, 1970). A phase shift was achieved in pea seedlings (Britz and Galston, 1982). However, counter to the predictions listed above, both seedlings of beans and peas continued to nutate while exhibiting gravitropism (Heathcote and Aston, 1970; Britz and Galston, 1982).

The second theory that describes nutation is the theory of internal oscillations. This theory states that nutations are movements induced by internal oscillations within plants. Major predictions of this theory are that: 1) initiation of nutation is independent of external stimulation and; 2) different periods of nutation

may occur within a single plant and within a population (reviewed in Johnsson, 1979).

One model supporting the theory of internal oscillations proposes that the oscillators from which nutations originate could be individual cells. If a cell within the growing portion of the stem underwent some volumetric change such as those associated with growth, that change could spread to neighbouring cells. Also, if the direction of this change is unidirectional, for example clockwise around the transversal section of the stem, nutation would result (Heathcote and Aston, 1970).

Another model supporting the internal oscillation theory is based on local control of growth. In this model, the main role was ascribed to symplastically transported IAA. The presence of IAA in a patch of tissue somewhere on the growing portion of a stem would, at one moment, represent an inducing rather than a maintaining factor of growth. As a result of locally induced growth, breakage and regeneration of plasmodesmata responsible for transport of IAA would subsequently control the growth without any input from the outside environment (Brown, 1992).

The interaction of multidirectional movements of nutation with the unidirectional movements of phototropism and gravitropism provides a view into the complexity of the control of growth of seedlings. Although nutations were not apparent during gravitropism of sunflower seedlings (Johnsson, 1965), bean and peas continued to nutate during gravitropic response indicating that these processes were independently controlled. Britz and Galston (1983), concluded that

nutation, phototropism and gravitropism were distinctly different, based on their magnitudes in pea seedlings which had hooks and buds removed. Removal of the hooks and buds according to Britz and Galston resulted in depletion of auxin which further resulted in differential effect on magnitude of each of the studied movements. When auxin was added to cut surfaces of peas seedlings, they responded by exhibiting differentially altered nutation, phototropism, and gravitropism (Britz and Galston, 1983).

Although the ecological advantage of nutation, if any, is unknown, phenomena which occur across a wide range of biological systems have frequently been found to serve some function. Finding an answer to the questions why and how nutations happen will help better understanding of overall control of growth in plants.

OBJECTIVES

The objective of this work was to contribute to the body of knowledge about the elongation growth of seedlings of *Arabidopsis thaliana* and role of growth in different type of movements exhibited by these seedlings. Phototropism, a blue-light-induced directional movement, was most extensively studied. Kinetics and growth distribution during phototropism were measured in seedlings under various conditions. The interactions of phototropism with gravitropism, phototropism with

nutation, and gravitropism with nutation were also examined.

Arabidopsis seedlings have proven to be a good experimental object for this research project. The sensitivity of these seedlings to different types of stimulation enabled them to exhibit tropistic responses that were easy to observe and score. With appropriate magnification of images of seedlings, elongation rates of seedlings were recorded without difficulties.

In recent years, a large number of mutants have been isolated and characterized in *Arabidopsis*. The availability of mutagenesis methods and the percentage of mutants obtained with desired mutations were taken into consideration when *Arabidopsis* was chosen as the object of this study. Separation and characterization of the signal transduction pathways of different types of movements exhibited by *Arabidopsis* seedlings, that would follow physiological description of these movements would be facilitated by the existence of specific mutants. Ideally, seedlings from these mutant strains would have an alteration in only one type of movement or growth control mechanism, while the other functions would be unchanged. This would allow for the study of the particular process involved in growth control.

The feasibility of description and the prospect of further characterization of sensory physiology processes at the molecular level by using genetic tools, emphasized *Arabidopsis* seedlings as suitable experimental object for this research.

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CHAPTER II

Kinetics for phototropic curvature by etiolated seedlings of *Arabidopsis thaliana*

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ABSTRACT

An infra-red (IR) imaging system has been used to study the influence of gravity on the kinetics of first positive phototropism. The development of phototropic curvature of etiolated seedlings of *Arabidopsis thaliana* was measured in the absence of visible radiation. Following a pulse of blue light, stationary seedlings curved to a maximum of approximately 16° about 80 min after stimulation. The seedlings then curved upward again or straightened by about 6° over the subsequent 100 min. Seedlings rotated on a clinostat reached a similar maximum curvature following photostimulation. These seedlings maintained that curvature for 30-40 min before subsequently straightening to the same extent as the stationary seedlings. It is concluded that straightening is not a consequence of gravitropism, although gravity has some effect on the phototropism kinetics.

INTRODUCTION

Since the seminal experiments of Darwin (1896), it has been recognized that phototropic curvature of a plant shoot toward a unilateral light source results in the shoot receiving an opposing gravitational stimulus. Thus, it would be expected that the shoot would grow upward again or straighten as a result of gravitropism during or following the phototropic curvature.

Because gravitropic curvature may confound phototropic curvature, the kinetics of phototropism have been of interest to a number of investigators over the last 100 years. The kinetics of phototropism have been measured in a variety of experimental systems from the fungus, *Phycomyces* (Galland, 1983), to monocotyledonous plants such as maize (Nick and Schafer, 1988) and *Avena* (Shen-Miller and Gordon, 1967; Steinitz *et al.*, 1988) and dicotyledonous plants such as cress (Hart and Macdonald, 1981). In many of these experiments, seedlings were placed onto a clinostat following photostimulation in order to minimize the effect of gravity on the development of the phototropic response. The generally accepted conclusion of these studies is that the seedling will curve upward as a consequence of negative gravitropism following curvature toward a unilateral light source (Nick and Schafer, 1988; Steinitz *et al.*, 1988).

Our original intentions were two-fold. First we sought to study the kinetics of the phototropic curvature induced by a pulse of blue light (BL) in etiolated seedlings of the dicotyledonous species, *Arabidopsis thaliana*. Second, these studies were expected to show that the reversal of curvature or straightening of the

seedling following phototropic curvature was a consequence of gravitropism. We have characterized the kinetics of phototropic curvature of seedlings on and off a clinostat. Our results indicate that gravitropism has little effect on this straightening.

MATERIALS AND METHODS

Growth conditions

The Estland race of *Arabidopsis thaliana* was used in this study. Seedlings were grown as previously described (Steinitz and Poff, 1986) with some modifications. Strips of micro-assay wells containing 0.7% (w/v) agar supplemented with 1mM KNO_3 were sown with 2 seeds per well. The strips were then placed in transparent plastic boxes sealed with Parafilm and kept for 3 days at 5 $\pm$ 1 C° in darkness. The boxes with strips were then moved into continuous white light for 19 hours at 25 $\pm$ 1 C° to potentiate germination. The white light treatment was followed by a period of 44-48 hours of growth in darkness at 25 $\pm$ 0.5 C° and >90% relative humidity until the experiments were started. Immediately prior to video recording, the strips with plants were removed from the plastic box, and placed on a stationary stand or mounted on a clinostat in complete darkness. All manipulations of the seedlings were performed in complete darkness.

Light sources

White light (65 $\mu molm^{-2}s^{-1}$) used to potentiate germination was provided by

General Electric (Cleveland, OH, USA) DeLux Cool-white fluorescent tubes. The actinic light source for phototropism consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA, USA) 300W ELH tungsten halogen lamp, and a 450 nm interference filter with a half band width of 10 nm (PTR Optics, Waltham, MA, USA). The duration of actinic irradiation was controlled with a Uniblitz shutter (Vincent Associates, Rochester NY, USA). In all experiments, the phototropic response was induced with a BL pulse of 0.9 s at a fluence rate of 0.34 $\mu molm^{-2}s^{-1}$ for a fluence of 0.3 $\mu molm^{-2}$. This fluence is within the range of fluences inducing the peak response in first positive phototropism of etiolated seedlings of *A. thaliana* (Konjević *et al.*, 1989; Steinitz and Poff, 1986). The IR light source consisted of a Leitz Prado-Universal (Ernst Leitz Gmbh, Wetzlar, Germany) projector with a 250-W tungsten halogen lamp, and a Kodak Wratten 87c gelatin filter (Eastman Kodak, Rochester, N.Y., USA) transmitting light >800nm (transmission <1.5% at wavelengths lower than 800 nm, measured using a Perkin-Elmer Lambda 7 spectrophotometer). Red light (at 0.6 $\mu molm^{-2}s^{-1}$) used for preirradiation was obtained from 2 gold fluorescent tubes (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp., Highland, IL, USA). This source provides radiation from 560 to 720 nm with maximum output at 620 nm. Fluence rates were measured with a Li-Cor (Lincoln, NE, USA) Li-190 SA quantum sensor in combination with a Li 1000 Data Logger.

Infra-red imaging system

An IR-imaging system (Fig. 2.1.) was used to monitor the seedlings under radiation to which the seedlings are physiologically blind (*i.e.*, in "physiological darkness"). This system consisted of two spatially separated stations, one for recording and one for monitoring. The recording station, which was situated in a dark room, consisted of an IR-sensitive Cohu Solid State Camera 4815-2000 (Cohu, San Diego, CA, USA) and IR light source. The camera was equipped with an extension tube and an 85mm, f2 lens (Minolta Co., Osaka, Japan) to magnify the seedling images. Equipment in the monitoring station included a Panasonic AG 6300 video cassette recorder-VCR (Matsushita Corp.,N.J., USA) and Electrohome V-6 type (Electrohome Ltd, Ontario, Canada) monitor. Images of the seedlings were recorded throughout an experiment while being monitored in "real time". The camera was focused onto a particular plane. The strip containing the seedlings was subsequently positioned in darkness on a stand such that the seedlings were in focus for the camera.

Following an experiment, the angles of curvature were measured while playing back the recording. The VCR was connected to an IBM AT computer with a Java video analysis program (Jandel Scientific, Corte Madera, CA, USA). Recorded images of seedlings were displayed on a high resolution Electrohome ECM 1312U (Electrohome Ltd., Ontario, Canada) monitor.

Angles of curvature were measured and recorded using the Java software. In this procedure, one straight line was made along the lower part of the image of

the seedling frozen on the screen. A second line was then made as a tangents to the curved portion of the hypocotyl below the hook. The angle of curvature was the angle, θ , between these intersecting lines (Fig. 2.2.) The initial curvature of each seedling was measured from recordings made approximately 5 min before the actinic BL irradiation. Subsequent curvatures for stationary seedlings (*i.e.*, not placed on the clinostat) were then measured at 10-min intervals for 180 min following photostimulation. Seedlings mounted on the clinostat were oriented with their longitudinal axes perpendicular to the axis of rotation (0.48 rpm). For these, curvatures were measured at approximately 15-min intervals following photostimulation. (Images of the seedlings were recorded when they entered the field of view of the camera, so the intervals were 15 $\pm$ 3 min). If the seedling was curved at the beginning of the experiment, that curvature was subtracted from the curvatures measured subsequently. Thus, all curvatures are degrees of curvature during the course of the experiment.

The repeatability of the angle measurement was assessed by measuring the same curved seedling 15 times. The repeatability error ranged from $\pm$ 2 for a 5° angle to $\pm$ 5 for a 55° angle. This source of error is largely a consequence of variability in positioning the straight line tangents.

Line tracings of entire representative seedlings were also recorded from the video images using the Java software. By tracing a seedling image at various times, image sequences showing curvature as a function of time were generated.

RESULTS

The time courses for phototropic curvature to a BL pulse by three individual seedlings demonstrate the considerable variability observed in the responses of different seedlings (Fig. 2.3.) An average time course for phototropic curvature can be constructed from the average curvature of a number of seedlings at each time. Such an average time course (Fig. 2.4.) shows a lag time of about 10-20 min following the BL pulse during which the seedling shows no measurable curvature. Following this lag period, curvature toward the light source increases with time to a maximum of about 16° at 80 min after photostimulation. Curvature then decreases in a straightening phase, resulting in a loss of about 6° of curvature by 180 min after the BL pulse. Control seedlings, treated in the same manner as the experimental seedlings but lacking the BL pulse, showed no significant curvature (Fig. 2.4.) Some seedlings exhibited no curvature to the unilateral BL (data not shown).

A sequence of line images of a single seedling (Fig. 2.5.) demonstrates the curvature and straightening which are shown graphically in figure 2.4. for the average of many seedlings. The seedling for figure 2.5. was selected as representative of the kinetics of curvature. However, this seedling's curvature was greater in amplitude than the mean curvature of the population. The image sequence shows that seedling growth continues throughout the experiment. Curvature appears to begin just below the hook region, and progresses down the hypocotyl. In addition, straightening is not limited to any single portion of the

previously curved hypocotyl.

The average time course for phototropic curvature of seedlings rotated on a clinostat (Fig. 2.6.) is very similar to the average time course for stationary seedlings (Fig. 2.4.) The lag phase of about 15 min, the average maximum curvature of about 17°, and the average final curvature of 10° for the seedlings on the clinostat are similar to the corresponding values for the stationary seedlings. However, a slight difference was observed between stationary seedlings and seedlings on a clinostat. The maximum curvature was maintained for a longer time by the seedlings on the clinostat before straightening. Control seedlings, which were rotated on the clinostat but which had not been photostimulated, showed no significant curvature (Fig. 2.6.)

The effect of red light on the curvature and straightening was examined by measuring the time course for phototropic curvature of seedlings which had been preirradiated from above with 60 min of red light (RL) immediately before the BL pulse. The results (Fig. 2.7.) show a curve shape which does not appear to be different from that for seedlings not RL-preirradiated (Fig. 2.4.) The lag phase, time to maximum curvature, and straightening are all similar. However the amplitude of the curvature is greater for the RL-preirradiated seedlings than for the non-preirradiated controls.

DISCUSSION

It has previously been noted that kinetic measurements are important for an understanding of sensory responses (Iino *et al.*, 1984; Galland and Lipson, 1987). However, in order to obtain such data for the phototropism of higher plants, it is necessary to measure curvature in physiological darkness. For this reason, an infrared system has been used, such that the response of the seedlings lacks the confounding effect of visible light. This is of particular importance since no wavelength in the visible region of the spectrum can be considered "safe", blue and green inducing phototropism (Steinitz *et al.*, 1985) and red light causing the enhancement of phototropism (Janoudi and Poff, 1991). For these reasons, we agree with Iino and Carr (1981) that sensory responses of plants to light should be observed under IR radiation.

The time courses for phototropic curvature by different seedlings showed significant differences in lag time, the amplitude of the response and the time required for maximum response. These variations would result in considerable noise for the curvature of a number of seedlings measured at a particular time. At least part of that variation results from variable position of the seedling with respect to the light source. It has previously been reported that phototropic curvature is low if the side of the hook with the cotyledons attached is positioned toward the source of the light (Khurana *et al.*, 1989). It is possible that much of the variability in the kinetics is a consequence of this dependence of curvature on hook

position. However, considerably more data would be necessary to document any effect of the hook position on the extent of the lag phase or on the amplitude of the final response. We believe that the oscillations in curvature of an individual seedling are a consequence of nutation of that seedling. This is based on observations of seedlings from above (data not shown).

If the variability is minimized by averaging the response at each time for a number of seedlings, several general conclusions become possible. First, the maximum curvature induced by a pulse of BL is transient for both stationary seedlings and seedlings rotated on a clinostat (Figs. 2.3., 2.4. and 2.6.) Second, the time course for phototropism of stationary seedlings is quite similar to that of seedlings rotated on a clinostat. In particular, the lag phase and the final curvature are the same (Figs. 2.4. and 2.6.) Third, stationary seedlings exhibit straightening immediately after reaching their maximum curvature, while seedlings on the clinostat maintained their maximum curvature for about 30 min before exhibiting straightening.

In several previous papers (Nick and Schafer, 1988; Steinitz *et al.*, 1988), plants were reported to develop a stable curvature which was maintained for six or more hours. In contrast, we see a phase of straightening beginning 2 h after photostimulation. These differences observed between the response of *A. thaliana* and the response of *Zea* (Nick and Schafer, 1988) or *Avena* (Steinitz *et al.*, 1988) could be due to their different taxonomic status. However, this difference also could be a consequence of differences in the RL irradiation. Red light is known to affect

both phototropism (Janoudi and Poff, 1991) and gravitropism (Willkins, 1966). It is clear from this study that RL preirradiation increases the amplitude of the response, but does not significantly affect the straightening (Fig. 2.7.) However, the red light irradiation protocol used here differed substantially from that of Nick and Schafer (1988). Thus, there are insufficient data to assess the possibility that the straightening could be eliminated by a continuous red light irradiation. However, it is interesting to note that the time of maximum curvature for RL preirradiated seedlings seems later than that for the non-preirradiated seedlings. The differences in reported data could also be a consequence of different experimental procedures such as the positioning of seedlings on the clinostat. Shen-Miller and Gordon (1967) reported complete straightening of oat coleoptiles on a clinostat following unilateral BL irradiation. Their clinostat protocol was similar to that which we used, with vertical mounting of the seedlings on a clinostat with horizontal axis of rotation.

It should be noted that the amplitude and kinetics for gravitropism vary from species to species and depend on the other environmental conditions of the plant material (Willkins, 1966; Firn *et al.*, 1978). Since an earth-based experiment must inexorably confront the ubiquitous 1 g force of gravity, any apparent phototropic curvature in seedlings not maintained on a clinostat must be considered to be an equilibrium between phototropism and gravitropism. Thus, it would not be surprising if the amplitude or kinetics of apparent phototropic curvature varied from species to species.

What is the cause of the straightening? Our results demonstrate that gravity

is not the primary cause for the straightening. However, there is a small effect of gravity on the straightening. Since the final curvature developed by stationary seedlings is the same as that developed by seedlings on the clinostat, we can conclude that there is no significant gravitropism during the 180 min duration of the experiment. Moreover, since the maximum curvature developed by stationary seedlings is the same as that developed by seedlings on the clinostat, we can conclude that there is no significant influence of gravity on the development of phototropic curvature for 80-100 min. Thus, there appears to be no gravity-dependent interference with the translation of the full amount of photoproduct into curvature. However, gravity does have a relatively subtle effect, causing straightening about 40 min earlier than the straightening observed in seedlings on the clinostat. The growth rate of the seedling may be of importance in evaluating these data. Preliminary measurements of seedling growth rates show that seedlings placed on the clinostat grow slightly faster than comparable stationary seedlings. Such an increase in growth rate has been noted before (Nick and Schafer, 1988). If a more detailed analysis of growth rates substantiates this measurement, then the subtle effect of gravity on straightening may not be a gravity effect on curvature, but instead may be a consequence of the altered growth rate.

One can also conclude that straightening is not a consequence of a decay in any component in the signal process leading to curvature. Such a decay would be expected to result in a cessation of the development of curvature, resulting in the maintenance of a stable curvature. In contrast, we observe a decrease in the

angle of curvature or curvature in the opposite direction, which we refer to as straightening.

One possible hypothesis to explain the straightening is based on a limited pool of material required for or affecting growth on each side of the seedling. Depletion of this pool would be expected first on the side experiencing the greatest rate of curvature. Given a relatively slow replenishment of the pool, depletion of the pool on one side would result in a lower growth rate on that side, and thus curvature away from the original stimulus or straightening. The spatial distribution of straightening is consistent with this hypothesis. Straightening occurs throughout the curved region unlike the phototropic curvature itself which is initiated just below the hook region and progresses down the hypocotyl (Fig. 2.5.) The spatial distribution of gravitropism is similar to that of phototropism (data not shown), being initiated below the hook region and progressing down the hypocotyl. The fact that straightening occurs throughout the curved portion of the hypocotyl supports the conclusion that straightening is not a consequence of gravitropism.

Straightening has also been described in the tropistic curvature of maize roots (Ishikawa *et al.*, 1991). However, interpreting the results of that study is complicated since the stimulus employed was gravity and the presentation was continuous. An attractive hypothesis was suggested for straightening by Ishikawa *et al.* (1991). They base this hypothesis on differences in kinetics on two sides of the root for a Cholodny-Went hormone redistribution and changes in the sensitivity of the cells to the auxin. At present, there are inadequate data to indicate which

hypothesis is correct, if either.

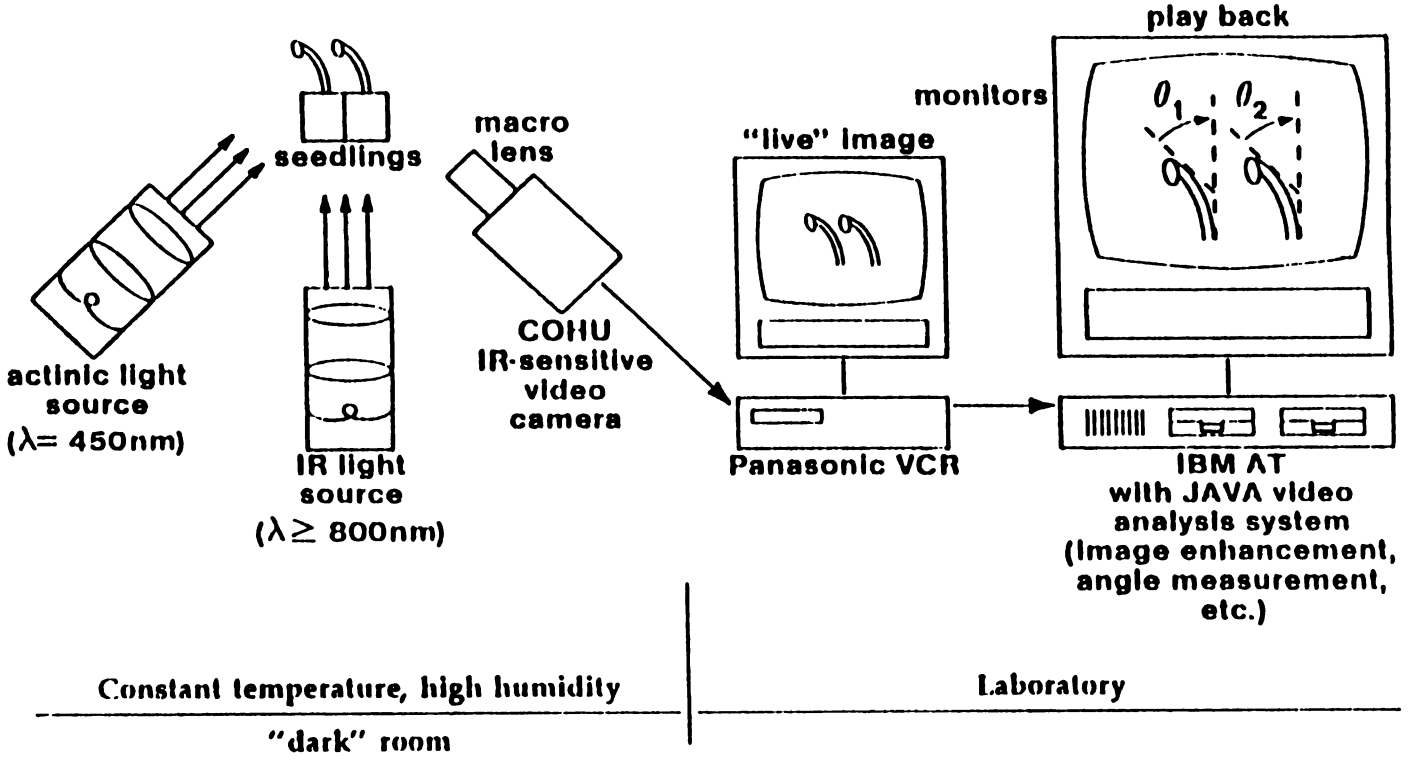

Figure 2.1. Block diagram of an IR imaging system. Seedlings are imaged using an IR-sensitive camera with IR radiation at wavelength longer then 800nm. The camera is aligned orthogonal to the long axis of the seedlings and orthogonal to the actinic light (λ= 450nm). This portion of the system is located in a dark room with controlled temperature and humidity. The camera is connected to a computer station in a separate room.

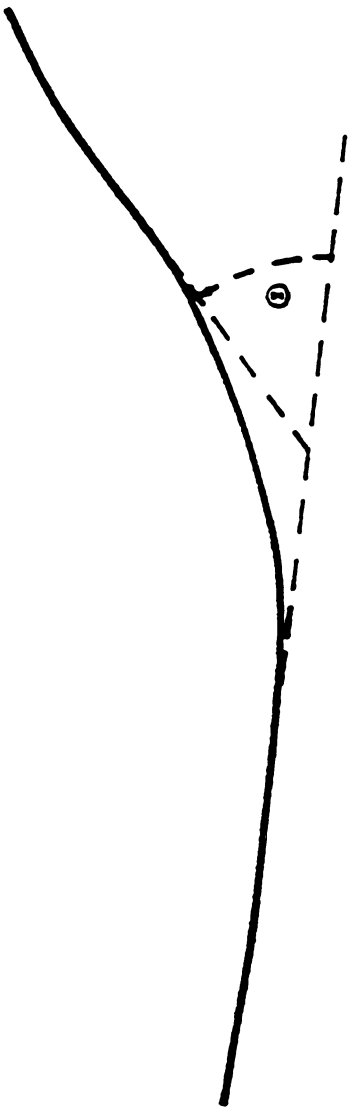

Figure 2.2. Diagram illustrating the measurement of curvature by hypocotyls of *Arabidopsis thaliana*. The bold line represents the seedling hypocotyl; the dashed lines represent splines tangent to the curved and uncurved hypocotyl portions. The angle, θ, between the splines is measured as the angle of curvature.

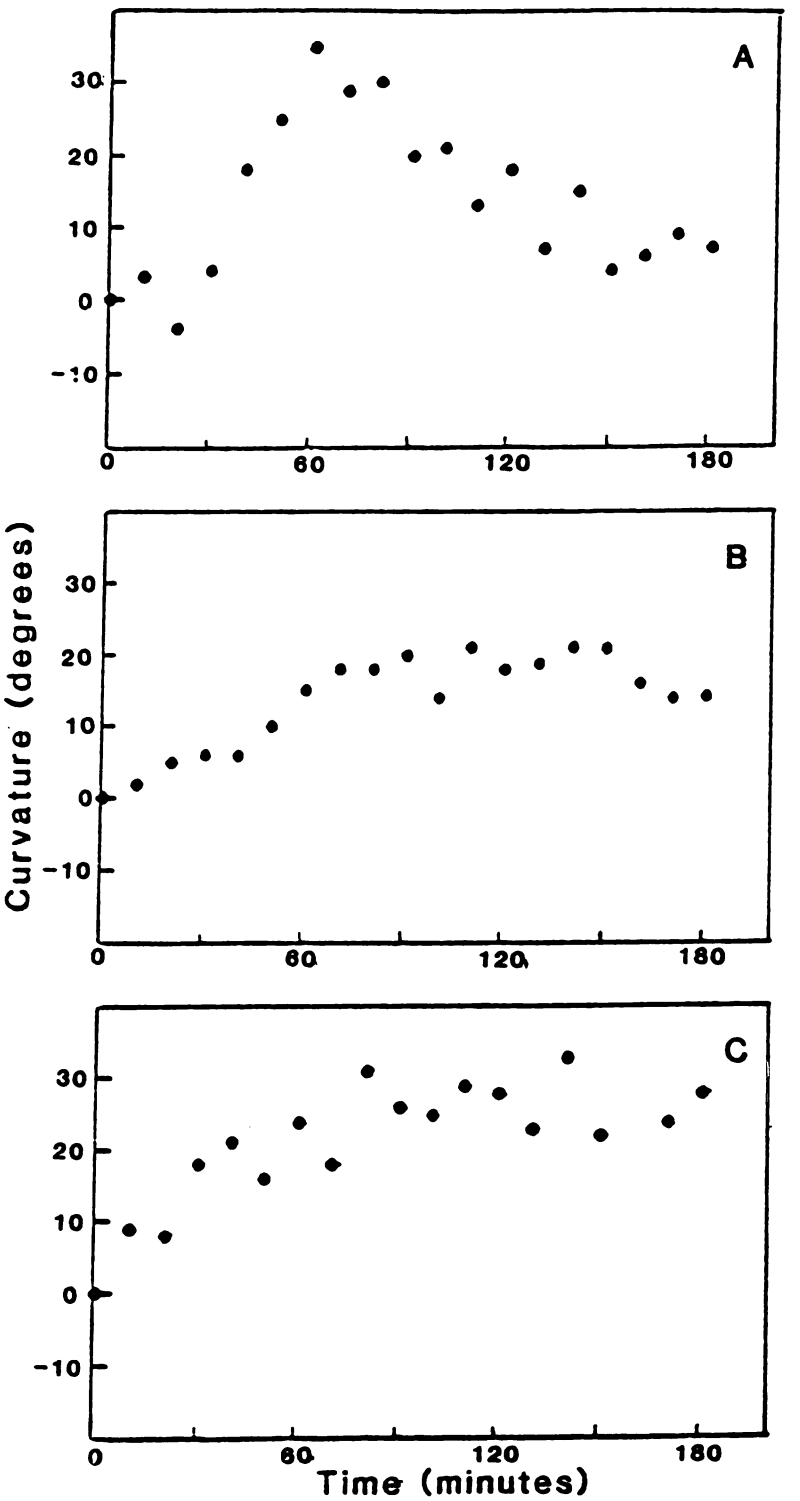

Figure 2.3. Time course for development of curvature by three individual seedlings (A,B,C).

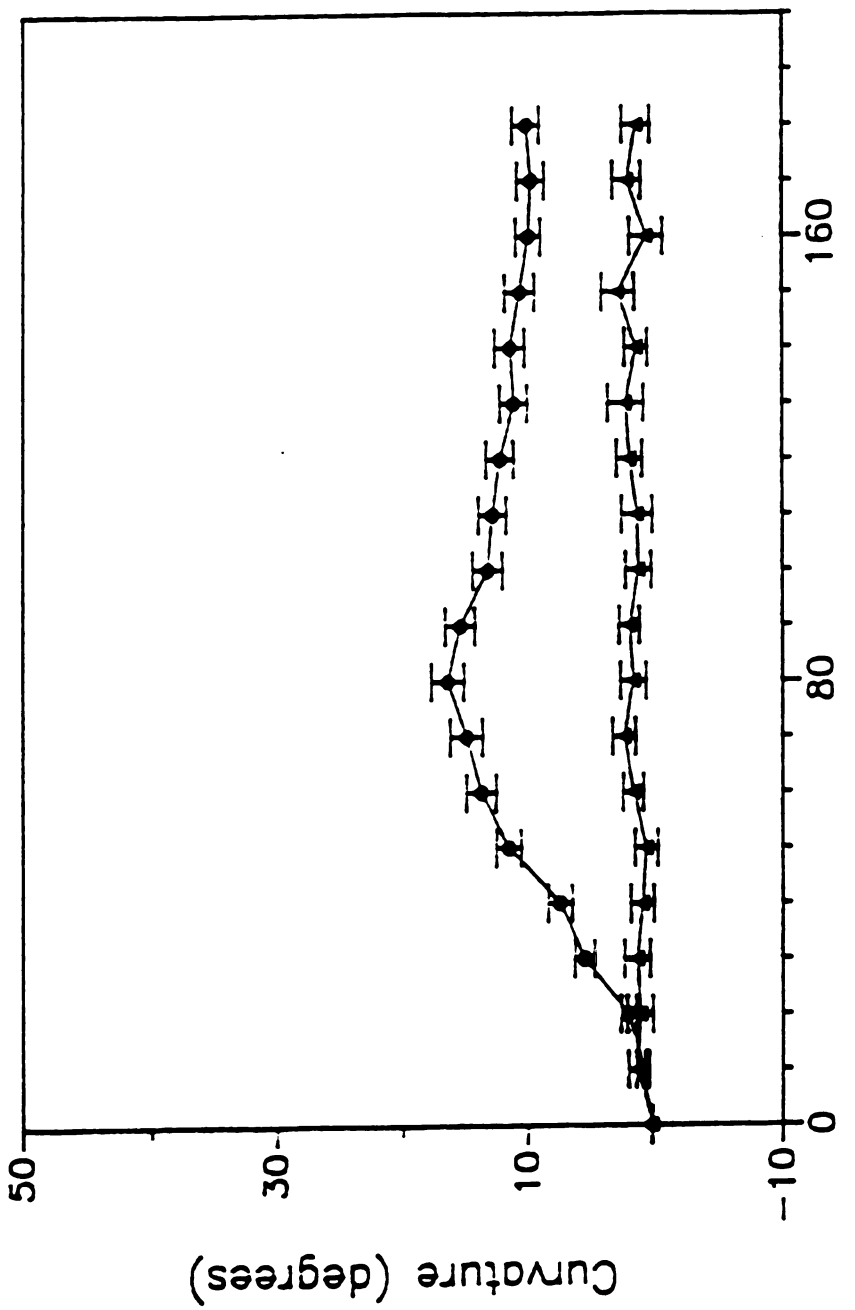

Figure 2.4. Time course for development of average curvature to a BL pulse by stationary seedlings: ●, stimulated, n=55; ▲,non-stimulated, n=18; vertical bars represent ± 1 SE.

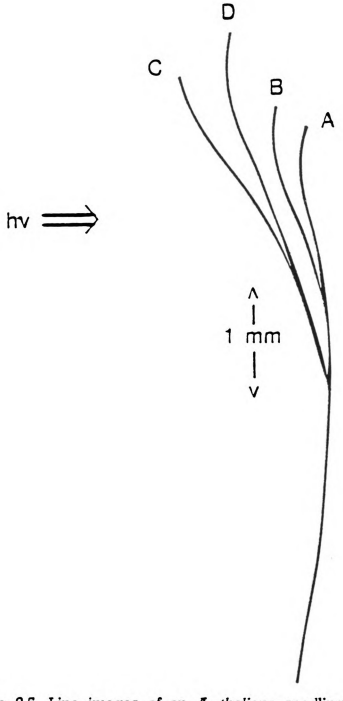

Figure 2.5. Line images of an *A. thaliana* seedling during phototropic curvature and straightening. Each line represents the seedling at the indicated times following the blue light stimulus: A, 20 min; B, 40 min; C, 80 min; D, 130 min.

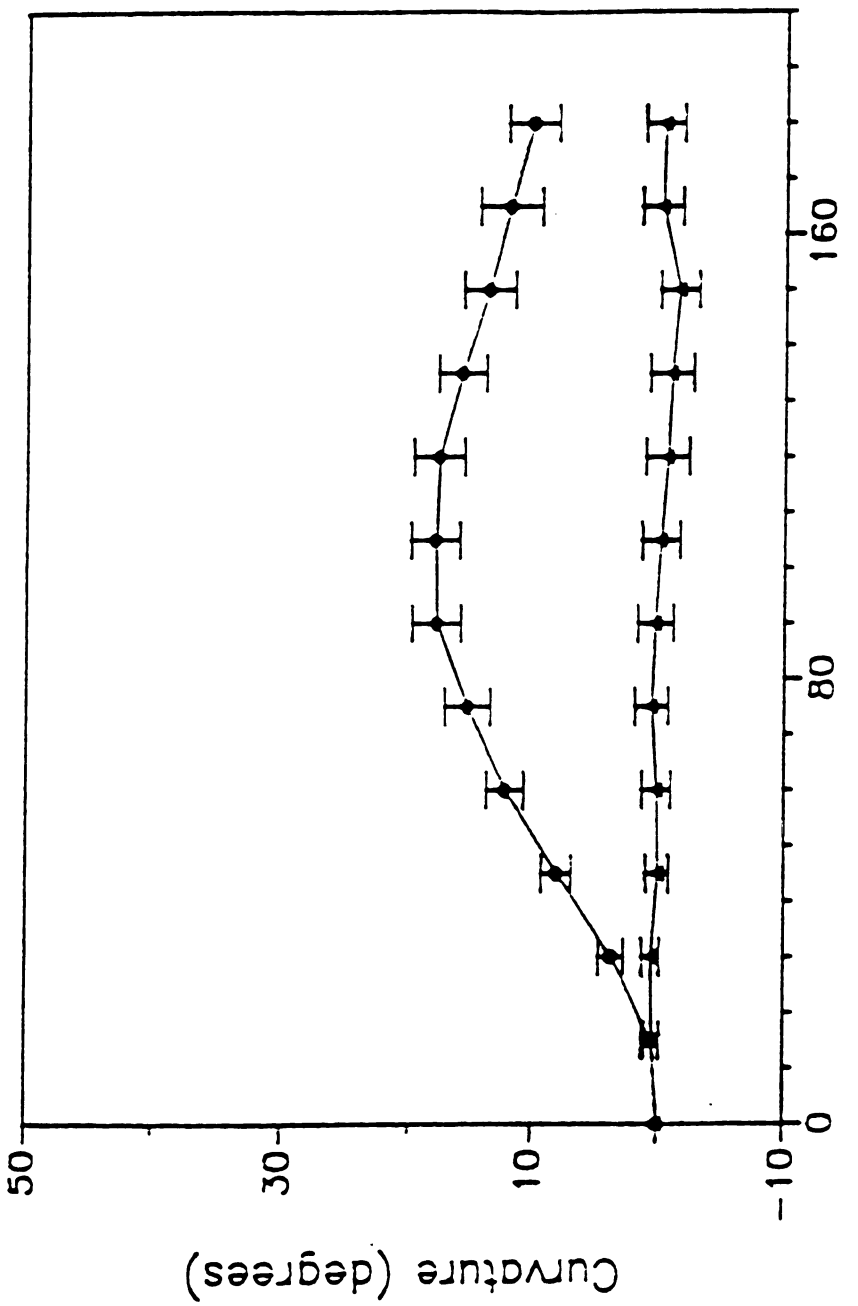

Figure 2.6. Time course for development of average curvature to a BL pulse by seedlings rotated on clinostat: ●, stimulated, n=32; ▲, non-stimulated, n=28; vertical bars represent ± 1 SE.

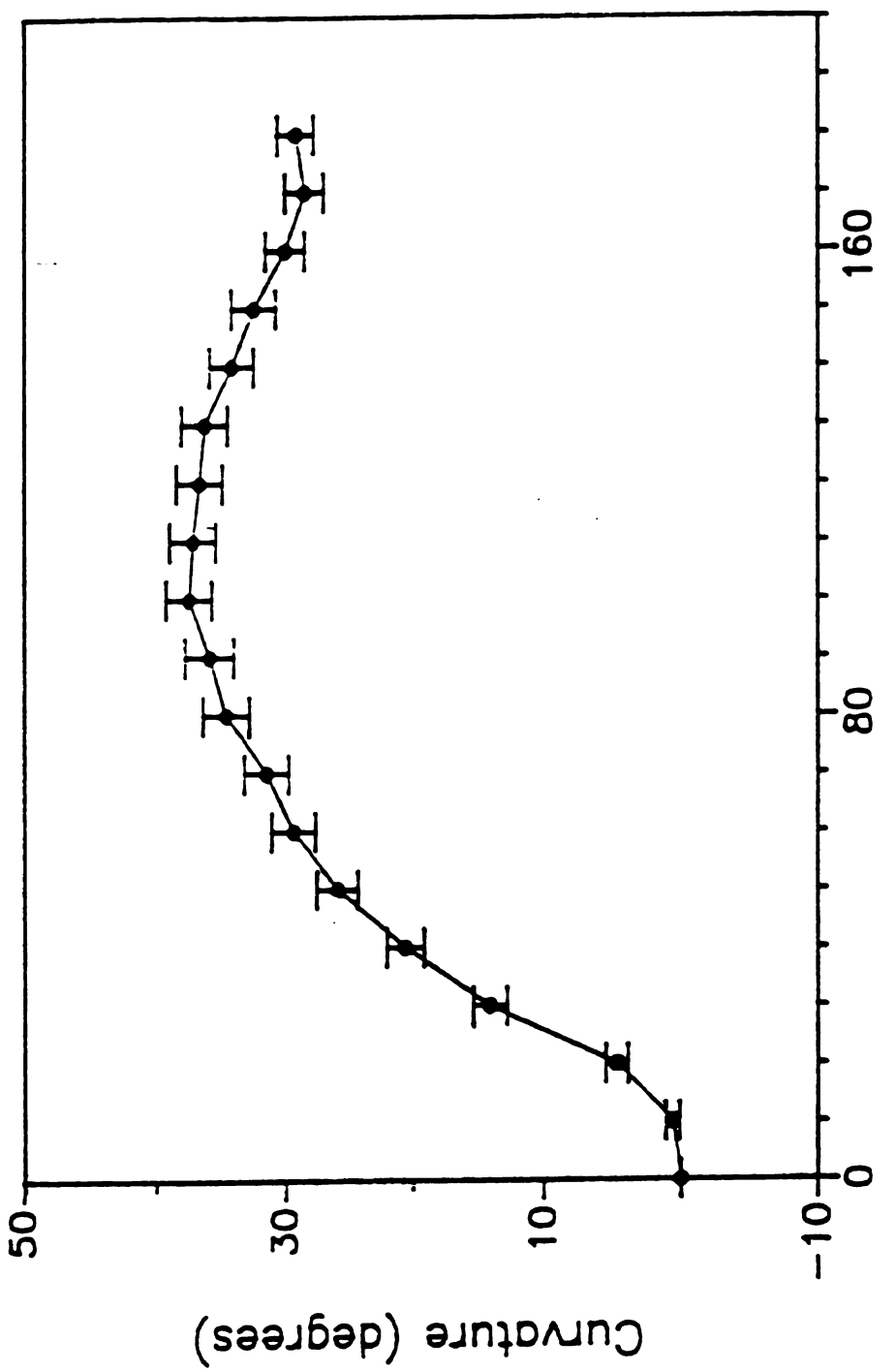

Figure 2.7. Time course for development of average curvature to a BL pulse by seedlings pre-irradiated with red light: n=32; vertical bars represent ± 1 SE.

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CHAPTER III

Growth distribution during phototropism of *Arabidopsis thaliana* seedlings

Previously submitted and accepted for publication in Plant Physiology. Referred to throughout the dissertation as CHAPTER III.

ABSTRACT

The elongation rates of two opposite sides of hypocotyls of *Arabidopsis thaliana* seedlings were measured during phototropism by using an infra-red imaging system. In first positive phototropism, second positive phototropism and in red-light-enhanced first positive phototropism curvature toward the light source was the result of an increase in the rate of elongation of the shaded side and a decrease in the rate of elongation of the lighted side of the seedlings. The phase of straightening that followed maximum curvature resulted from a decrease in the elongation rate of the shaded side and an increase in the elongation rate of the lighted side. These data for the three types of blue light induced phototropism tested in this study and for the phase of straightening are all clearly consistent with the growth rate changes predicted by the Cholodny-Went theory.

INTRODUCTION

It has been known for over a century that phototropism by a higher plant seedling results from different rates of elongation by the two sides of the shoot (Darwin, 1896). Blaauw (Went and Thimman, 1937) postulated that the gradient of light-induced different growth rates in the cells by which it was absorbed. Because the light was attenuated as it passed through the shoot, growth of the cells was inhibited more on the lighted side than on the shaded side (Went and Thimman, 1937). However, this localized light effect was not consistent with the demonstration of Boysen Jensen that there is stimulus transmission down the shoot (Went and Thimann, 1937). Three major theories consistent with the stimulus transmission were advanced to explain the differential growth during phototropism. These theories, Cholodny-Went (C-W; Went and Thimman, 1937), Boysen Jensen (BJ; Went and Thimman, 1937) and Paál (Pohl and Russo, 1984) suggested that unequal quantities of GF (growth factor) on opposite sides of seedling are responsible for the changes in growth rates that result in curvature. The history of this aspect of phototropism is covered by Went and Thimann (1937) and Pohl and Russo (1984).

A controversy persists in the literature over the validity of the C-W theory (Trewavas, 1992). Much of this controversy has resulted from a concentration on the identification and role of the growth factor. However, it is possible to directly study the applicability of the generalized C-W theory without studying auxin

distribution. As has been noted by Pohl and Russo (1984), the C-W theory predicts an increase in growth rate on the shaded side and concomitant decrease in growth rate on the lighted side of the seedling as the result of a lateral movement of GF from the lighted to the shaded side (Went and Thimann, 1937). In direct contrast, the B-J theory predicts an increase in growth rate on the shaded side but no change in growth rate on the lighted side (Pohl and Russo, 1984). Finally, Paál's theory predicts a general inhibition of growth rate by light and a greater inhibition on the lighted side than on the shaded side (Pohl and Russo, 1984). It is interesting that the prediction of Paál's theory is much the same as the prediction of Blaauw's hypothesis for differential growth, although the basic premise is different for the two. Paál's theory is predicated on the inhibition by light of a transmissible substance whereas Blaauw's hypothesis is predicated on direct photo-inhibition of growth of the individual cells. It follows that one should be able to distinguish between these possibilities (C-W, B-J and Paál/Blaauw) with the careful measurements of growth rates on the two sides of the tropistic organ.

As has been noted in the C-W forum (Trewavas, 1992), if the C-W theory is to be generally accepted, the conditions for its validity must be carefully characterized. As the study of tropisms has been refined, their complexities have come to be recognized. For example, multiple photoreceptor pigments are involved in the induction of phototropism (Galland and Lipson 1987, Konjević *et al.*, 1989). The amplitude of phototropic curvature is increased by the process of adaptation (Janoudi and Poff, 1991); and the kinetics of phototropic curvature may

be quite complex (Orbović and Poff, 1991). This places increased importance on the careful characterization of the conditions for which the C-W theory is valid.

Because of the emergence of *Arabidopsis thaliana* as a model system for the study of tropisms and because of difficulties of characterizing growth regulator(s) gradients across the extremely small *Arabidopsis* hypocotyls, we have measured growth rates of the hypocotyls during phototropism. Here we report growth rate changes on opposite sides of the hypocotyl during first and second positive phototropism, during the straightening following phototropism, and for both, dark grown and red light pre-irradiated seedlings. These growth rate changes are all clearly consistent with the C-W theory.

MATERIALS AND METHODS

Growth conditions

Arabidopsis thaliana seedlings were grown as described previously (Orbović and Poff, 1991) with slight modifications. Two seeds of the Estland ecotype were sown per well of microassay strips containing 0.7% (w/v) agar supplemented with 1 mM KNO_3. The strips were placed in Parafilm-sealed plastic boxes and germination was potentiated by chilling for 3 d at 4±1 °C in darkness followed by an exposure to the white light for 20 h at 25±1 °C. Following the white light treatment, the boxes containing the strips were placed in darkness for 42 h until the start of the experiment. Just before the beginning of each experiment, strips with

the seedlings were removed from the boxes and positioned such that the seedlings were be imaged by the video camera. All manipulations of the seedlings were performed at 25±1 °C and RH >90% in complete darkness except for those experiments in which the seedlings were pre-irradiated with red light.

Light sources

White light (65 μmol $m^{-2}s^{-1}$) for the potentiation of germination was provided by General Electric (Cleveland, OH) DeLux Cool-white fluorescent tubes. The blue light source consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA), a 300 W ELH tungsten halogen lamp and a 450 nm interference filter (PTR Optics, Waltham, MA) with a half band-width of 10 nm. First positive phototropism was induced by a 0.9 s pulse of blue light at 0.34 μmol $m^{-2}s^{-1}$ to give a fluence of 0.3 μmol m^{-2}. This is the fluence required to induce the peak in first positive phototropism for *A. thaliana* (Steinitz and Poff, 1986). The red light that was used for the 1-h irradiation of seedlings from above (0.5 μmol $m^{-2}s^{-1}$) was obtained from a single gold fluorescent tube (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp. Highland, IL). This source provides radiation from 560 to 720 nm with a maximum output at 620 nm. The infra-red light source for the imaging system consisted of a Leitz Prado-Universal (Ernst Leitz Gmbh, Wetzlar, Germany) projector with a 250 W tungsten halogen lamp and a Kodak Wratten 87c gelatin filter (Eastman Kodak, Rochester, NY) with <1.5% transmission at wavelengths lower than 800 nm (measured using a Perkin-Elmer Lambda 7

spectrophotometer). The infra-red source supplied radiation at 10 W m^2. Fluence rates were measured with a Li-Cor (Lincoln NE) Li-190 SA quantum sensor in combination with a Li 1000 Data Logger, or with a model 68 Kettering radiometer (Laboratory Data Control, Riviera Beach, Fl). The duration of blue light irradiation was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY).

Data collection

The infra-red imaging system described previously (Orbović and Poff, 1991) was used to record images of seedlings in the absence of visible radiation. Images were recorded every 10 min for 2-3 h and subsequently played back from the video tape for analysis using the Java (Jandel Scientific, Corte Madera, CA) video analysis program. Single frame images of each seedling were displayed on the screen and the lengths of the two sides measured. The tip of the seedling was not used in the measurements because of the difficulty of finding a single point on the hook from which to measure. The lowest portion of the seedling (approx. one eighth of the total length) was not used in the length measurement as it does not contribute to the elongation of the entire seedling (data not shown). Instead, the length of the hypocotyl was measured from a point approximately 0.5 mm above the well. The top of the hypocotyl was operationally defined as the apex of the hypocotyl on the inside of the hook (Fig. 3.1.) Because the seedling and camera were fixed in place, and magnification held constant, this measurement was quite reproducible (see below).

The length of un-irradiated seedlings and seedlings given blue or red light from above was measured along the central axis of the image of seedlings (Fig. 3.1. doted line). For these three treatments, the average length of the opposite sides of a single plant for any particular time point was not significantly different from the length measured along the central axis. For this reason, the length of these seedlings was measured along the central axis. This increment from the initial length was used for subsequent analysis.

The angle of curvature was measured from the frozen image of seedling on the screen as previously described (Orbović and Poff, 1991), and any change from the initial curvature was recorded as the increment or decrement in curvature.

The repeatability of length and angle measurements was assessed by 10 repetitions of length and curvature measurement for several seedlings. The repeatability error for length measurements of the 4.6 mm long representative seedling ranged from 0.02% to 1.4% of total length. The repeatability error for angle measurements ranged from $\pm 2°$ for 5° angles to $\pm 5°$ for 40° angles.

Sigma plot software (Jandel Scientific, Corte Madera, CA) was used for fitting curves to the data.

RESULTS

First positive phototropism

The elongation rate of un-irradiated seedlings was approximately 50 μm 10 min^{-1} over the 3-h measurement period (Fig. 3.2. A). These un-irradiated seedlings showed no significant curvature over the 3 hour monitoring period (Fig. 3.2. B).

Seedlings which received a 0.3 μmol m^{-2} blue light pulse from above showed no significant curvature over the 3 hour period (Fig. 3.3. B). Following the BL pulse, the growth rate of these seedlings was similar to that of the un-irradiated seedlings (Figs. 3.2. A and 3.3. A). Throughout the 3-h monitoring period, the elongation rate of seedlings given the BL pulse from above was about 50 μm 10 min^{-1} (Fig. 3.3. A).

For the first 80 min following a unilateral blue light pulse, the shaded side of seedlings elongated more rapidly than the lighted side (Fig. 3.4. A). Throughout this time, the curvature of the seedlings steadily increased (Fig. 3.4. B). About 30 min following the unilateral irradiation, the elongation rate of the shaded side reached its highest value while the elongation rate of the lighted side started decreasing. The elongation rate of the shaded side subsequently decreased and reached its lowest value about 150 min following the blue light pulse. By 80 min after the blue light pulse, the elongation rates of the two sides of the seedlings were approximately equal again. The elongation rate of lighted side of seedlings then exceeded the elongation rate of the shaded side. This corresponds very well

with the point of maximum curvature at 80 min following the blue light pulse and subsequent straightening (Fig. 3.4. B).

Because the elongation rate of the lighted side of the seedlings during straightening did not reach a level as high as the elongation rate of shaded side during bending (Fig. 3.4. A), the seedlings retained a degree of curvature at the end of experiment (Fig. 3.4. B).

Second positive phototropism

Second positive phototropism was induced by 30 min of blue light at $4x10^{-4}$ $\mu mol\ m^{-2}s^{-1}$ for a fluence of 0.72 $\mu mol\ m^{-2}$. Curvature and the elongation rates of the lighted and shaded side were measured for 120 min beginning with the initiation of the unilateral irradiation. The elongation rate of the shaded side of the seedlings increased rapidly to a maximum about 30 min following the start of the irradiation and subsequently decreased (Fig. 3.5. A). In contrast, the elongation rate of the lighted side decreased below the control level to a minimum at about 50-80 min and subsequently increased (Fig. 3.5. A). At about 100 min following the initiation of the irradiation, the elongation rate of the lighted side became greater than that of the shaded side (Fig. 3.5. A).

Second positive curvature was evident 20 min after the beginning of the irradiation. From 20 min to 70 min, curvature of seedlings increased linearly, reached its maximum at about 90 min, and then decreased until the end of experiment (Fig. 3.5. B).

Red light enhanced first positive phototropism

Elongation of the red light pre-irradiated seedlings was similar to but slightly higher than that of the un-irradiated plants (Fig. 3.6. A). The red light pre-irradiation by itself induced no significant curvature in these seedlings (Fig. 3.6. B).

When first positive phototropism was induced by a blue light irradiation of 0.3 μmol m^{-2} in the red light pre-irradiated seedlings, the growth rates on both sides of the seedlings changed rapidly (Fig. 3.7. A). Within 10 min following the blue light pulse, the shaded side of seedlings elongated more rapidly than the lighted side. The elongation rate of the shaded side reached its maximum 20 min after the blue light pulse and subsequently started decreasing. The elongation rate of lighted side decreased steadily following the blue light irradiation, reached a minimum 40 min after the blue light pulse and subsequently increased. The elongation rate of the lighted side became higher than that of the shaded side by 80-90 min following the blue light pulse and remained so until the end of the 2 h monitoring period (Fig. 3.7. A).

Curvature to the blue light pulse by the red light pre-irradiated seedlings was evident within 10 min following the blue light (Fig. 3.7. B). Curvature increased to a plateau by about 70 min following the blue light irradiation. Approximately 90 min after the blue light irradiation, the seedlings began to straighten and continued to do so for the remaining 30 min in which they were monitored (Fig. 3.7. B).

DISCUSSION

These data, taken together, show growth patterns which are predicted by the Cholodny-Went theory and not those predicted by either the B-J theory or the Paál/Blaauw theories. The B-J theory specifically addressed second positive phototropism and suggested that there is acceleration of growth on the shaded side with no effect on growth rate of the lighted side (Pohl and Russo, 1984). We observe an inhibition of growth on the lighted side (Figs. 3.4. A, 3.5. A and 3.7. A) of Arabidopsis seedlings irrespective of the duration of blue light photostimulation (0.9 sec or 30 min) or pretreatment of seedlings (-RL or +RL). Similarly, the accelerated growth of the shaded side of the *Arabidopsis* seedlings following the beginning of unilateral blue light irradiation (Figs. 3.4. A, 3.5. A and 3.7. A) is inconsistent with both the Paál (Pohl and Russo, 1984) and Blaauw (Went and Thimman, 1937) theories. Our measurements show a significant increase in growth rate of the shaded side and a decrease in growth rate of the lighted side of seedlings during the development of curvature under all conditions of phototropism, first positive, second positive and red light enhanced first positive. Thus, these data are in agreement with the predictions of the C-W theory (Went and Thimman, 1937).

The patterns of distribution of growth reported here, for first and second positive phototropism are very similar (Figs. 3.4. A and 3.5. A). This similarity is consistent with a single mechanism controlling the two responses. However, a red

light pre-irradiation of the seedlings changes the pattern of growth distribution during the response to a subsequent blue light pulse (Fig. 3.7. A) in comparison with that of etiolated seedlings (Figs. 3.4 A and 3.5 A). The effect of the red light pre-irradiation, probably acting through phytochrome (Janoudi and Poff, 1992), is to shorten the lag phase of the response thereby permitting the elongation rates of the shaded and lighted side to reach their maximum and minimum, respectively, earlier than in the seedlings not pre-irradiated with red light (Fig. 3.7. A).

The same growth rate changes that we see during phototropic curvature are also seen in reverse during the straightening phase in which the seedling loses a portion of this curvature towards the light. The mechanism of straightening is unknown but it clearly results from a decrease in growth rate on the shaded side and an increase in growth rate on the lighted side. This is opposite to the changes in growth rate that were responsible for the curvature toward the light.

It is interesting to note that our conclusions might have been different had we measured growth at only one time or under only a single condition of phototropism. Under some conditions, the kinetics of growth rate changes appear to be different on the shaded and lighted side of the seedlings. Because of this, individual time points can be found for which growth rate is increased on the shaded side and unchanged on the lighted side. Other individual time points can be found for which growth rate is unchanged on the shaded side and decreased on the lighted side. Thus, these time points, if taken individually, appear to be consistent with the B-J or Paál/Blaauw theories. However, all of the data together

are consistent only with the predictions of the C-W theory.

Measurements of growth rates during phototropism have been reported for both monocotyledonous and dicotyledonous species. It was shown for maize coleoptiles that there is a redistribution of growth following unilateral irradiation with blue light such that the shaded side started elongating while the lighted side slowed down simultaneously (Iino and Briggs, 1984; Baskin *et al.*, 1985). Similar findings have been reported for pea epicotyls for both first and second positive phototropism (Baskin, 1986; Briggs and Baskin, 1988). In contrast, Bruinsma and Hasegawa (Bruinsma and Hasegawa, 1989) reported inhibition of growth on the lighted side of the sunflower and radish seedlings in response to unilateral irradiation while the shaded side maintained a constant growth rate. Hart *et al.* (1982) have worked with de-etiolated seedlings of cress and concluded that, although growth was inhibited on the lighted side following unilateral irradiation, this inhibition was not always the major factor mediating phototropic curvature. Finally, Macleod *et al.* (1984) suggested that a complex pattern of acceleration and inhibition of growth in different zones of the coleptile is responsible for bending to unilateral light. Moreover, they reported that this pattern was dependent on the light pretreatment of the coleoptiles as well as on the point of administration of the actinic light pulse (Macleod *et al.*, 1984).

To our knowledge this is the first report of a systematic measurement of growth rates under many of the different conditions of phototropism. In all of these conditions, curvature was the consequence of an increase in growth rate on one

side and a decrease in growth rate on the other side, although the kinetics for these changes appeared not to be simultaneous.

The apparent difference in the kinetics of growth rate changes on the lighted and shaded side of the seedlings (Figs. 3.4. A, 3.5. A and 3.7. A) could result from any one or combination of a number of factors. For example, a growth factor may be redistributed within the seedling (Iino, 1991) and the tissue may respond more slowly to withdrawal than to addition of that factor (Evans and Hokanson, 1969; dela Fuente and Leopold, 1970). Alternatively, the different kinetics could be a consequence of, or complicated by, changed tissue sensitivity to the growth factor (Ishikawa *et al.*, 1991). However, if there is a chemical message transported across the bending organ, there should be a time delay before the response (Macleod *et al.*, 1986). It should be noted that a red light pre-irradiation decreases the delay time for the response such that the increased growth rate on the shaded side appears concomitantly with the growth rate decrease on the lighted side (Fig. 3.7. A). However, we have no data which would permit us to identify the source of the difference in kinetics or the removal of that difference by the red light pre-irradiation.

A growth response which is separate from the tropic response is induced in dark-adapted sporangiophores of *Phycomyces* (Galland *et al.*, 1985). Could the blue light irradiation itself introduce a transient growth response in *Arabidopsis* seedlings? To answer that question, seedlings were irradiated from above with blue light at the same fluence as that administered unilaterally to induce first

positive phototropism. The results (Fig. 3.3. A) show a growth rate comparable to that measured for un-irradiated seedlings (Fig. 3.2. A). Blue light given from above to the seedlings in our experiment therefore is not inducing growth response. It should be noted however that Rich *et al* (1987) have shown that an irradiation from above may not be equivalent to a bilateral irradiation.

In summary, following a unilateral blue light irradiation inducing phototropic curvature, the growth rate of the shaded side of *Arabidopsis thaliana* hypocotyl is increased while the growth rate of the lighted side is decreased. Regardless of the type of the response being monitored, whether first or second positive phototropism, red light-enhanced first positive phototropism or the phase of straightening following phototropic curvature these results are consistent with predictions based on the C-W theory and not with those based on either the B-J, Paál or Blaauw theories.

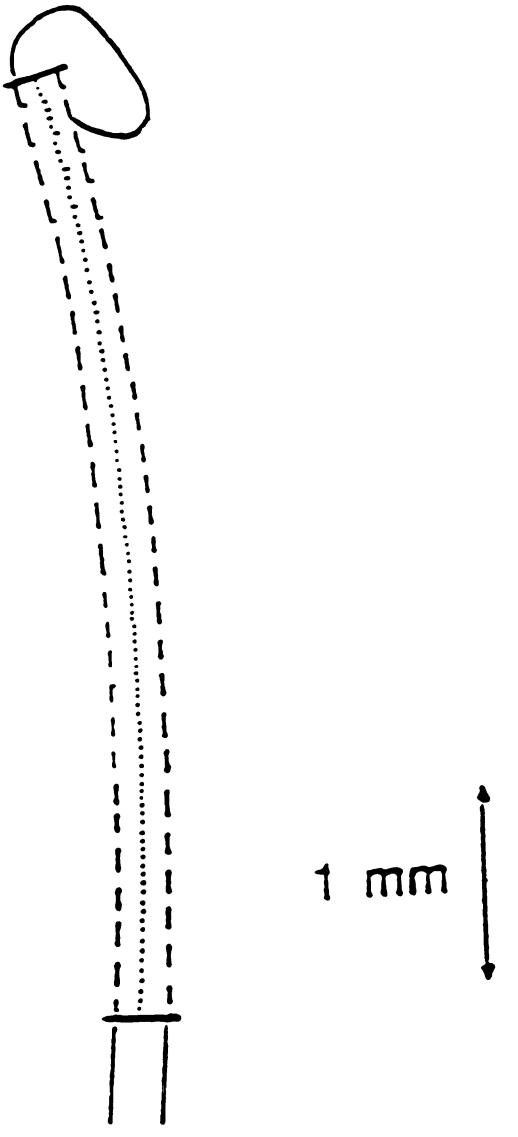

Figure 3.1. The image of a representative seedling "frozen" on the monitor screen. Dashed lines represent the length measured along opposite sides of seedling. The doted line represents the length measured along the central axis.

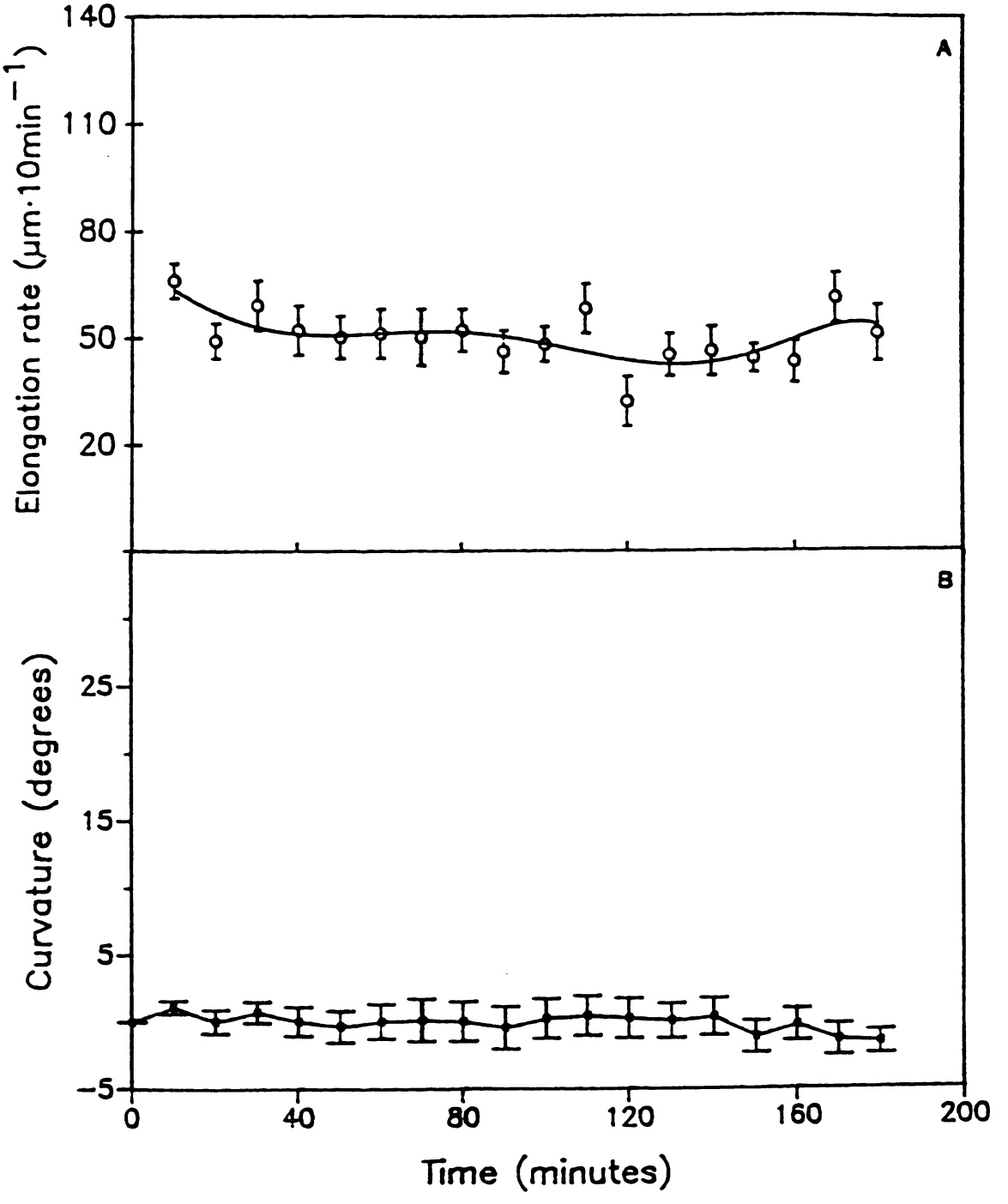

Figure 3.2. The elongation rate-(a) and the time course for development of average curvature-(b) of un-irradiated seedlings; a) elongation measured along the central axis; n=25-30; vertical bars represent ± 1 SE. b) n=25-30; vertical bars represent ± 1 SE.

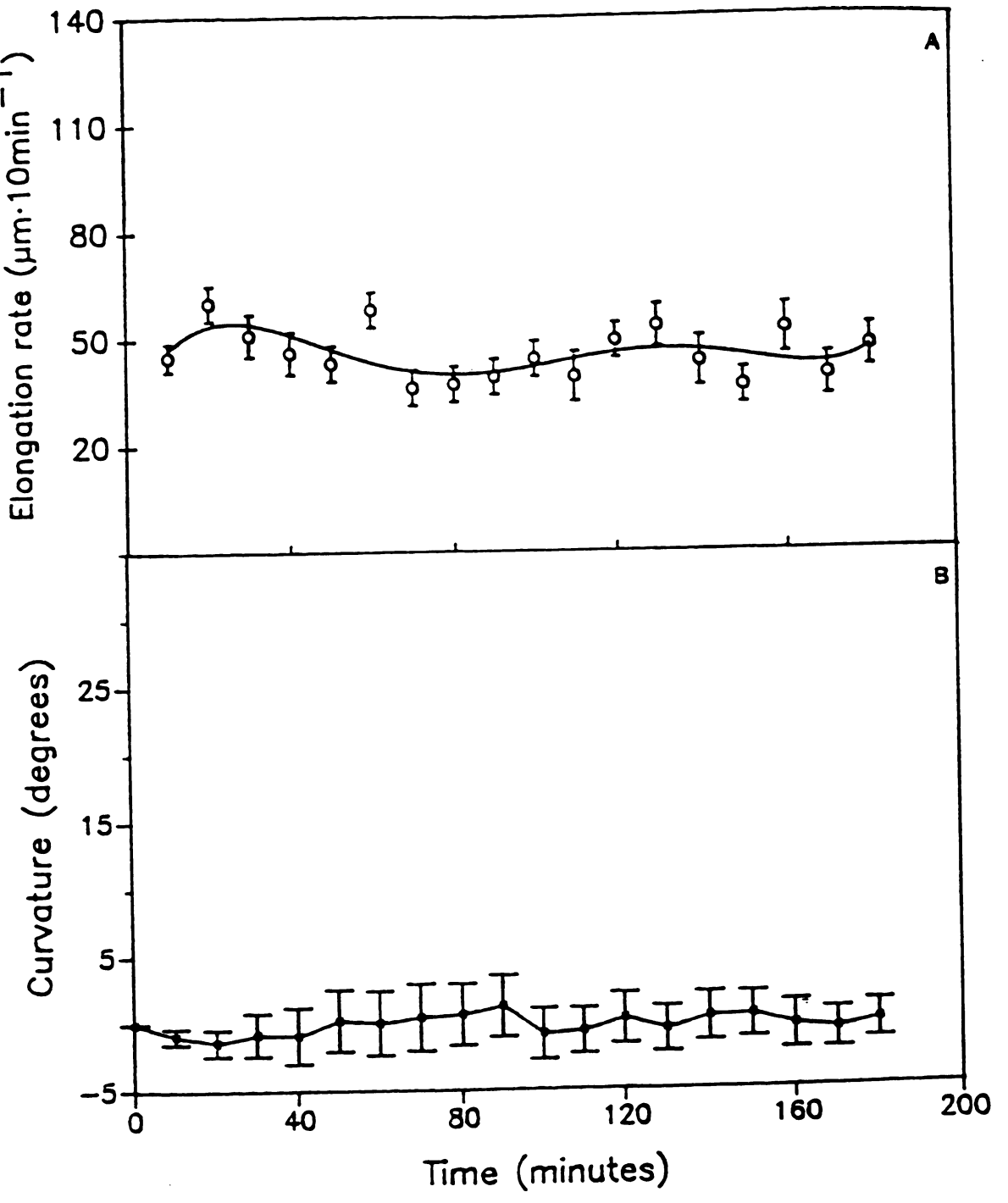

Figure 3.3. **The elongation rate-(a) and the time course for development of average curvature-(b) of seedlings irradiated with a BL pulse (0.3 μmol m^{-2}) from above at time zero;** a) elongation measured along the central axis, n=18-34; vertical bars represent ±1 SE. b) n=18-34; vertical bars represent ±1 SE.

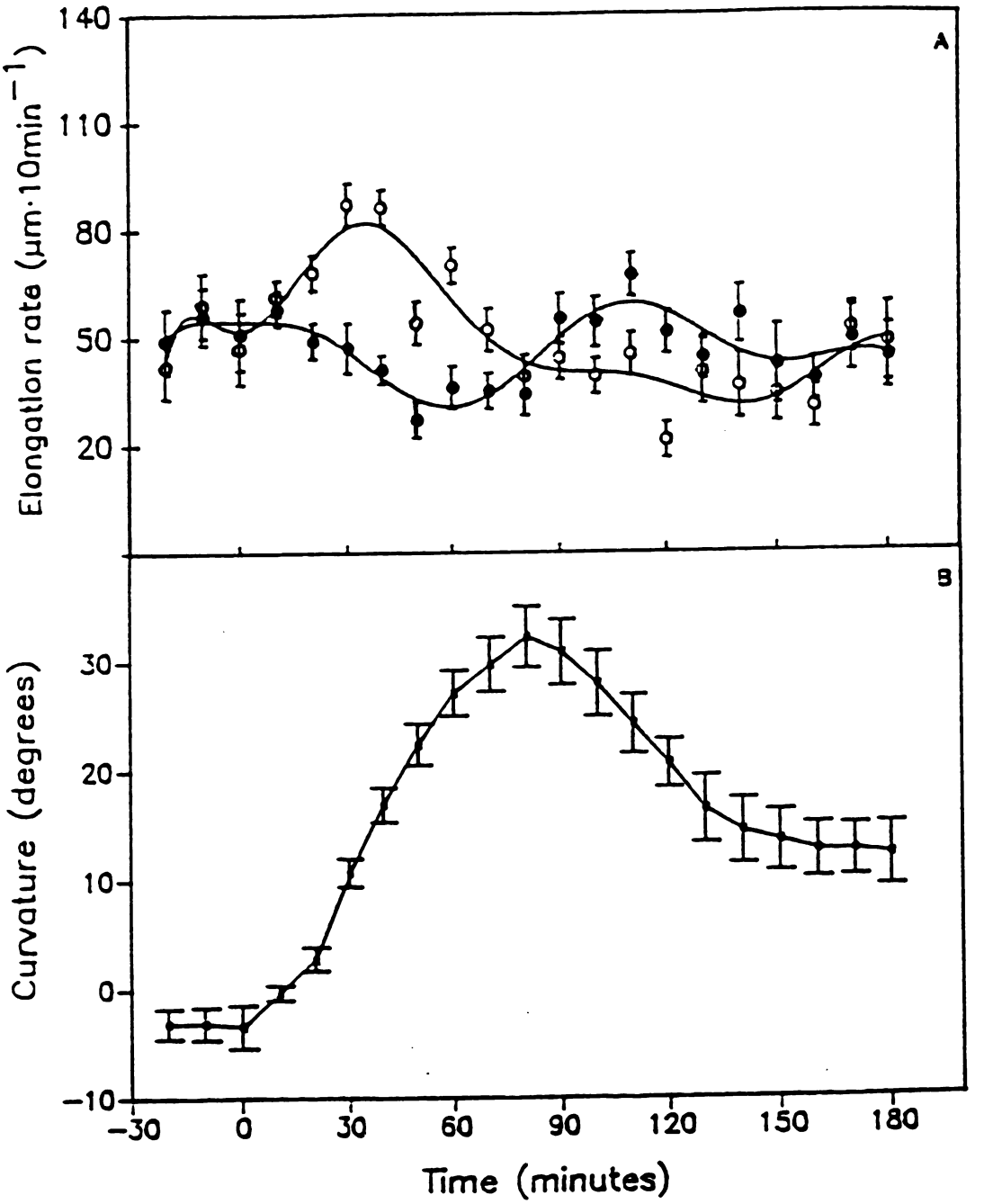

Figure 3.4. The elongation rate of lighted and shaded side-(a) and the time course for development of average curvature-(b) of seedlings during first positive phototropism (unilateral BL pulse, 0.3 μmol m⁻²); a) o, shaded side, n=12-39, •, lighted side, n=12-39; vertical bars represent ±1 SE. b) n=12-39; vertical bars represent ±1 SE.

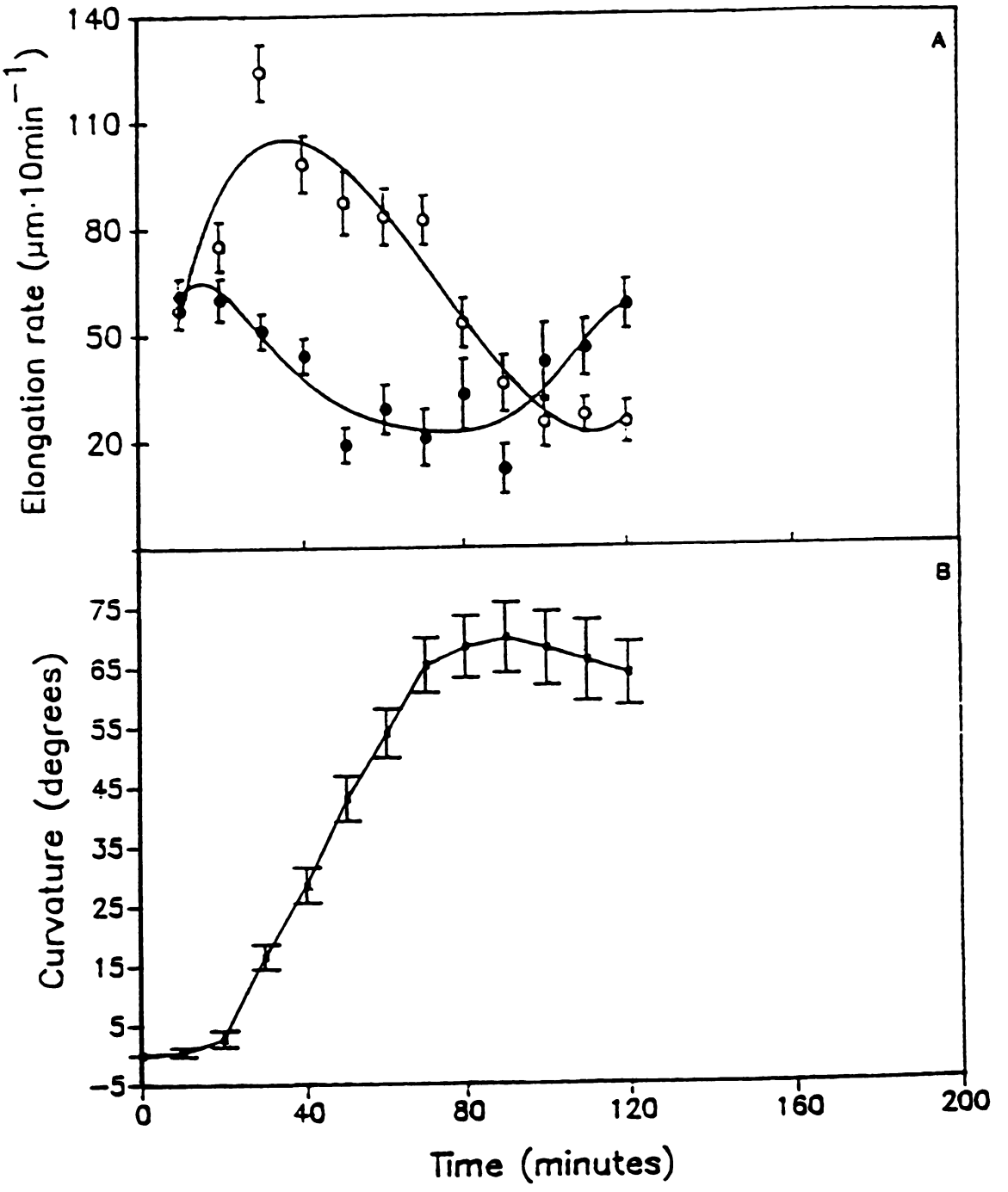

Figure 3.5. The elongation rate of the lighted and shaded side-(a) and the time course for development of average curvature-(b) of seedlings during second positive phototropism (unilateral BL for 30 min, 0.72 μmol m⁻²); a) o, shaded side, n=19-26; •, lighted side, n=19-26; vertical bars represent ±1 SE. b) n=19-26; vertical bars represent ±1 SE.

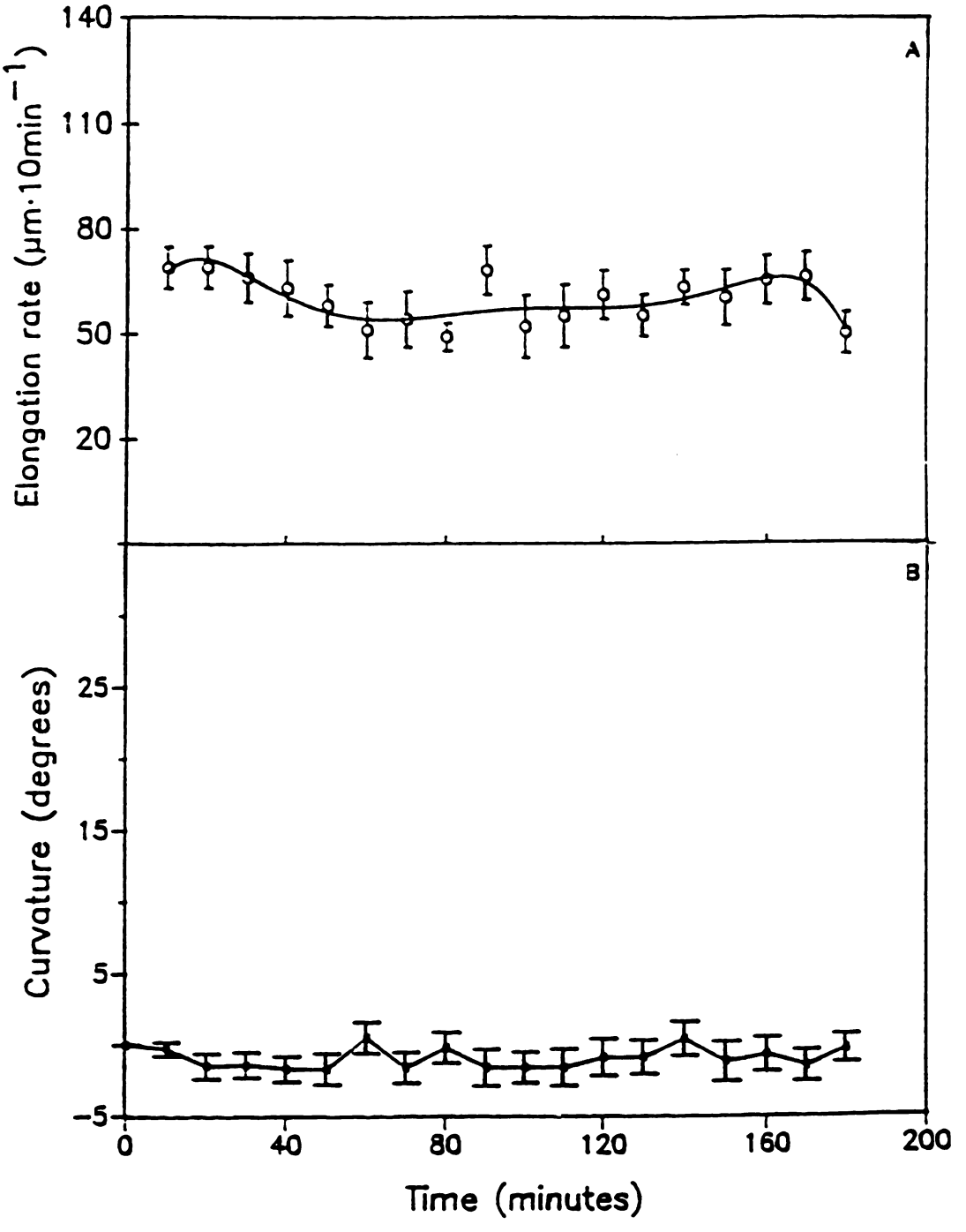

Figure 3.6. The elongation rate-(a) and the time-course for development of average curvature-(b) of seedlings irradiated with the RL for 1 h from above; a) elongation measured along the central axis, n=21-26; vertical bars represent ±1 SE. b) n=21-26; vertical bars represent ±1 SE.

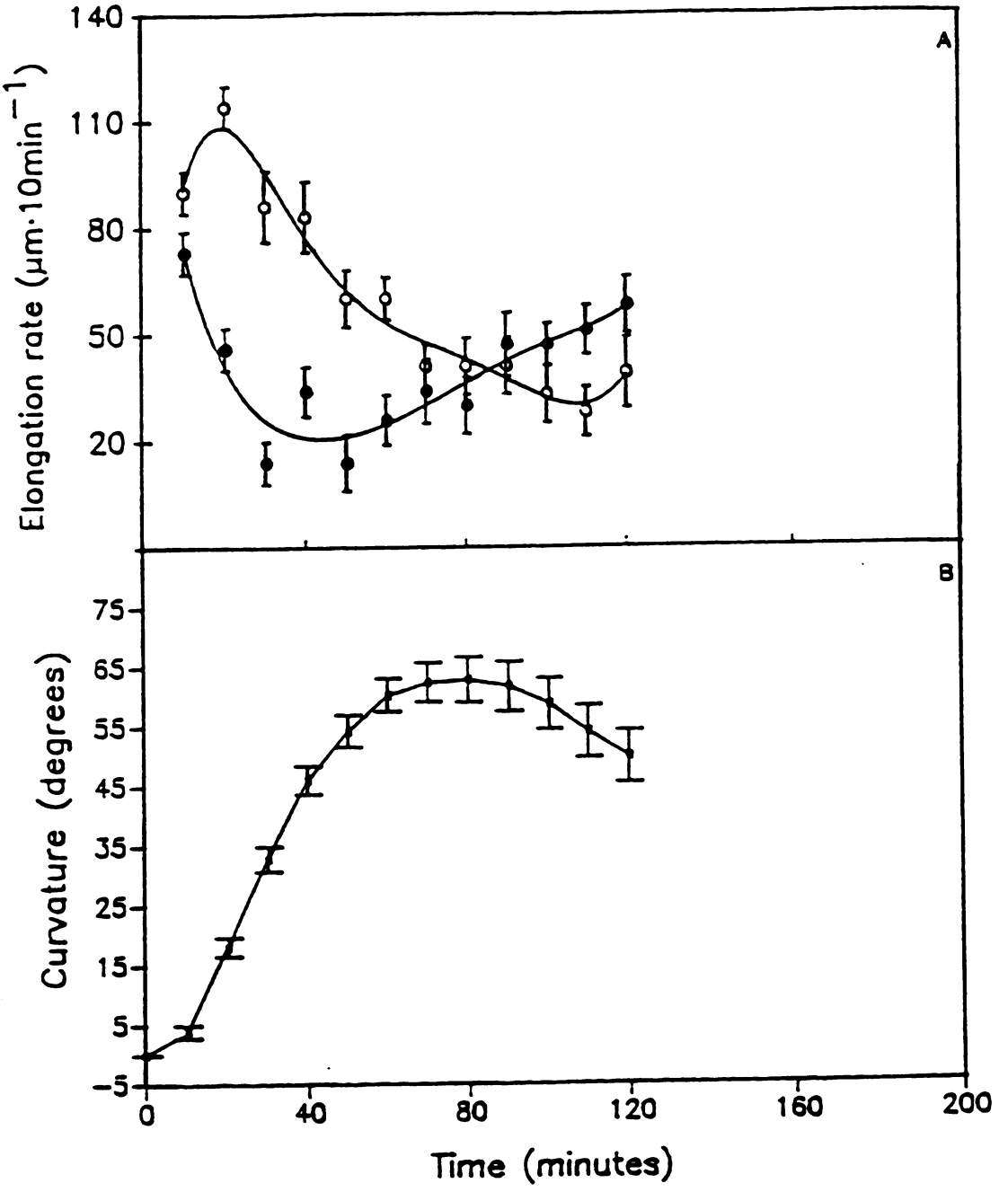

Figure 3.7. The elongation rate of lighted and shaded side-(a) and the time-course for development of curvature-(b) of seedlings during red light enhanced first positive phototropism (1 hour of RL followed by the BL pulse 0.3 μmol m^{-2}); a) o, shaded side, n=18-25; ●, lighted side, n=18-25; vertical bars represent ±1 SE. b) n=18-25; vertical bars represent ±1 SE.

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CHAPTER IV

Effect of temperature on growth and phototropism of *Arabidopsis thaliana* seedlings

ABSTRACT

Seedlings of *Arabidopsis thaliana* grown for 42-44 hours at 25 °C respond to a change in that growth temperature by changing their elongation rate. Regardless of whether the new temperatures were higher or lower than 25 °C, the seedlings grew slower after the transfer at all tested temperatures. The kinetics of first positive phototropism in seedlings transferred from 25 °C to 15 °C differed from the kinetics exhibited by seedlings transferred to 28 °C. At 15 °C, measurable curvature began 40-50 min after the BL pulse. No straightening was evident within 150 min after the BL pulse. Seedlings transferred to 28 °C exhibited a lag phase of 20-30 min, and straightening became evident as early as 70 min after the photostimulation. At all temperatures, the distribution of growth on the two opposite sides of seedlings exhibiting first positive phototropism, changed in a manner corresponding to the change in curvature. Kinetics of phototropism for populations of seedlings transferred from 25 °C to both 15 °C or 28 °C differed from the kinetics of phototropism exhibited by the seedlings grown and stimulated at 25 °C (Chapter III). Based on these results, it is concluded that changes in temperature conditions affect the elongation rate of seedlings and first positive phototropism.

INTRODUCTION

Heat energy is an environmental factor that is present without interruption throughout the lifetime of all biological systems. Furthermore, heat energy affects every molecule in the organisms. Since it is not possible to provide conditions without heat energy, experiments in this field of research were designed to test the effects of temperature gradients or temporal changes in temperature, a measure of heat energy.

The research on thermosensing and thermal responses has progressed to a considerable level in microorganisms (Poff *et al.*, 1984). Mutants with altered responses to some thermal stimulation have been characterized in bacteria, cilliates and slime molds. Based on results from studies of these mutants, it has been proposed that the receptor for serine acts as a thermoreceptor in bacteria (Poff *et al.*, 1984). This proposed existence of specific thermal receptor still requires additional confirmation. Another model based on the structural change within the cell organelle was put forward to describe the mechanism of thermosensing. This model proposes that a structural change in the cell membrane could signal a change in temperature. Since lipids comprise most of the cell membrane a phase transition due to the isomerization of chains of fatty acids at a specific temperature could alter the packaging of the lipids in the membrane. This structural change in the cell membrane has been proposed as the signal which would trigger the physiological response (Poff *et al.*, 1984).

Less is known about the perception of environmental thermal stimuli in plants. Phase transitions of lipids in plasma membranes have also been suggested to be the basis for the sensing of temperature changes in plants. Raison and Chapman (1976) used an Arrhenius plot to describe the elongation rate of mung bean plants at different temperatures. It was proposed that the temperatures at which the slope of the Arrhenius plot changed represented conditions at which phase transition occurred in the plasma membranes of the growing cells (Raison and Chapman, 1976). However, this model was disputed by Bagnal and Wolfe (1978) who claimed that process of plant elongation is more complex than processes which can be described with Arrhenius plots (Bagnal and Wolfe, 1978).

Due to the economic importance of photosynthesis, the effects of different temperatures on the rate of photosynthesis have been studied most extensively within this field. The results of these studies suggest that there is an optimum temperature at which the photosynthesis rate reaches a maximum while at other temperatures, the rate of photosynthesis is lower (Flore and Lakso, 1989). Other studies have focused in detail on the response of plants such as cytoplasmic streaming, turgor, exchange of ions between the cells and its wall, to rapid cooling (Minorsky, 1989). Recently, thermotropism and its interaction with gravitropism have been described in maize roots (Fortin and Poff, 1991).

This work was undertaken to describe the elongation rates of *A. thaliana* seedlings upon transfer from one temperature to another. The effects of changes in temperature conditions on kinetics and growth distribution during first positive

phototropism were also examined.

MATERIALS AND METHODS

Growth conditions

Seedlings of the Estland race (WT) of *A. thaliana* were grown as previously described (Chapter III) in strips of microassay wells containing 0.7% (w/v) agar. The strips were incubated at 4±1 °C for 3 days and then transferred to WL at 22±1°C for 19 hours. Both the chilling and the WL treatments were employed to potentiate and synchronize germination. Following the WL treatment, the strips were transferred into darkness at 25±1 °C for 42 to 44 hours. At the end of this period, seedlings were put into a chamber set to the experimental temperature. Seedlings were permitted to equilibrate to the temperature in the chamber for 40 min before video recording began.

A custom-built chamber was used to provide the different temperature conditions. The chamber consisted of a double-walled plexiglass container enclosed in styrofoam for insulation. Copper tubing inserted between the walls of the chamber carried circulating liquid from a waterbath. The desired temperatures within the chamber were obtained by altering the temperature of the circulating liquid. The temperature inside the chamber was measured using a thermistor element connected to a electronic tele-thermometer YSI-42 SC (Yellow Springs

Instruments, Co. Inc., Yellow Springs, OH). Temperature variation was within $\pm$0.5 °C for individual experiments and within $\pm$1.0 °C for the group of experiments.

Light sources

White light (65 $\mu mol.m^{-2}s^{-1}$) used to potentiate germination was provided by General Electric (Cleveland, OH) DeLux Cool White fluorescent tubes. Phototropism was induced by a unilateral BL pulse. The BL source consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA) 300 W ELH tungsten Halogen lamp and a 450 nm interference filter (PTR Optics, Waltham, MA) with half-band width of 10 nm. The fluence of BL used to induce phototropism was 0.3 $\mu mol.m^{-2}$ obtained in a single 0.9 sec pulse at a fluence rate of 0.34 $\mu mol.m^{-2}s^{-1}$. Fluence rates were measured with a Li-Cor (lincoln, NE) Li-190 SA quantum sensor in combination with a Li 1000 Data Logger. The duration of BL was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY). The infra-red irradiation used video recording was described previously (Orbović and Poff, 1991).

Data collection

The video analysis system used for data collection and the method for data collection were previously described (Orbović and Poff, 1991; Chapter III). Length and curvature of seedlings were measured from the successive video recordings. Changes in length and curvature calculated from these measurements and used for statistical analyses. Lines were fitted to the data points in figure 4.1. by inspection.

Data describing the growth of seedlings at 25 °C were taken from the Chapter III.

RESULTS

Upon transfer from one temperature to another, etiolated seedlings of *Arabidopsis* exhibit a change in elongation rate. Transfer from 25 °C to both lower and higher temperatures resulted in decrease of elongation rate (Fig. 4.1.) The elongation rate of seedlings was lowest at 9 °C (Fig 4.1. a). When seedlings were transferred to 15 °C and 36 °C (Fig. 4.1. b and g) the elongation rates were similar but higher than the elongation rate measured at 9 °C. Although still lower, the elongation rate of seedlings transferred to 20 °C, 28 °C and 31.5 °C were closest to the elongation rate of seedlings both grown and measured at the same temperature of 25 °C (Fig. 4.1. c,d,e and f). The elongation rate of seedlings at 20°C was higher than the elongation rate at 31.5 °C, but lower than the rate at 28 °C.

The data points defining the elongation rates after transfer from 25 °C to each temperature were averaged to obtain a single value representing growth rate at that temperature. A plot of the average elongation rate as a function of temperature shows a bell shaped curve (Fig. 4.2.)

During first positive phototropism in seedlings transferred to 15 °C, the

elongation rates of the lighted and shaded sides of the seedlings changed simultaneously about 40 min after the BL pulse (Fig. 4.3. a). The elongation on the lighted side began to slow and reached its lowest value 80 min after the BL pulse. Subsequently, the elongation rate of the lighted side increased to the elongation rate of the shaded side 2 hours after photostimulation. The changes in the elongation rate on the shaded side were opposite and nearly symmetrical to the changes observed on the lighted side. That is, the elongation rate on the shaded side increased between 40-80 min, then decreased to elongation rate of the lighted side 2 hours after the BL pulse. Over the last 30 min of the experiment, the elongation rates on the two sides of the seedlings were similar (Fig. 4.3. a).

The kinetics of phototropism of seedlings transferred to 15 °C corresponded to the recorded changes in elongation rates (Fig. 4.3. a and b). The seedlings first exhibited a curvature between 40-50 min following the BL pulse and then the curvature increased in a linear fashion for the next 50 min (0.44 deg.min^{-1}). Curvature proceeded at a slower rate for an additional 30 min. Between 120-150 min after the BL, pulse curvature remained unchanged (Fig. 4.3. b).

When the seedlings were transferred to 28 °C and irradiated with a unilateral BL pulse they exhibited first positive phototropism (Fig. 4.4.) The small sample of seedlings (14) makes it difficult to estimate if the change in elongation rates on the opposite sides of the seedlings occurred simultaneously. The difference in the elongation rates on the lighted and shaded sides became evident 20-30 min after the BL pulse (Fig 4.4. a). The elongation rate on the shaded side

increased for 30 min, and then decreased for 50 min at which time it reached its lowest value. The elongation rate remained at this value for additional 20 min. On the other hand, the elongation rate on the lighted side initially decreased, and then increased. At 80 min after the BL pulse, the elongation rate on the lighted side became equal to the elongation rate on shaded side. In the following 40 min the elongation rate on the lighted side exceeded the elongation rate on the shaded side (Fig. 4.4. a).

Phototropic curvature at 28 °C became evident between 20-30 min after the BL pulse. Curvature increased linearly to its maximum over the next 40 min (0.62 $deg.min^{-1}$). These seedlings subsequently started straightening and within the next 50 min reversed the previously attained curvature by about two thirds (Fig. 4.4. b).

DISCUSSION

Research in the field of thermosensing and thermal responses is hampered by the difficulties centered around the nature of heat as a stimulus. First, heat energy non-specifically affects all the molecules that constitute living systems. Second, it is impossible to provide conditions in which heat energy is absent. Therefore, spatial and temporal changes in temperature have most frequently been used as thermal stimuli (Poff *et al.*, 1984). In this work, elongation rates and phototropism of *A. thaliana* seedlings were examined upon transfer from one

temperature to another.

When etiolated seedlings of *Arabidopsis* were transferred from one temperature to another the elongation rate was reduced. The relationship between temperature and elongation rate is described by a bell- shaped curve (Fig. 4.2.) There are two possible explanations for these results.

One possibility is that the seedlings were adapted to the first temperature at which they grew (25 °C) since germination. The growth conditions changed upon transfer to the second temperature, and the seedlings responded by slowing their elongation rate. To test this hypothesis, initial temperatures other than 25 °C should be tested in conjunction with a variety of second temperature conditions. If the curves obtained from such experiments are also bell-shaped with maximum elongation rates at temperatures corresponding to the initial temperatures, adaptation would be likely factor controlling growth response to thermal stimuli. However, it has been shown that maize roots transferred from 16 °C and 26 °C to a range of other temperatures grew fastest at about 30 °C (Fortin and Poff, 1991).

A second possible explanation for the shape of the curve in figure 4.2. is to that the major processes controlling the elongation rate have optimum temperatures close to 25 °C. In this case it would be expected that any change in temperature from the optimum would result in decrease in elongation rate. The data presented here support this hypothesis.

Seedlings transferred from 25 °C to other temperatures also exhibited an altered phototropic response (Figs. 4.3., 4.4. and 3.4.) The difference was

pronounced in the kinetics of the response, while magnitude appeared not to be affected strongly. Seedlings transferred from 25 °C to 15 °C required more than 40 min to exhibit measurable curvature toward the light source. The bending rate of these seedlings was also lower than the rate observed in seedlings kept at 25 °C (Figs. 3.4 and 4.4.) These alterations in lag time and bending rate point to multiple effects of temperature.

At 15 °C, the redistribution of growth regulators mediating phototropism may be slower, resulting in longer lag phase. Once redistribution is achieved, the reduced bending rate could be due to temperature effects on processes directly involved in cell extension.

In Chapter III, it was suggested that during phototropism at 25 °C, the kinetics of initial changes in elongation rates of lighted and shaded side of the seedlings were consequence of different multiple processes. If the distribution of growth during phototropism was mediated by a single process, the initial changes in elongation rate on the two sides of responding seedling at two different temperatures should occur at different times after the BL pulse but the kinetics of these changes should be the same. The data presented on figure 4.3. argue in favour of multiple processes. Apparently, the process responsible for the faster response of the shaded side of the seedlings at 25 °C is diminished, so that only the slower process remains. That is, the slower process results in simultaneous distribution of growth which controls curvature at 15 °C (Figs. 3.4. and 4.3.)

Several reports have described the effect of different temperatures on IAA-

induced growth responses (Nissl and Zenk, 1969; Murayama and Ueda, 1973). In oat coleoptiles segments, it was shown that an increase in ambient temperature from 21 °C to 40 °C resulted in a considerable decrease and near disappearance of the lag phase in response to $5x10^{-3}$ M of exogenous IAA (Nissl and Zenk, 1969). However, the effects of lower temperatures were not examined. Murayama and Ueda (1978) measured the growth response of pea stem segments to 10 $\mu g.ml^{-1}$ of IAA at temperatures between 15 °C and 40 °C. They showed that the latent period of the response to applied auxin, increased from about 2 min at 40 °C to about 22 min at 15 °C (Murayama and Ueda, 1973). It is possible that one of the processes involved in growth distribution during phototropism of *Arabidopsis* seedlings is the redistribution of IAA within the hypocotyl, such that there is a different concentration of IAA on the two sides of the seedling. The sensitivity of IAA-induced responses to different temperatures may be the cause of the difference in the kinetics of growth distribution during phototropism at 15 °C and 25 °C. Lower temperature can affect the rate of transport of auxin across the hypocotyl and delay the response of tissue to incoming auxin. At this time, there are no data to support this hypothesis or to suggest what other processes are involved in the distribution of growth during phototropism.

Comparison of the lag phases preceeding the initiation of curvature at 25 °C and 15°C yields a ratio of about 1:2. At 25 °C, seedlings began to straighten almost immediately after they reach maximum curvature (Fig. 3.4. b). However, seedlings 15 °C do not exhibit any straightening within 30 min after attaining maximal

curvature. Although it is not possible to estimate what the time requirement is for initiation of straightening of seedlings at 15 °C, it is obvious that it is different from the time requirement at 25 °C. Since the straightening is delayed much more than initiation of curvature at 15 °C, it is suggested that these two processes may be controlled differently (Figs. 3.4. and 4.3.)

Kinetics and growth distribution during phototropism in seedlings transferred to 28 °C from 25 °C were similar to those measured at 25 °C (Figs. 3.4. and 4.4.) However, the kinetics of straightening appeared different. This difference further supports the previous suggestion (see above) that initiation of curvature and initiation of straightening may not be controlled by the same mechanism.

On the basis of these data it can be concluded that temperature affects both undirected and directed growth of *Arabidopsis thaliana* seedlings. The reduction in elongation rate in etiolated seedlings transferred from 25 °C to different temperatures suggests that 25 °C may be at, or near the optimum temperature for growth. Transfers of seedlings to temperatures different from 25 °C resulted in an altered phototropic response. Temperature affected phototropism by changing the initial lag phase, the bending rate and the time required for initiation of straightening.

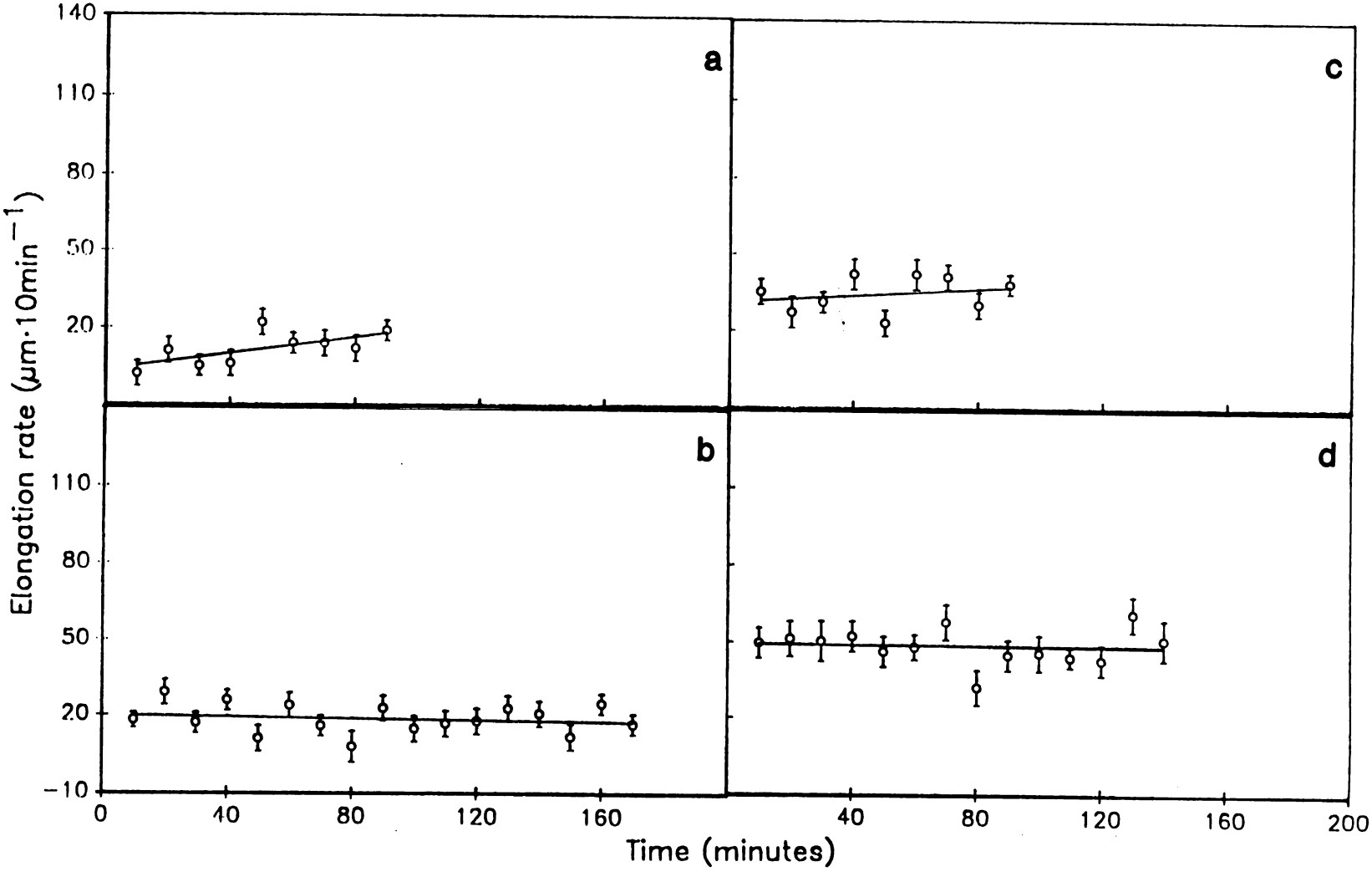

Figure 4.1. Average elongation rate of seedlings transferred from 25 °C to: a-9 °C, n=29; b-15 °C, n=32-35; c-20 °C, n=29; d-25 °C, n=26-30; transfer of plants was done 40 min before the 0 min time point; vertical bars represent ±1 SE.

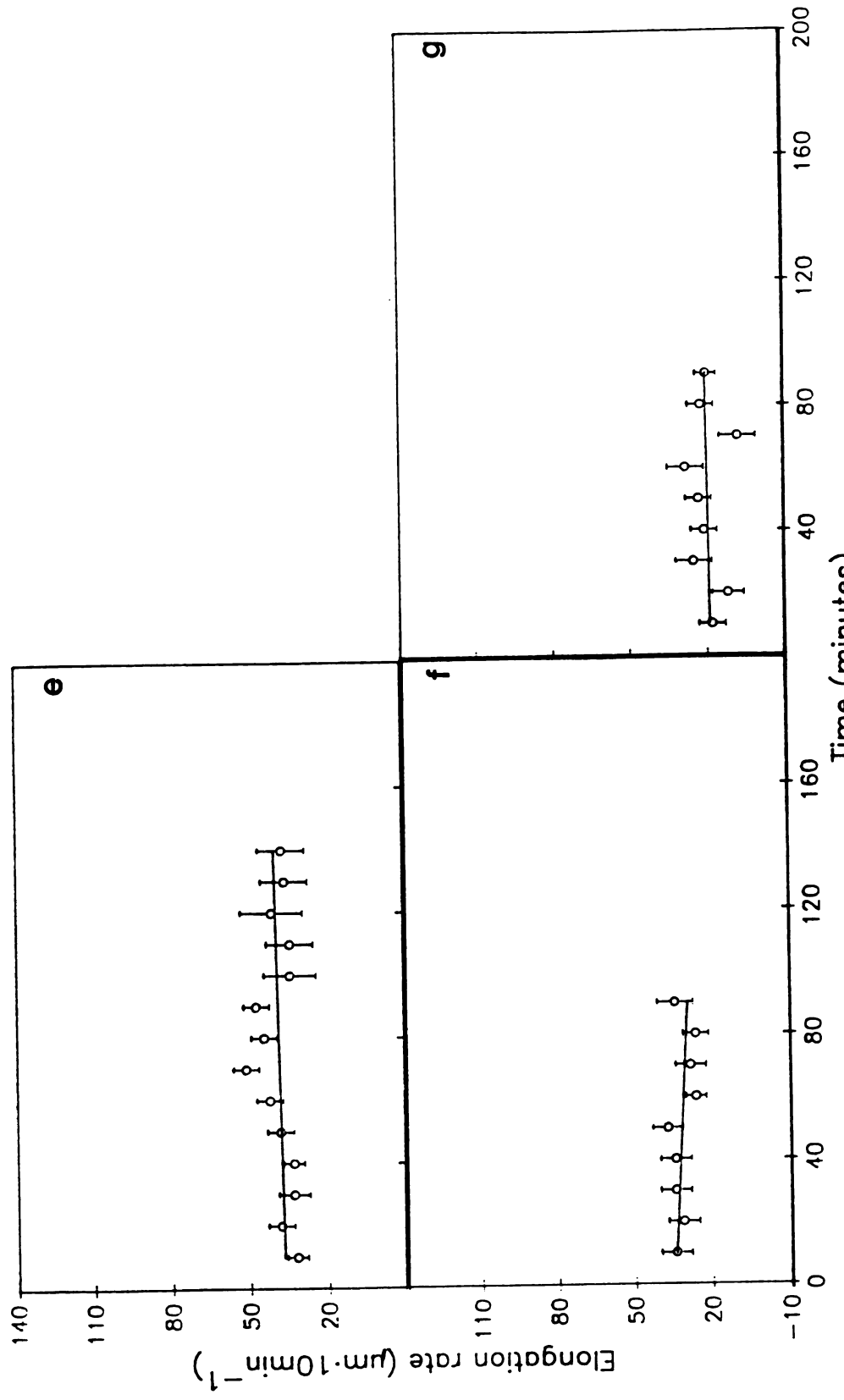

Figure 4.1. (cont'd) Average elongation rate of seedlings transferred from 25 oC to: e-28 °C, n=11-35; f-31.5 °C, n=26; g-36 °C, n=21; transfer of plants was done 40 min before the 0 min time point; vertical bars represent ±1 SE.

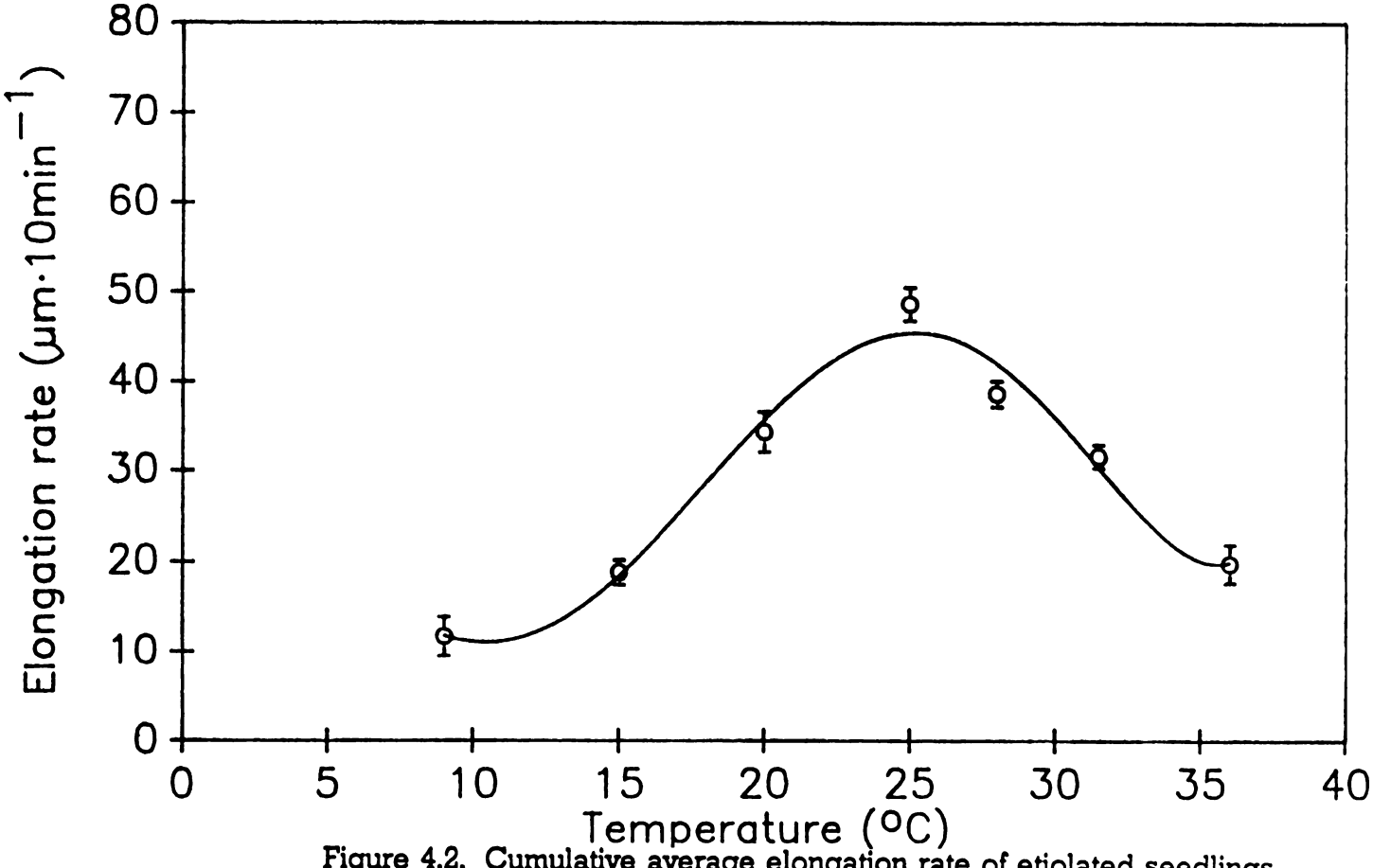

Figure 4.2. Cumulative average elongation rate of etiolated seedlings incubated at 25 °C and then transferred to different temperatures; at 9 °C n=9; at 15 °C n=18; at 20 °C n=9; at 25 °C n=14; at 28 °C n=15; at 31.5 °C n=9; at 36 °C n=9; vertical bars represent ±1 SE.

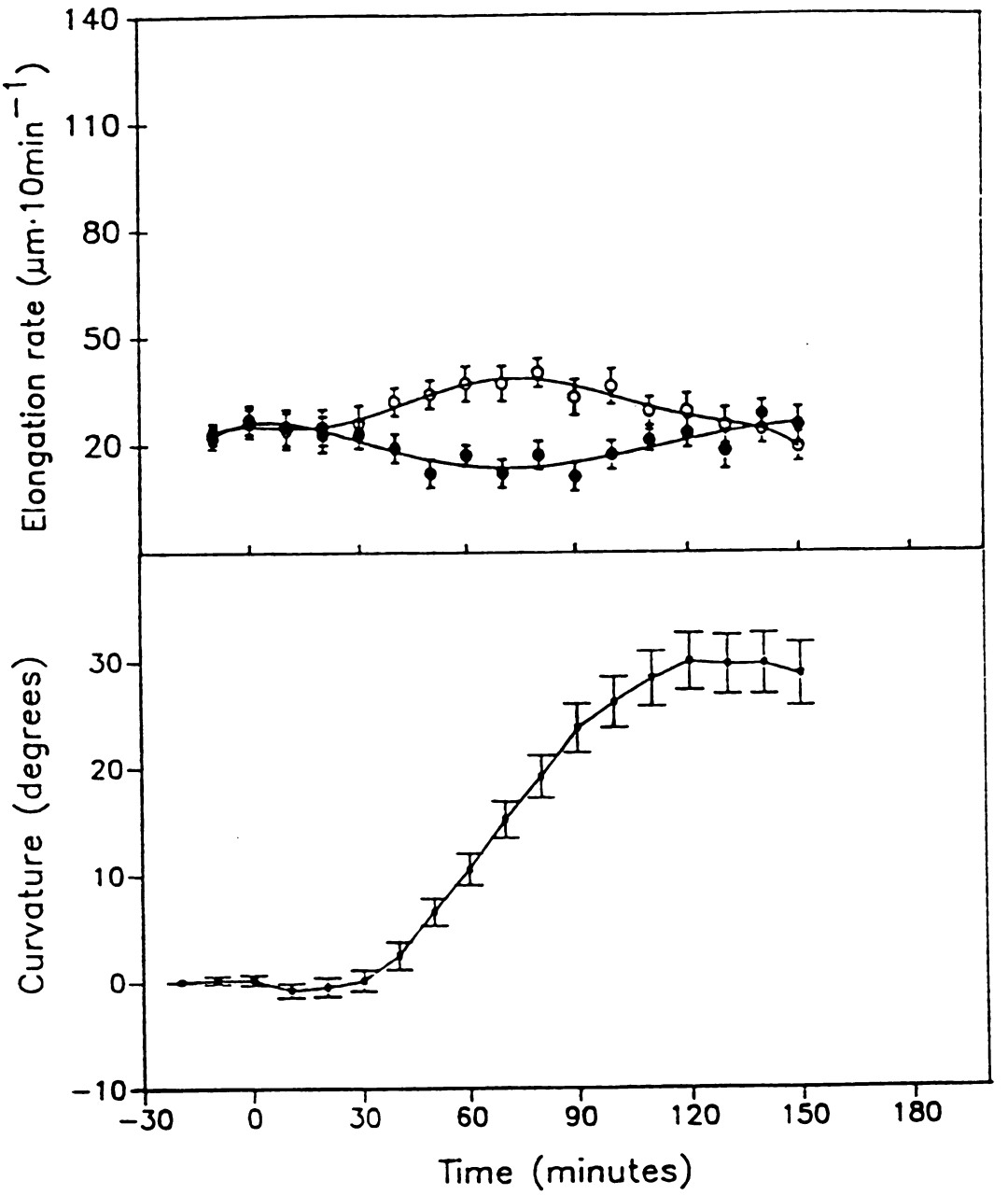

Figure 4.3. The elongation rate of lighted and shaded side-(a) and the time-course for development of average curvature-(b) of seedlings during first positive phototropism upon transfer from 25 °C to 15 °C; BL pulse administered at 0 min time point; a) o, shaded side, n=22-26, •, lighted side, n=22-26; b) n=22-26; vertical bars represent ± 1 SE.

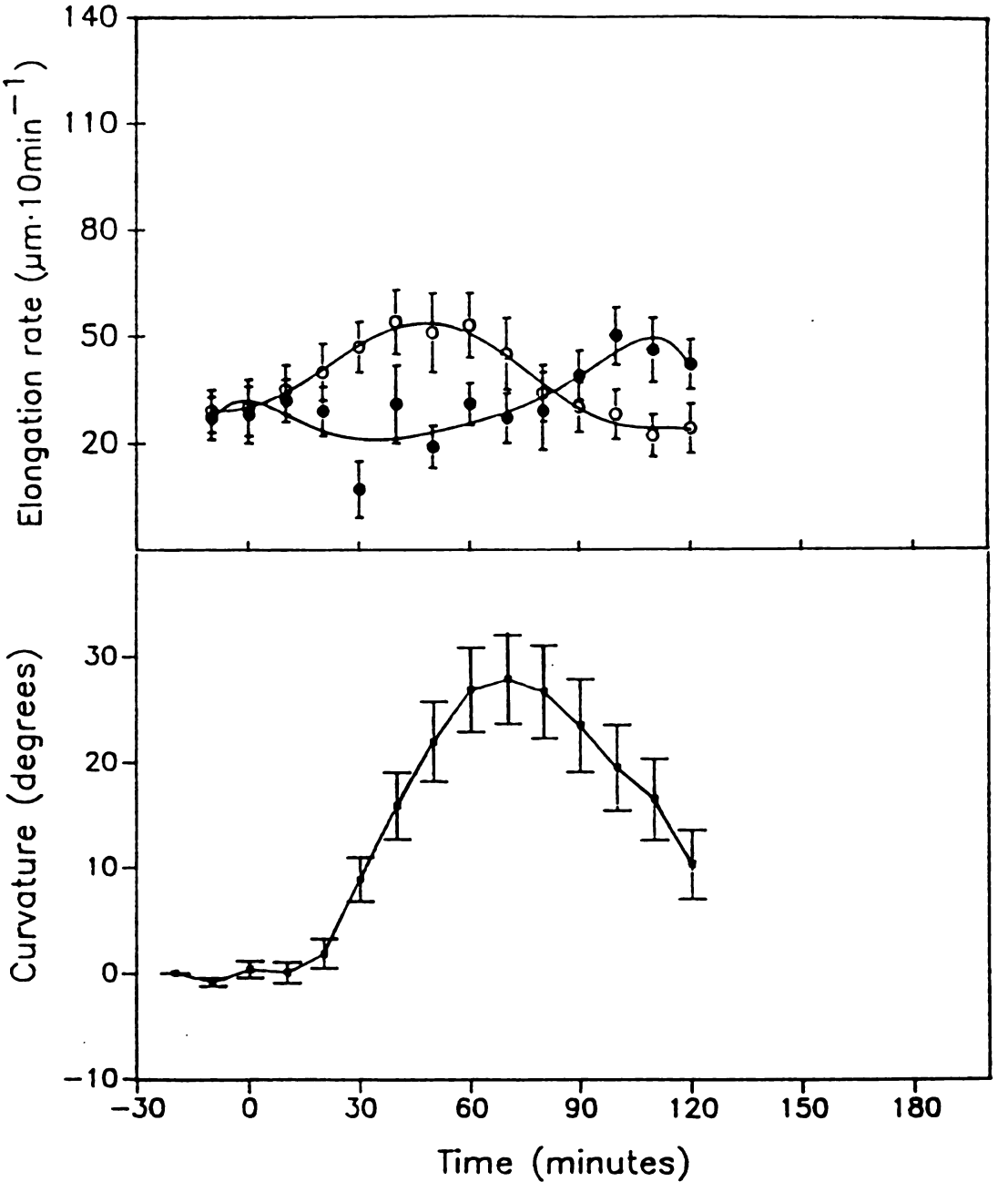

Figure 4.4. The elongation rate of lighted and shaded side-(a) and the time-course for development of average curvature-(b) of seedlings during first positive phototropism upon transfer from 25 °C to 28 °C; BL pulse administered at 0 min time point;a) o, shaded side, n=14, •, lighted side, n=14; b) n=14; vertical bars represent ±1 SE.

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CHAPTER V

Nutation in hypocotyls of *Arabidopsis thaliana* seedlings

ABSTRACT

Etiolated seedlings of *Arabidopsis thaliana* exhibited nutation under conditions of physiological darkness. The population appeared heterogeneous regarding both pattern and period of nutation. About ten percent of the monitored individuals exhibited regular elliptical nutation, circumnutation. Pre-irradiation for 1 hour with RL prevented occurrence of circumnutation without having an effect on the average rate of the nutational movement. Phototropic stimulation appears to be superimposed over the mechanism controlling nutation. Seedlings that were given a unilateral blue light pulse to induce first positive phototropism appeared to nutate during development of curvature only perpendicular to the direction of light stimulation. Throughout the gravitropic response, some seedlings continued to exhibit nutation suggesting that these two processes are independently controlled. Based on these results, we suggest that nutation in *Arabidopsis* probably is not controlled by the mechanism predicted by the theory of gravitropic overshoots.

INTRODUCTION

Many plant organs move about the longitudinal axis as they grow. These movements are named nutation and can be rhythmic or irregular, and can take place in one plane or in many planes. Circumnutation describes the special circumstance when nutational movements proceed in a rhythmic elliptical fashion. Nearly a century ago Darwin described nutation in more than 100 plant species (Darwin, 1896). Darwin proposed that nutation is universal and autonomously induced, and that both gravitropism and phototropism are extended and augmented nutations (Darwin, 1896). Although the phenomenon of nutation has received much attention in the intervening years, the mechanism controlling nutation is still undiscovered.

Several investigators have examined the relationship between gravity and nutation in sunflower and cress seedlings (Johnsson and Israelson, 1969; Volkmann *et al.*, 1986; Brown *et al.*,1990). Johnson and Israelson claimed that gravity is necessary for induction of nutational movements and on the other hand Brown's and Volkmann's group suggested nutation to be independent from gravity. Only a few investigations of the effects of light on nutation have been reported. Studies using pea seedlings and *Avena* coleoptiles suggested that light treatment increases the amplitude and regularity of nutational movements (Galston *et al.*, 1964). However, additional studies of the relationship between light and nutation are clearly needed.

This work was undertaken to describe nutation in hypocotyls of seedlings of *Arabidopsis thaliana* and systematically examine the interaction of nutation with phototropism and gravitropism. Nutation was monitored in both dark and red light pre-irradiated seedlings.

MATERIALS AND METHODS

Growth conditions

Arabidopsis seedlings were grown as previously described (Orbović and Poff, 1991). Two seeds were planted per well of microassay strips containing 0.7% (w/v) agar supplemented with 1 mM KNO_3. Germination of seeds was potentiated by chilling for 3 d at 4±1 °C in darkness followed by an exposure to white light for 20 h at 22±1 °C. After the white light treatment, the strips with seeds were placed in darkness for 42 h to allow germination and growth of seedlings. The strips with seedlings were then positioned in a way that could be imaged by a camera(s). All manipulations of the seedlings were performed in complete darkness at 25±1 °C and relative humidity higher than 90%.

Light sources

White light (65 $\mu molm^{-2}s^{-1}$) provided by General Electric (Cleveland, OH) DeLux Cool-white fluorescent tubes was used for the potentiation of germination.

The blue light source consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA) 300 W ELH tungsten halogen lamp and a 450 nm interference filter (PTR Optics, Waltham, MA) with a half-band width of 10 nm. Phototropism was induced by a 0.9 s pulse of blue light at 0.34 $\mu molm^{-2}s^{-1}$ to give a fluence of 0.3 $\mu molm^{-2}$. Red light was obtained from single gold fluorescent tube (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp. Highland, IL). This source provided radiation from 560 to 720 nm with a maximum output at 620 nm and a fluence rate of 0.5 $\mu molm^{-2}s^{-1}$ (at a distance of 0.5 m). Red light pre-irradiation was administered from above for a period of 1 h. The infra-red light source for the imaging system consisted of a Leitz Prado-Universal (Ernst Leitz Gmbh, Wetzlar, Germany) projector with a 250 W tungsten halogen lamp and a Kodak Wratten 87c gelatin filter (Eastman Kodak, Rochester, NY) transmitting light >800 nm (transmission <1.5% at wavelengths lower than 800 nm, measured using a Perkin-Elmer Lambda 7 spectroradiometer). Fluence rates were measured with a Li-Cor (Lincoln NE) Li-190 SA quantum sensor in combination with a Li 1000 Data Logger. The duration of blue light irradiation was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY).

Data collection

The infra-red imaging system used to record images of seedlings in the absence of any visible radiation was described previously (Orbović and Poff, 1991). In experiments where the pattern of nutation of seedlings was monitored, the

camera recorded images of the seedlings from above. Recordings were made every 10 min for 3- 4 h and subsequently played back from the video tape for analysis using the Java (Jandel Scientific, Corte Madera, CA) video analysis program. Single frame images of the seedling recorded from above were displayed on the monitor, the cursor positioned on the top of the image of the hook and that point recorded by computer. Consecutive points were then connected with lines to obtain a pattern of nutation.

Distances between two consecutive points representing the position of the top of the hook of seedling were measured to calculate average length of nutational movement per 10 min. Nutation in etiolated seedlings was followed for 4 h and in RL pre-irradiated seedlings for 3 h. The error in this type of measurement was 0.05mm10min^{-1}.

The interaction of nutation and phototropism was examined by following nutation of etiolated seedlings was followed for 1 h after which a blue light pulse was administered unilaterally to induce first positive phototropism. Nutation of seedlings was recorded from above.

The angle of curvature was measured from the frozen image of seedling on the screen as previously described (Orbović and Poff, 1991). Any change from the initial curvature was taken as the increment or decrement in curvature.

The period of nutation was estimated as depicted in figure 5.2.

RESULTS

Etiolated seedlings of *Arabidopsis thaliana* exhibited high variability in the pattern of nutation (Fig. 5.1.) with only five out of forty eight (10%) exhibiting circumnutation (Fig. 5.1. B). Two etiolated seedlings, out of forty eight, moved at average rate of 0.05mm10min^{-1} which is within the error of the measurement (Fig. 5.1. C) . The period of nutation varied from 150 min to 240 min in a small pool of seedlings (8) exhibiting oscillatory movements.

Etiolated seedlings monitored for 1 h prior to an exposure to a blue light pulse exhibited irregular nutation (Fig. 5.3.) The blue light stimulation induced seedlings to curve toward the light source. During steady development of phototropic curvature that started 10 and 20 min after the BL pulse in seedlings analyzed on panels A and B of figure 5.3. respectively, there was little evidence of nutation. Movements of two representative seedlings recorded from above show characteristic pattern of movement following blue light irradiation (Fig. 5.3. A and B).

Red light pre-irradiation of seedlings also affected nutation. None of the forty six seedlings pre-irradiated with 1 h of red light exhibited circumnutation. However, a comparison of the average rate of nutational movement in etiolated and in red light-irradiated seedlings (Fig. 5.4.) revealed they were not significantly different (T-test). Population variance was also not significantly affected with the red light pre-irradiation (F-test).

A time-course for the gravitropic response of a population of etiolated seedlings to a constant 90 degree gravi-stimulation is shown in figure 5.5. Gravitropic curvature became evident 20 min after the beginning of stimulation and reached a maximum at 90 min. After 90 min, seedlings began straightening or bending downward until the end of the experiment at the 180 min after the beginning of stimulation (Fig. 5.5. A). Some seedlings from this population continued to nutate while exhibiting gravitropism as exemplified in the figure 5.5. B.

DISCUSSION

Two major theories have been advanced to explain the mediation of nutation:1) the theory of gravitropic overshoots and, 2) the theory of internal oscillations.

Gravitropic overshoot theory views nutation as a continuing gravitropic response to gravity stimulation. This theory predicts: 1) the rate of curvature during gravitropism and nutation should be similar; 2) the relationship between the period of nutation and the gravitropic response time should be constant; and 3) gravitropic stimulation should have phase-shifting and entrainment effects on nutation. It was found that all these predictions are satisfied for seedlings of Helianthus anuus (Israelson and Johnsson, 1967; Johnsson and Israelson, 1969; Andersen and

Johnsson, 1972). In bean seedlings however, the rate of gravitropism was reported to be slower than the rate of nutation and gravitropic stimulation did not induce phase shift of nutation (Heathcote and Aston, 1970). On the other hand, gravitropic induction resulted in phase-shift of nutation in pea seedlings (Britz and Galston, 1982).

In *Arabidopsis* seedlings, the rate of bending during gravitropic response appeared similar to the rate of nutation (Figs. 5.2. and 5.5. A). It should be noted, however, that in this case, curvature of a single seedling was compared to the average curvature of the population (Figs. 5.2. and 5.5. A). The response time for gravitropism, which is the time between the onset of gravitropic stimulation and first evident response of seedlings was 20 min (Fig. 5.5. A). To establish the constant relationship between gravitropic response time and the period of nutation was very difficult because of high variability of the latter (150-240 min). The theory of gravitropic overshoots (Israelson and Johnsson, 1967) predicts ratio of 1:4 of response time for gravitropism and period of nutation, respectively, and the data obtained for *Arabidopsis* seedlings do not fit the predicted ratio (1:4 vs. 1:7.5-12).

Based on the gravitropic overshoot theory, nutation should stop in the absence of gravity (Israelson and Johnsson, 1967). However, hypocotyls of sunflower and roots of cress exhibited nutations throughout the space flight in the conditions of microgravity (Volkmann *et al.*, 1986; Brown *et al.*,1990). These descriptions of continued nutation in microgravity strongly argue against the gravitropic overshoots theory.

The persistence of nutation during the gravitropic response of seedlings is inconsistent with the gravitropic overshoot theory (Johnsson, 1979). In both, *Pisum* and *Phaseolus,* plants continued to nutate while exhibiting gravitropism which was used as an argument against the theory of gravitropic overshoots (Heathcote and Aston, 1970; Britz and Galston, 1982). In the population of etiolated seedlings of *A. thaliana,* some plants also nutated during the gravitropism (Fig. 5.5. B). These results are compatible with the idea of nutation as process independent of gravity stimulation but disagree with the gravitropic overshoots theory.

The theory of internal oscillations postulates the existence of oscillators at the cellular or tissue level. This theory predicts:1) oscillators driving nutation may be individual cells which would allow for different periods within the same species and within a single plant, and 2) initiation of nutation is independent of external stimuli and is achieved internally (Johnsson,1979 and references therein).

Regular, rhythmic nutational movements with the period close to constant were previously reported for stems and epicotyls of pea that were not simulated gravitropically (Britz and Galston, 1982; Baskin, 1986). We have observed multiple periods of nutation within the single plant and within the population of etiolated *Arabidopsis* seedling (data not shown). The ability of seedlings of *Arabidopsis* to exhibit multiple periods of nutation is consistent with the theory of internal oscillations.

The mechanism controlling phototropic curvature apparently is superimposed over nutational control in *Arabidopsis.* Phototropic stimulation may

influence the distribution of growth substances within the seedling by transversely polarizing distribution in one direction. This could then induce a differential growth larger in magnitude than the differential growth resulting in nutation. As a consequence, once seedlings start curving towards a light source they would appear to lose one dimension of nutation. Nutation that would take place in the plane of light stimulation would be hidden by the larger movement of phototropic bending.

In pea epicotyls, the magnitude of differential growth has been reported to be larger during phototropism than during nutation (Baskin, 1986). *Arabidopsis* seedlings exhibits vigorous movement in the direction of the photostimulation with only minor deviations to left or right (Fig. 5.3.)

Some etiolated seedlings of *A. thaliana* started curving away from the light source within the first 10-20 min after the BL pulse (Fig. 5.3.) Subsequently, these seedlings reversed the direction of bending. The reason for this behaviour could be that these seedlings were irradiated as they nutated away from the light source. Since the lag phase for the phototropic response is about 20 min (Orbović and Poff, 1991), immediately after photostimulation, differential growth that is resulting in nutation has not yet been obscured by the differential growth responsible for phototropism. Although this phenomenon is similar to initial downward bending of WT corn coleoptiles after the beginning of gravity stimulation (Hild and Hertel, 1972) it is unclear at the moment whether these two processes have the same basis.

Red light appears to abolish circumnutation in etiolated *Arabidopsis* seedlings, thereby making nutational movements more irregular. However, red light does not appear to change the average rate of nutational movements in the population (Fig. 5.4.) These results contradict previous reports in which red light and white light appear to increase the regularity and amplitude of nutations in peas and *Avena* (Galston *et al.*, 1964).

In summary, we conclude that etiolated seedlings of *Arabidopsis thaliana* nutate with about ten percent exhibiting circumnutation. Red light pre-irradiation abolishes the ability of etiolated seedlings to circumnutate. Phototropism obscures nutation while gravitropism proceeds over a measurable background of nutation. Thus, the data reported here argue against the gravitropic overshoot theory as an explanation for initiation of nutation in *Arabidopsis* seedlings and suggest that nutation in this species may be independent of external stimulation.

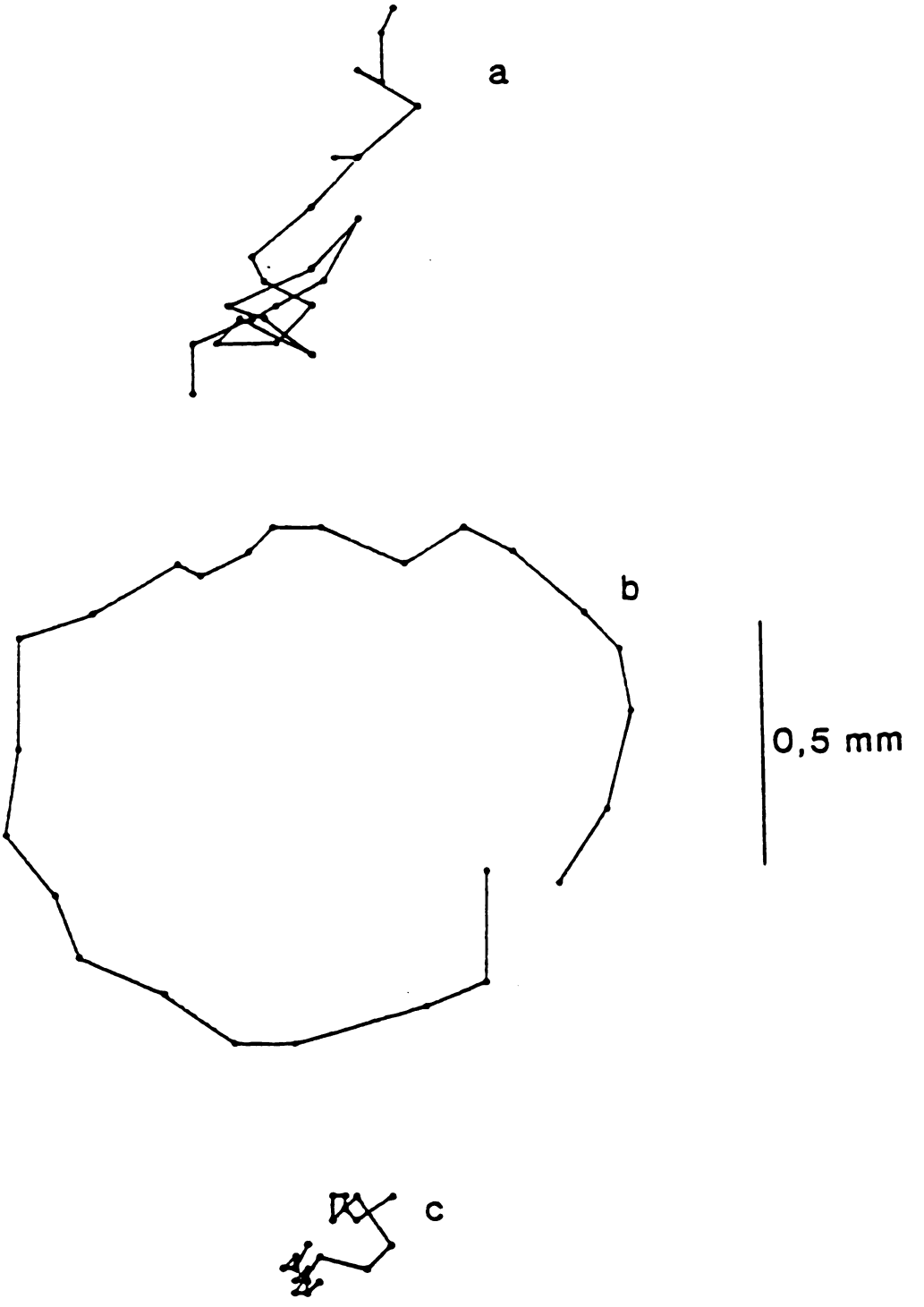

Figure 5.1. Pattern of nutation of three individual seedlings observed from above (A, B, C).

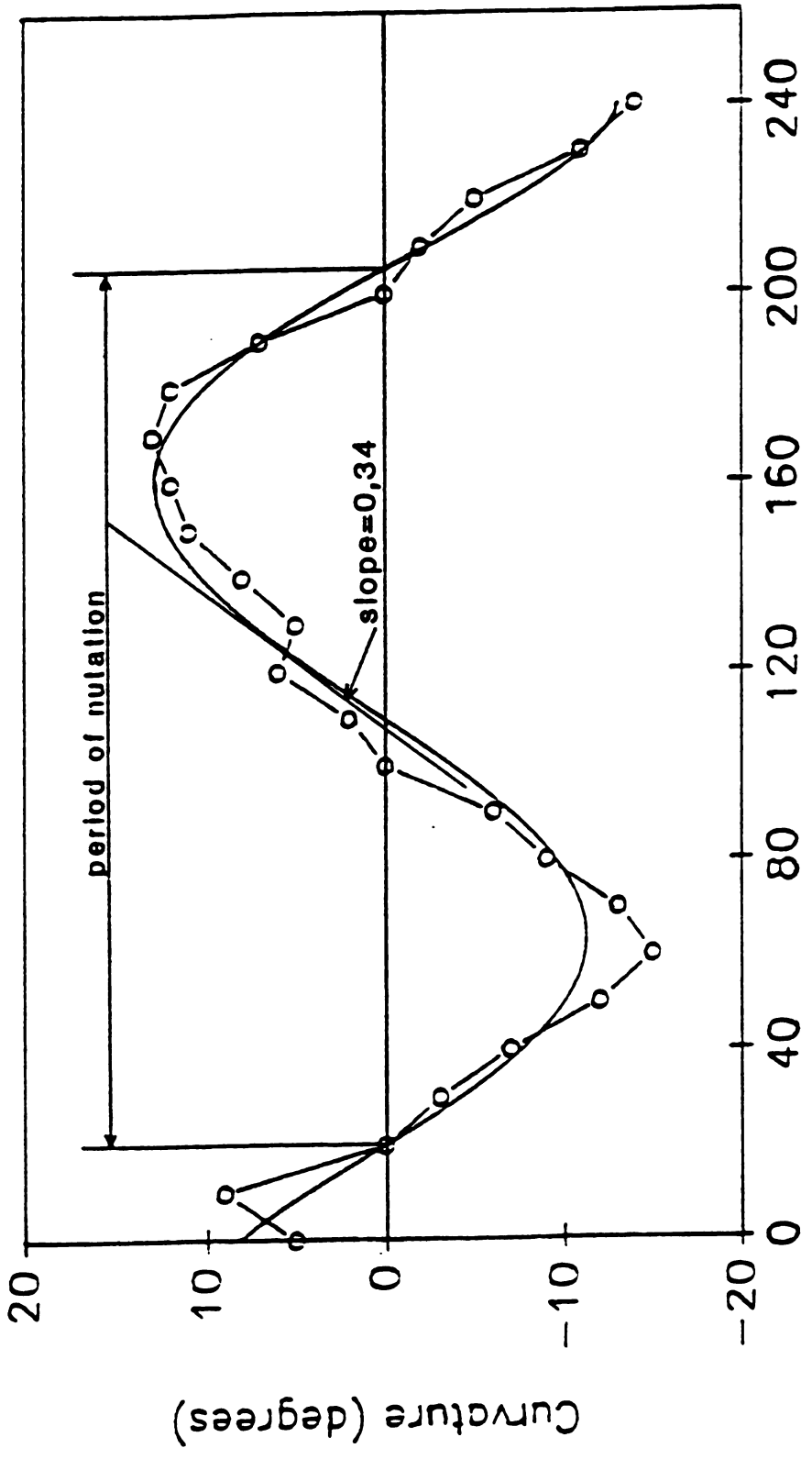

Figure 6.2. Nutation of individual seedling expressed as the change of curvature of the hypocotyl in one plane. Observed with the camera from the side.

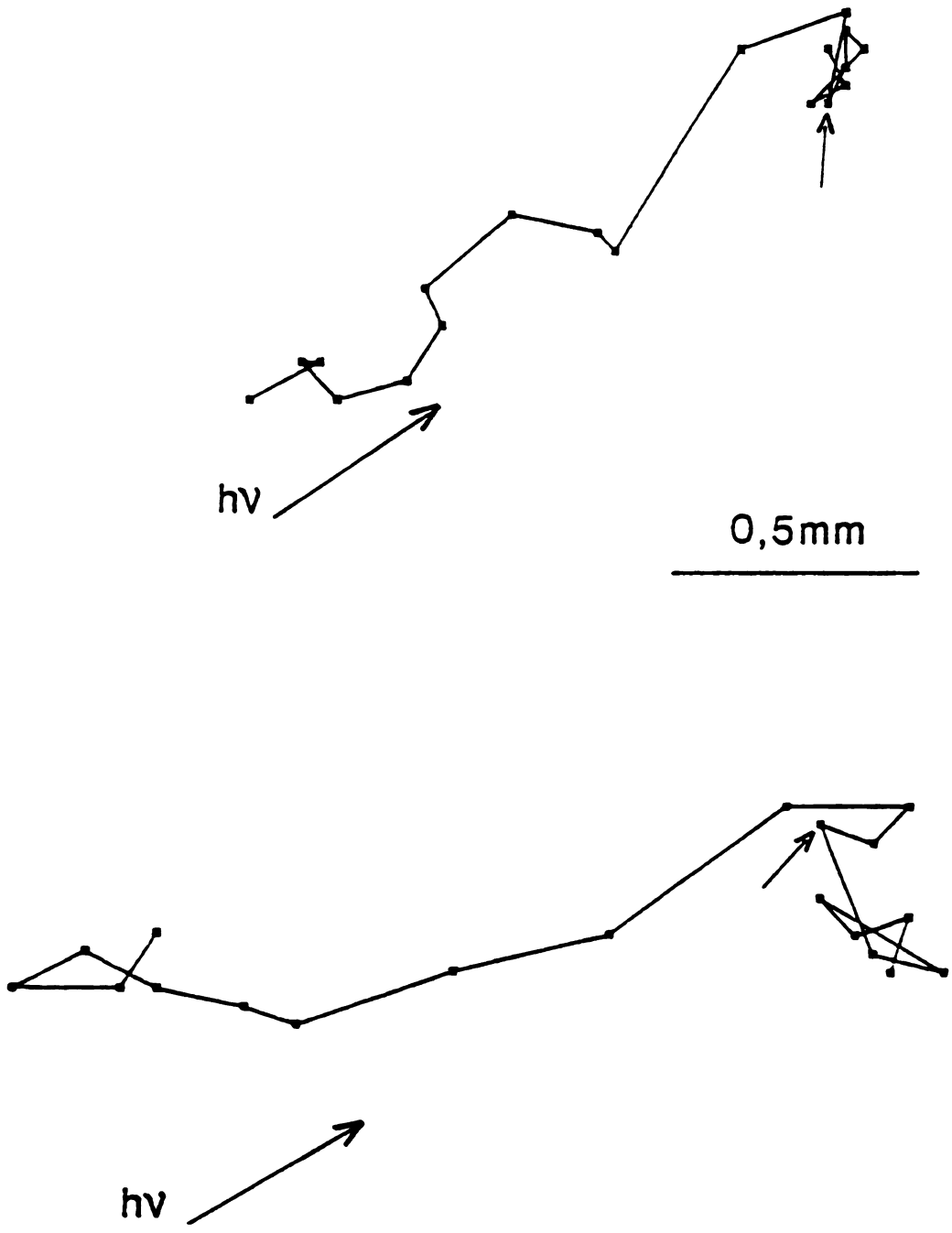

Figure 5.3. Pattern of nutation of two etiolated seedlings given a BL pulse at 60 min time point. Long arrows designate the direction of photostimulation and short arrows designate the position of the seedling at the time the BL was administered.

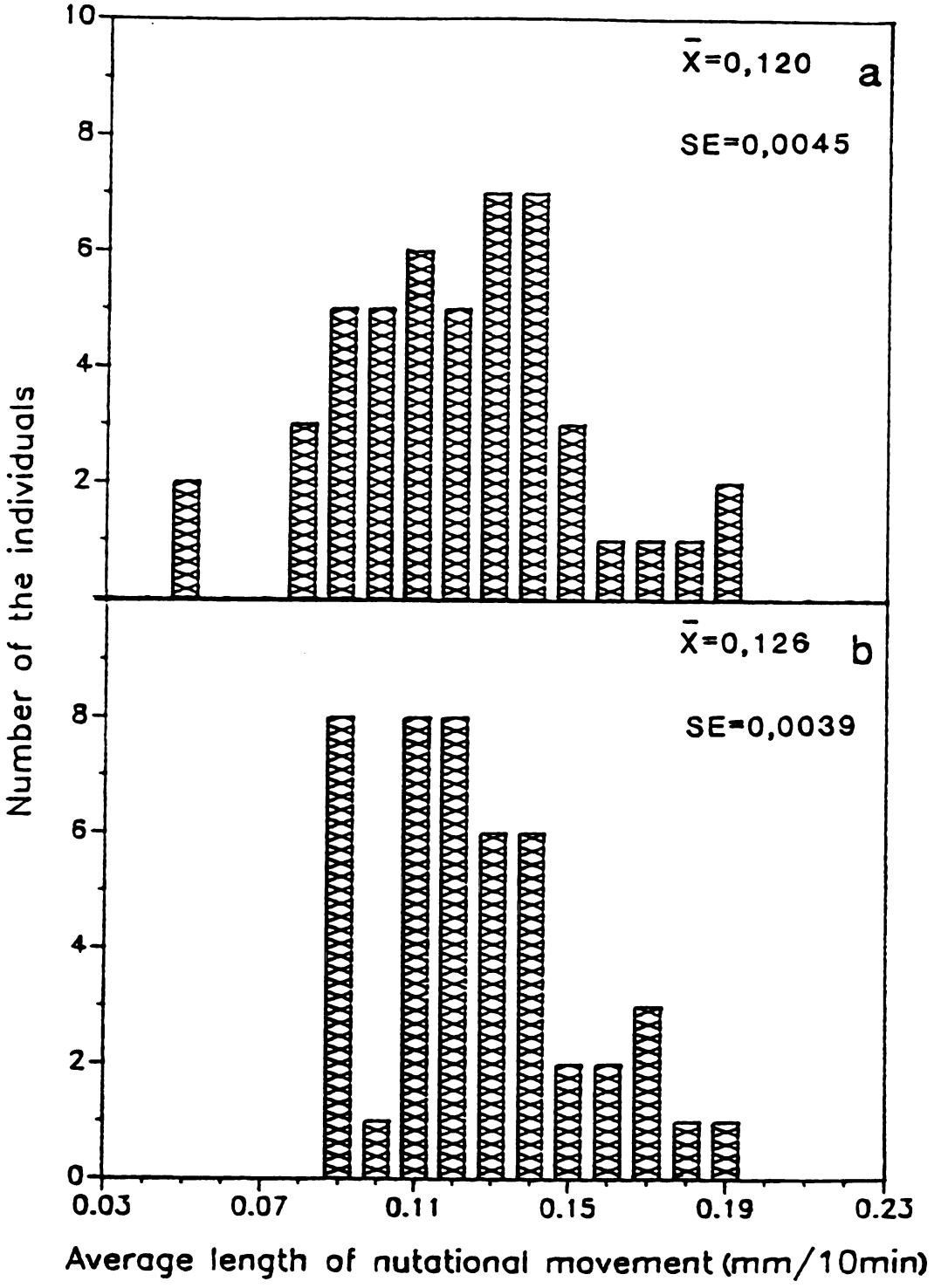

Figure 5.4. Histogram distribution of average lengths of nutational movement per 10 min; a-etiolated seedlings, n=48; b-RL pre-irradiated seedlings, n=46.

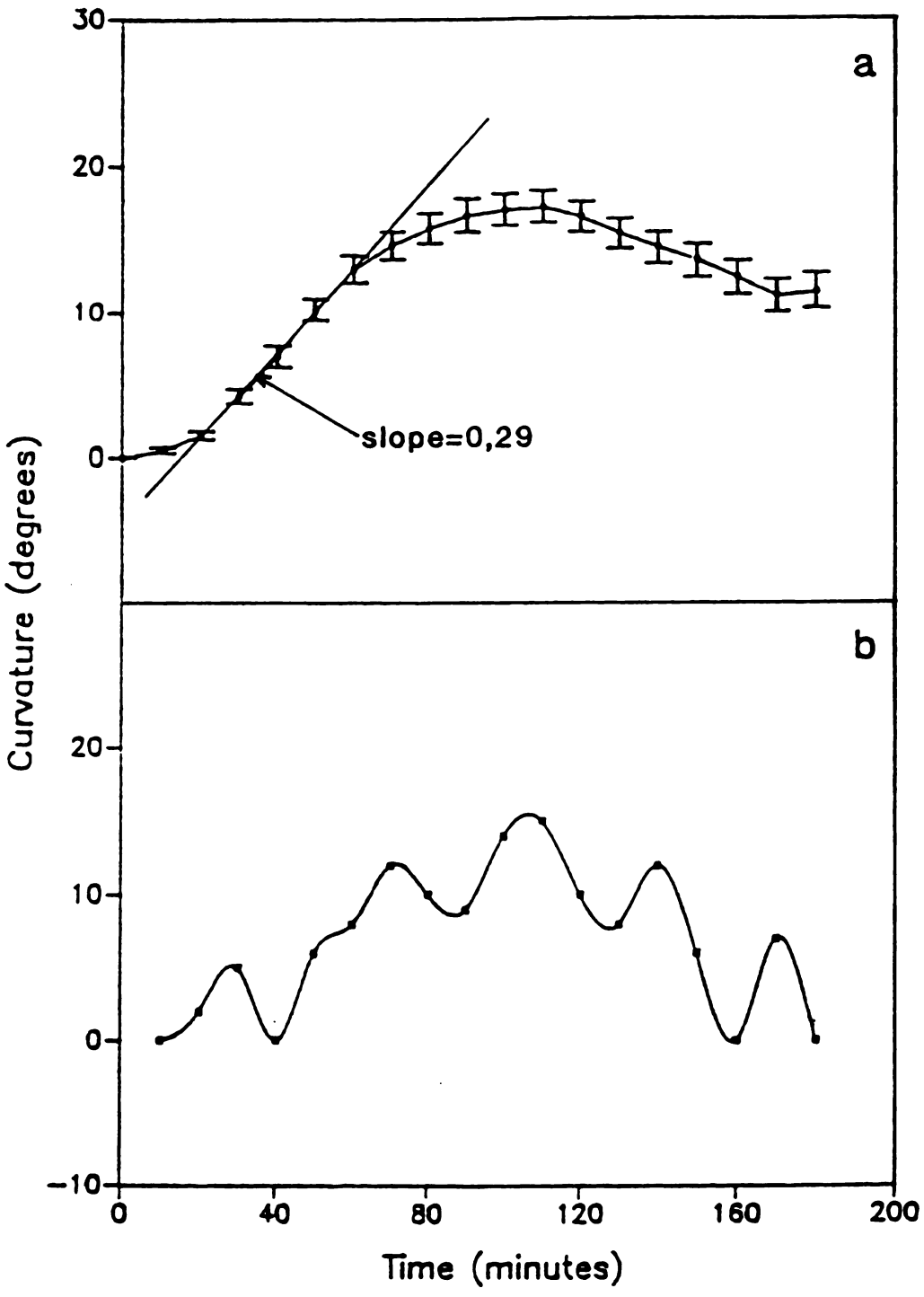

Figure 5.5. Time-course for the development of gravitropic curvature to constant 90 degrees stimulation in etiolated seedlings ; a-population, n=62, vertical bars represent ±1 SE; b- individual seedling.

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CHAPTER VI

Summary and conclusions

Seedlings of *Arabidopsis thaliana* exhibit various types of unidirectional and multidirectional movements. In response to short- or long-term unilateral BL irradiation, *Arabidopsis* seedlings exhibit phototropism toward the light source. Short-term irradiation with unilateral BL results in first positive phototropism while long-term irradiation results in second positive phototropism. The types of phototropism tested in this study, first and second positive, RL enhanced first positive and first positive phototropism of seedlings rotated on a clinostat are all transient but have different magnitudes. Irradiation of seedlings for one hour with RL prior to the BL pulse decreases the lag phase and increases the magnitude of first positive phototropism. However, neither RL pre-irradiation nor rotating the seedlings on the clinostat affects the process of straightening which takes place after the seedlings reach their maximum curvature.

Phototropism is the result of unequal elongation rates on opposite sides of the seedling. Regardless of the duration of BL photostimulation, the previous history of seedlings (-RL or +RL) or temperature conditions, the shaded side of the seedlings grew faster while the lighted side of the seedlings grew slower producing phototropic curvature. The kinetics with which elongation rate on opposite sides of a seedling change are similar during first and second positive phototropism, following BL photostimulation. However, the elongation rate of the shaded side of seedlings increases earlier than the elongation rate of lighted side starts decreasing. This trend of changes of elongation rates continues for some time but is subsequently reversed so that the shaded side start growing slower and

the lighted side faster. As the elongation rate of lighted side becomes equal to and then exceeds the elongation rate of shaded side, seedlings stop bending toward the light source and start straightening.

When seedlings are pre-irradiated with RL enhancing the first positive phototropism, the initial phase of the growth distribution is different from the distribution in etiolated seedlings. The elongation rates on the two sides of RL treated seedlings are changing simultaneously and earlier than in the etiolated seedlings. In contrast, during first positive phototropism at 15 °C, the change in elongation rates on two sides of the seedling is simultaneous but 20-30 min later than in seedlings at 25°C.

In the absence of any stimulation, etiolated *Arabidopsis* seedlings do not exhibit unidirectional movements and grow at the rate of about 50 $\mu m.10min^{-1}$. Neither RL pre-irradiation for one hour or low fluence of BL (0.3 $\mu mol.m^{-2}$) from above affect the growth rate of seedlings at 25 °C. However, transfer of seedlings from 25 °C to both, lower and higher temperature resulted in decrease of growth rate.

Non-stimulated, etiolated seedlings of *Arabidopsis* exhibit nutational movements. About 10% of the etiolated seedlings circumnutated, while after one hour of RL irradiation, no seedlings exhibited circumnutation. Nutation of *Arabidopsis* seedlings appeared to be independent from gravitropism and phototropism.

In spite of the small size of *Arabidopsis thaliana* seedlings, growth and

movement of this plant can be studied productively. These seedlings exhibit a variety of movements and a uniform growth which are relatively easy to observe and quantitate. Independence of the different type of movements points toward complexity of control of plant growth. These movements may be advantageous for successful survival in particular environment. Taking the pieces of this network of transduction chains apart and trying to explain them on cellular and molecular level is the future task.

Mutants that are altered in their phototropism but have normal gravitropism have already been isolated and characterized in *Arabidopsis* (Khurana and Poff, 1989). Existence of separate transduction pathways has been suggested for BL-induced inhibition of hypocotyl elongation and phototropism on the basis of studies with mutants altered in only one of these two BL responses (Liscum *et al.*, 1992). Based on the results reported herein, it should be possible to isolate mutants that lack the ability to exhibit nutational movements but still could respond to light and gravity stimulation. Additional efforts should be made to isolate more mutants with alterations at different points in the transduction pathway for movements in *Arabidopsis*.

Once mutants are available, their mutations could be mapped within the genome on the basis of cosegregation of the trait of interest with available genetic markers. Succesful mapping of mutations would facilitate cloning of the genes that code for proteins involved in signal transduction pathways.

However, gene mapping may be difficult for traits that exhibit wide variation

within the population, as is the case for phototropism. For this reason, mutagenesis should be employed such that individual seedlings could be tested for the presence of a mutation. Gene interuption as the method of mutagenesis could be accomplished by T-DNA insertion into the plant genome and gene disruption or deletion as a consequence of high-energy irradiation (Straus and Ausebel, 1990; Feldman, 1991). Efforts are under way, some already succesful, to clone genes from mutants generated with these alternative mutagenesis methods (Sun *et al.*, 1992; Lisitsyn *et al.*, 1993; Rosen and Poff, unpublished).

Some genes that could be involved in growth control, such as Small Auxin Up Regulated (SAUR) genes, have already been cloned (McClure and Guilfoyle, 1987). These clones could be used for histological investigation of a possible role for auxins in any of the movements exhibited by *Arabidopsis* seedlings. Results from experiments with the SAUR genes should provide better insight into the terminal portions of signal transduction pathways for phototropism, gravitropism and nutation in *Arabidopsis*.

Cloning genes that are involved in control of movements in seedlings would allow for the study of their spatial and temporal expression during the straight and directional growth of *Arabidopsis* seedlings. Results from these experiments would offer a better understanding of the complex network of signal transduction pathways mediating different type of movements in *Arabidopsis* seedlings at the cellular and molecular level. It is my hope that the results obtained in this study will provide the basis for this future work.

APPENDICES

APPENDIX A

A role for carotenoids in phototropism of *Arabidopsis thaliana* seedlings

ABSTRACT

A carotenoid deficient mutant of *Arabidopsis thaliana* (Am 45-3) was used to investigate the role of carotenoids in phototropism and adaptation. The mutant seedlings appeared pale and contained about 2.5-3% of the amount of carotenoids present in the WT when grown in light. Phototropism of pale seedlings in response to unilateral BL pulse was similar to that of WT seedlings except that the amplitude of the response was lower. Pale seedlings retained their ability to undergo desensitization by BL irradiation as a part of adaptation. These seedlings also exhibited RL-induced enhancement of phototropism. Enhancement appeared to be associated with an increase of carotenoid content. These data are consistent with the conclusion that carotenoids are not the photoreceptor pigments for phototropism or desensitization, although the presence of carotenoids affects the amplitude of phototropism and mechanism for enhancement in *A. thaliana*.

INTRODUCTION

Blue light induces many physiological responses including phototropism in plants (Kaufman, 1993). The identity of the photoreceptor pigment for phototropism has not yet been elucidated and it was recently suggested that multiple photoreceptor pigments may mediate this process in *Phycomyces* and *Arabidopsis thaliana* (Galland and Lipson, 1987; Konjević *et al.*, 1989). In addition, the existence of a photoreceptor pigment was postulated that could control desensitization of *Arabidopsis* seedlings by BL to a subsequent unilateral photostimulation (Poff *et al.*, 1993)

Because of similarity of absorption spectra of carotenoids and the action spectrum for phototropism, carotenoid was suggested to be the photoreceptor pigment mediating this process (Curry, 1969). However, the absorption spectrum of β-carotene lacks the peak at about 370 nm present in the action spectrum for phototropism (Curry, 1969). Moreover, a mutant of *Phycomyces* that has less than $1x10^{-5}$ of the WT carotenoid content still displayed normal sensitivity to phototropic stimulation (Presti *et al.*, 1977). In addition, corn plants treated with a carotenoid synthesis inhibitor exhibit similar first and second positive phototropism to the response of untreated plants, the only difference being the amplitude of the response (Vierstra and Poff, 1981; Piening and Poff, 1988). These lines of evidence argued against carotenoids as the chromophores of the photoreceptor for the BL in phototropism. A flavoprotein is now thought to be a photoreceptor pigment for

phototropism as well as for other BL mediated processes (Song, 1984).

Adaptation in phototropism has been described in maize (Iino, 1988), *Phycomyces* (Galland, 1991) and *A. thaliana* (Janoudi and Poff, 1991). Irradiation of plants by light induces a change in their sensitivity and/or responsiveness to a subsequent irradiation (Galland, 1991; Janoudi and Poff, 1991). In maize, both RL and BL are capable of inducing desensitization in phototropism (Iino, 1988). In *Arabidopsis*, only BL can desensitize seedlings to a subsequent BL pulse (Janoudi and Poff, 1991). No attempt has yet been made to characterize the pigment responsible for desensitization in *Arabidopsis* seedlings.

A mutant strain of *A. thaliana* that has a pale phenotype was used to examine its carotenoid content and test for possible effects of decreased carotenoid levels on responses to BL. A mutant with a lower carotenoid content than the WT should be a good tool to test for carotenoid function in the BL absorption for phototropism or adaptation.

MATERIALS AND METHODS

Mutant population

A mixed population, Am 45-3, consisted of about 1/6 of seedlings that appeared pale (Fig. 1. b) and 5/6 that were normally pigmented (Fig. 1. a). This fits the expected distribution if the pale seedling is a homozygous recessive which is incapable of maturing and setting seed (Chi-square test on the expected ratio of

5 normally pigmented : 1 pale seedling has given probability value of 0.5). Two assumptions had to be made to allow comparison of observed ratio of two phenotypes in the Am 45-3 population with the predicted ratio 5:1. First, that the seeds obtained initially were progeny of a heterozygous plant and; second, that dominant homozygous and heterozygous plants have the same ability to set seeds. The high probability value obtained in the chi-square test is consistent with the hypothesis that Am 45-3 population probably consists of pale seedlings as homozygous recessive mutants, and normally pigmented seedlings representing a mixture of plants that are homozygous dominant and heterozygous for this genetic locus. Further in the text, the latter batch of seedlings is reffered to as normally pigmented, and carotenoid-deficient mutants as pale seedlings.

Growth conditions

Seedlings used in experiments for measurement of phototropism and gravitropism were grown as described previously (Khurana *et al.*, 1989) with one difference. Seedlings with the pale phenotype were grown for 4 h longer (43 h) than the normally pigmented seedlings (39 h) in order to bring them to approximately same size. The difference in pigmentation between the two phenotypes of etiolated seedlings is obvious and easy to score (Fig. 1. a and b). Pale plants that were light grown for 4 months also have a distinctive phenotype (Fig. 1. c). Although these plants in culture looked morphologically similar to WT plants, except being smaller in size, their stems always shriveled quickly after development of flowers which made it impossible to do any crossing experiment.

Light grown plants from figure 1. c are representative of those used as bulk tissue for HPLC analysis. These seedlings were grown on Murashige-Skoog 1X medium (Gibco BRL) supplemented with 3% (w/v) sucrose. Because the pale seedlings died under the light conditions in which the WT-Col seedlings were grown, the following procedure was used for their culture. Forty hours after they germinated in darkness, pale seedlings were selected from the mixed population according to the colour of their cotyledons and placed into plastic containers with nutrient medium. These containers were then covered with a green plexiglas (Rohm Gmbh Plexiglas gs, DIN 4102-B2) that had low transmittance throughout the visible part of the spectrum.

Light sources

White light that was used for growth of seedlings and to potentiate germination (60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was obtained from GE (Cleveland, OH) DeLux Cool-white fluorescent tubes. The BL source consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA) 300 W ELH tungsten halogen lamp and 450 nm interference filter with a half-band width of 10 nm (PTR Optics Waltham, MA). Red light (0.6 $\mu mol \cdot m^{-2} s^{-1}$) used for pre-irradiation of seedlings was obtained from one gold fluorescent tube (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp., Highland, IL). This source provides radiation from 560 to 720 nm with maximum output at 620 nm. The duration of actinic BL was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY). Fluence rates were measured with a Li-Cor (Lincoln, NE) LI-190 SA in combination with a LI 1000

Datalogger.

Measurement of curvature

Phototropic and gravitropic curvature of hypocotyls were measured as described by Khurana *et al.* (1989), although curvature was allowed to develop for 80 min in phototropism experiments.

High Performance Liquid Chromatography Analysis

HPLC analysis was performed as previously described (Rock, 1991), with the spectrophotometer set at 436 nm for detection of carotenoids. Identification of carotenoids was based on retention times of peaks on chromatograms. The area under the relevant peaks of absorbance on the chromatograms of the tested carotenoids was used as an indicator of their amounts. Quantification of individual carotenoids was done from the standard curves generated in the laboratory.

RESULTS

HPLC analysis

The content of 10 carotenoids was compared in light grown WT-Col and pale Am 45-3 seedlings. Chromatography revealed that the carotenoid content in the WT-Col plants was approximately 35-40 times higher than that in the pale mutant plants (Fig. 2. a and b). The amount of β-carotene was approximately 2.5 times higher in the WT-Col plants than in the pale plants when expressed as

percentage of total carotenoids while the amounts of lutein and antheraxanthin were lower when expressed on the same basis (Fig. 2. a and b).

Relative quantities of individual carotenoids as a percentage of total amount of 9 tested carotenoids in the RL-irradiated seedlings were not different from those in the etiolated seedlings of WT-Col (Table 1.) However, RL-irradiated WT-Col seedlings had approximately 40% more carotenoids than etiolated seedlings (Table 2.)

Fluence-response relationships

The fluence-response relationships for induction of first positive phototropism for the etiolated seedlings from Am 45-3 population with the normally pigmented phenotype and the WT-Col seedlings were similar (Fig. 3. a and b). Fluence requirements for initiation of phototropism as well as for maximum response and the descending arm of the curve corresponded very well in these two batches of seedlings (Fig 3. a and b). Based on these data, it was decided to further compare responses of pale seedlings to BL and responses of the normally pigmented seedlings to BL assuming that the normally pigmented seedlings have wild type ability to perceive BL. The fluence-response relationship for first positive phototropism to a BL pulse was also measured for pale seedlings (Fig. 3. c). The response of pale seedlings was lower than that of both WT-Col and normally pigmented mutant seedlings (Fig. 3. a, b and c).

Irradiation of seedlings with the RL for 1 hour resulted in a higher amplitude

of response to a subsequent BL pulse (Fig. 4.) This RL-induced enhancement of phototropism was used in all following experiments as the higher curvature was easier to score. The fluence-response curve describing first and second positive phototropism to the BL of the RL pre-irradiated seedlings was measured by varying the duration of pulses at constant fluence rate (Fig. 4.) For the normally pigmented mutant seedlings the threshold for first positive response was about 0.01 $\mu mol.m^{-2}$ and for second positive the threshold was about 100 $\mu mol.m^{-2}$ (Fig. 4. a). The region of the maximum for the first positive response shows two distinctive peaks followed by a descending arm (Fig. 4. a). The pale seedlings exhibited similar response to the unilateral BL pulses after the RL pre-irradiation (Fig. 4. b). The fluence requirements for initiation of the first and second positive responses as well as the fine structure for first positive phototropism corresponded well to those of the normally pigmented seedlings (Fig. 4. a). The major difference between the responses of these two batches of seedlings was the amplitude, with pale seedlings exhibiting a lower response than that of the normally pigmented seedlings (Fig. 4.)

The effect of a desensitizing irradiation pulse was examined for both phenotypes from Am 45-3 population (Fig. 5. a and b). Seedlings were first irradiated for 1 hour with RL, and then were given a BL pulse from above followed within 2.5 min by a unilateral BL pulse of 0.3 $\mu mol{\cdot}m^{-2}$ to induce phototropism. By varying the desensitizing BL from above, a fluence-response relationship for desensitization was measured. The fluence-response curves for desensitization in both phenotypes are similar (Fig. 5.) As the fluence of BL administered from above

increases, the response to the subsequent unilateral pulse decreases. Even the lowest fluence of BL applied from above (0.033 $\mu mol \cdot m^{-2}$), induced desensitization in both batches of seedlings. The highest fluence of BL applied from above (21 $\mu mol \cdot m^{-2}$) completely desensitized seedlings to the subsequent unilateral BL pulse (Fig. 5.) Pale seedlings in this type of experiment again exhibited a lower amplitude of response than the normally pigmented seedlings (Fig. 5.)

Gravitropism measurements

The ability of hypocotyls of pale seedlings to exhibit curvature was tested by exposing them to the constant 90 degrees gravity stimulation for 2 hours. Their response was not different from the response exerted by normally pigmented seedling (Table 3.) to the same stimulus, suggesting that the mechanism of differential growth in pale seedlings was not impaired.

DISCUSSION

The results of this study indicate that carotenoids do not play a major role in BL-induced phototropism and desensitization in *A. thaliana*. Although the level of 10 carotenoids in pale mutant seedlings was 35-40 times lower than in WT-Col plants they retained WT sensitivity to unilateral BL and their phototropic response was only about two times lower in amplitude (Figs. 2. and 4.)

The fluence requirements for the initiation of the first and second positive phototropic response as well as for induction of two peaks and descending arm in the range of first positive phototropism were similar in the pale seedlings and normally pigmented seedlings (Fig. 4. a and b). If any of the tested carotenoids were responsible for perception of unilateral BL that results in curvature, then some (or all) of the features of the fluence-response curve would be expected to move towards higher fluences according to the decrease in the amount of pigment(s).

Although carotenoids probably are not the photoreceptor pigments for phototropism in Arabidopsis they do affect the amplitude of the phototropic response (Figs. 2., 4. and 5.) In order to detect the direction of light stimulation and respond phototropically, the seedling must detect the difference in absorption of light on its lighted and shaded side. If carotenoids were a screening agent across the seedling then their absence or decreased presence would result in lack of, or decrease in the light gradient. Results describing a lower response to unilateral BL of the pale mutant seedlings when compared to the response of the normally pigmented seedlings (Fig. 4.) are consistent with such a role of carotenoids.

The data describing the difference in levels of carotenoids in etiolated and RL-irradiated seedlings of WT-Col seedlings also support the theory that carotenoids may play a role as screening agents. One of the possible ways of enhancing the phototropism could be by increasing the levels of carotenoids in the tissue, resulting in the steeper light gradient across the seedling. Higher levels of carotenoids are detected in RL pre-irradiated WT-Col seedlings than in etiolated

WT-Col seedlings (Table 2.) These results can be correlated with larger phototropic response of RL pre-irradiated normally pigmented mutant seedlings when compared to the etiolated WT-Col seedlings (Figs. 3. a and 4. a). Although these two strains differ genetically, this difference has not affected their first positive phototropic response (Fig. 3. a and b). Moreover, seedlings of the Estland ecotype showed the same difference in magnitude of phototropism with and without RL pre-irradiation (Janoudi and Poff, 1991). Enhancement of phototropism by RL may be due to the increased carotenoid content of the tissue (Figs. 3.a and 4.a).

However, there is a discrepancy between ratios of carotenoid levels in two different phenotypes and amplitudes of their responses to unilateral BL pulses. The explanation for this disagreement in the case of decrease of carotenoid content may be that carotenoids are not the only compounds that absorb the light from the blue part of the spectrum. When levels of carotenoids are very low, backscatter of light or the other molecular species such as pterins might prevent the disappearance of light gradient across the seedling which would result in complete absence of the response. Another explanation could be that the ratio of carotenoids in light grown pale vs. normally pigmented plants is not a good indicator of the ratio in etiolated tissue.

Therefore, the light gradient across the seedling established due to a presence of some molecular species that acts as a screening agent, will not necessarily be proportional to the magnitude of phototropism.

Pale mutant seedlings also retained the ability to undergo desensitization.

Adaptation in phototropism of *Arabidopsis* seedlings includes: desensitization, refractory period, recovery and enhancement (Janoudi and Poff, 1991). The complex shape of fluence-response curve for phototropism has been proposed to be due to adaptation (Poff *et al.*,1993). Fluence requirements for desensitization overlap with fluences that induce enhancement. Thus plant is responding to the BL as a stimulus that induces and enhances phototropism and simultaneously causes desensitization. Therefore, response of a plant to a phototropic stimulation is a function of all components in both induction and adaptation of phototropism. If carotenoids were responsible for the perception of light that induces desensitization, the pale seedlings would exhibit a threshold for desensitization changed corresponding to the changed amount of carotenoids. Since similar fluence requirements for desensitization were exhibited by the pale and normally pigmented seedlings (Fig. 5.), carotenoids probably are not responsible for mediation of this process.

The quantities of individual carotenoids as a percentage of total amount of carotenoids have not changed due to 1 hour of RL (Table 1.) These results indicate that the possible action of carotenoids in enhancement of phototropism is not mediated by single molecular species which would require the increased amount of that pigment.

Some carotenoids are known to be synthesized in plant organs in the absence of light (Britton, 1988). However, light is known to induce a large increase in carotenogenesis (Britton, 1988). For example, Oelmuller and Mohr have reported

an increase in β-carotene in milo seedlings grown for 72 hours under RL as opposed to the plants grown in darkness (Oelmuller and Mohr, 1985). We have measured the difference in carotenoid content between pale seedlings and WT-Col seedlings in light grown tissue and related this difference to the magnitude of phototropism of etiolated seedling. The assumption is that the inductive effect of white light on carotenogenesis is equal in both tested phenotypes. This is supported by the fact that the pale seedlings retain their sensitivity to BL (Fig. 4.) and responsiveness to RL (Figs. 3. c and 4. b).

In summary, we report that seedlings with the pale phenotype from the population of mutant Am 45-3 have 35-40 times lower level of 10 tested carotenoids and exhibit two times lower phototropism than the normally pigmented seedlings from the same population. Pale seedlings retained their ability to: undergo desensitization by BL, exhibit RL-mediated enhancement of phototropism and respond gravitropically in the WT fashion. Red-light-mediated enhancement of phototropism may be in part due to the increase of carotenoid content in the tissue. On the basis of these data it is concluded that carotenoids are not photoreceptor pigments for phototropism or desensitization in *A. thaliana* although they appear to affect the amplitude of the phototropic response.

Table 1. The quantity of individual carotenoid as percentage of total amount of nine tested carotenoids in etiolated (-RL) and red light- irradiated seedlings (+RL) of WT-Col. Values in the table are means ±1 SE; n=2.

	-RL (%)	+RL
β-carotene	4.1 ± 1.9	4.7 ± 0.5
lutein	42.7 ± 0.4	42.1 ± 1.5
zeaxanthin	0.6 ± 0.0	1.2 ± 0.6
antheraxanthin	3.2 ± 0.5	3.2 ± 0.1
lutein epoxide	7.7 ± 0.9	8.0 ± 0.2
all-trans violaxanthin	29.4 ± 0.5	28.5 ± 0.1
9 cis-violaxanthin	3.6 ± 0.5	3.5 ± 0.5
13 cis-violaxanthin	3.6 ± 0.8	3.6 ± 0.8
9 cis-neoxanthin	5.3 ± 0.1	5.1 ± 0.0

Table 2. The quantity of individual carotenoids in etiolated (-RL) and red light-irradiated (+RL) seedlings of WT-Col. Values in the table are means ±1 SE; n=2.

	-RL ($\mu g.gFW^{-1}$)	+RL
β-carotene	0.076 ± 0.011	0.097 ± 0.046
lutein	1.367 ± 0.008	2.014 ± 0.062
antheraxanthin	0.144 ± 0.008	0.205 ± 0.026
all-trans violaxanthin	1.707 ± 0.045	2.590 ± 0.047
9-cis violaxanthin	0.072 ± 0.013	0.106 ± 0.012
9-cis neoxanthin	0.287 ± 0.018	0.412 ± 0.002

Table 3. Gravitropic response to constant 90 degrees stimulation for 2 hours by two mutant, RL pre-irradiated phenotypes. Values in the table are means $\pm$1 SE; n>60.

	degrees of curvature
normally pigmented phenotype	12.8 $\pm$ 0.8
pale phenotype	12.6 $\pm$ 1.2

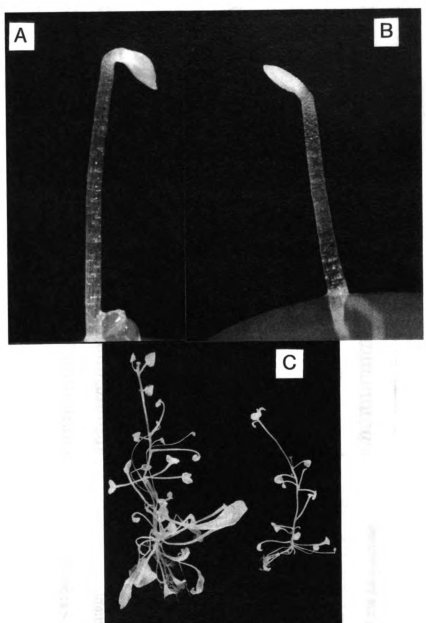

Figure 1. Photographs of etiolated: normally pigmented-(a) and pale-(b) seedling 39 h and 43 h old, respectively, and 4 month old light grown pale plants-(c).

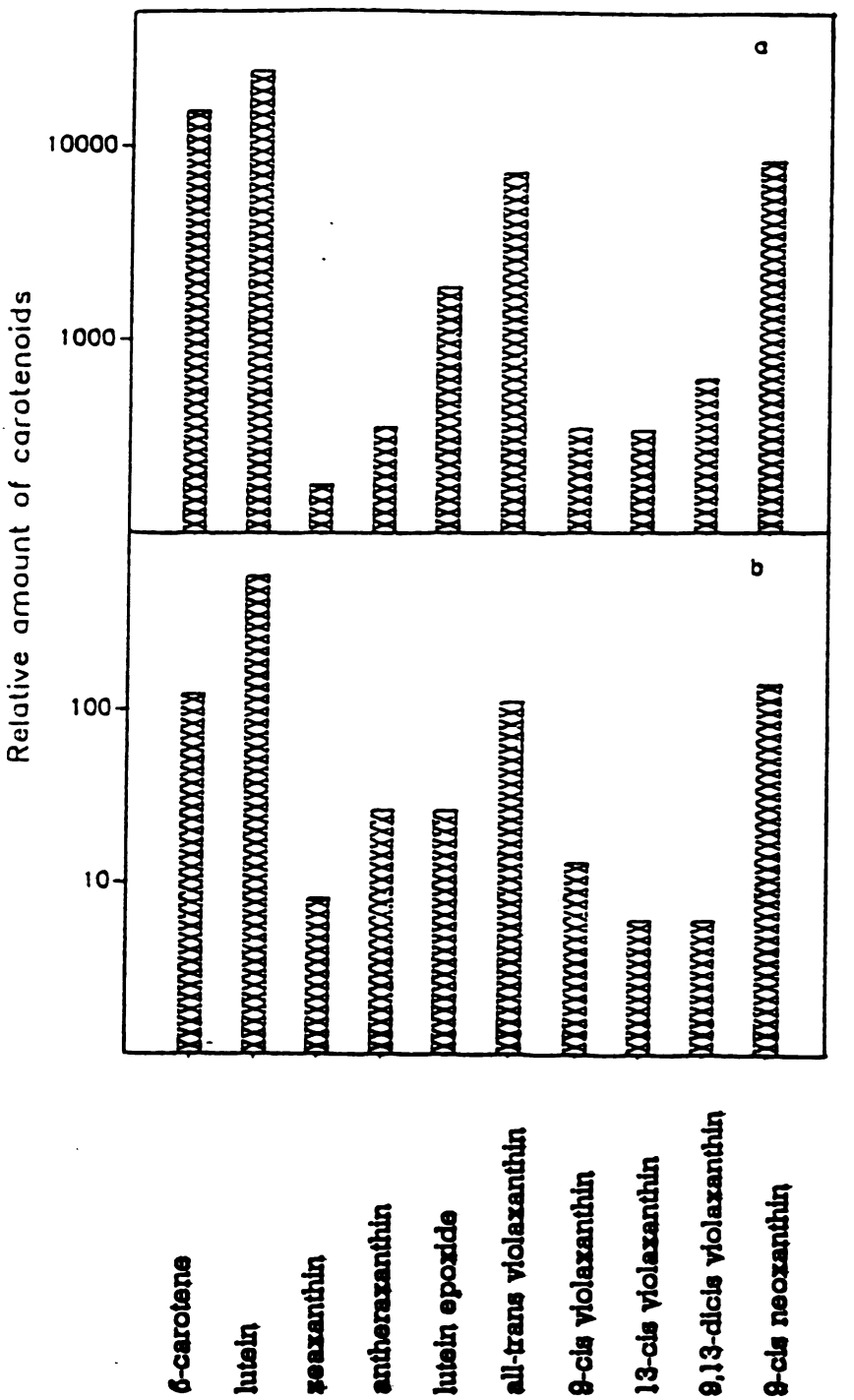

Figure 2. The relative amounts of 10 tested carotenoids in light grown WT-Col-(a) and pale-(b) seedlings.

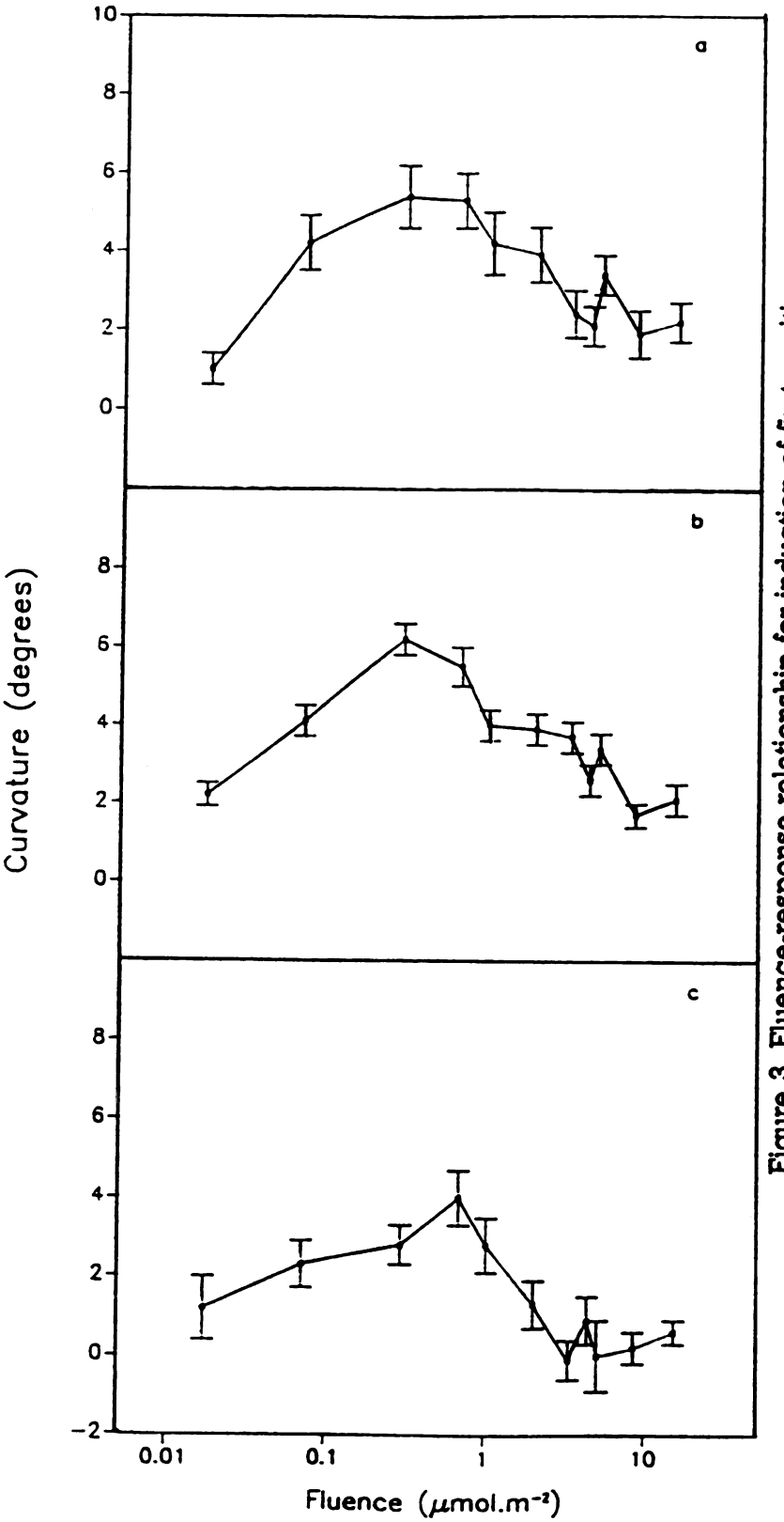

Figure 3. Fluence-response relationship for induction of first positive phototropism of etiolated WT-Col-(a), normally pigmented-(b) and pale- (c) seedlings; n>60; vertical bars represent ±1 SE.

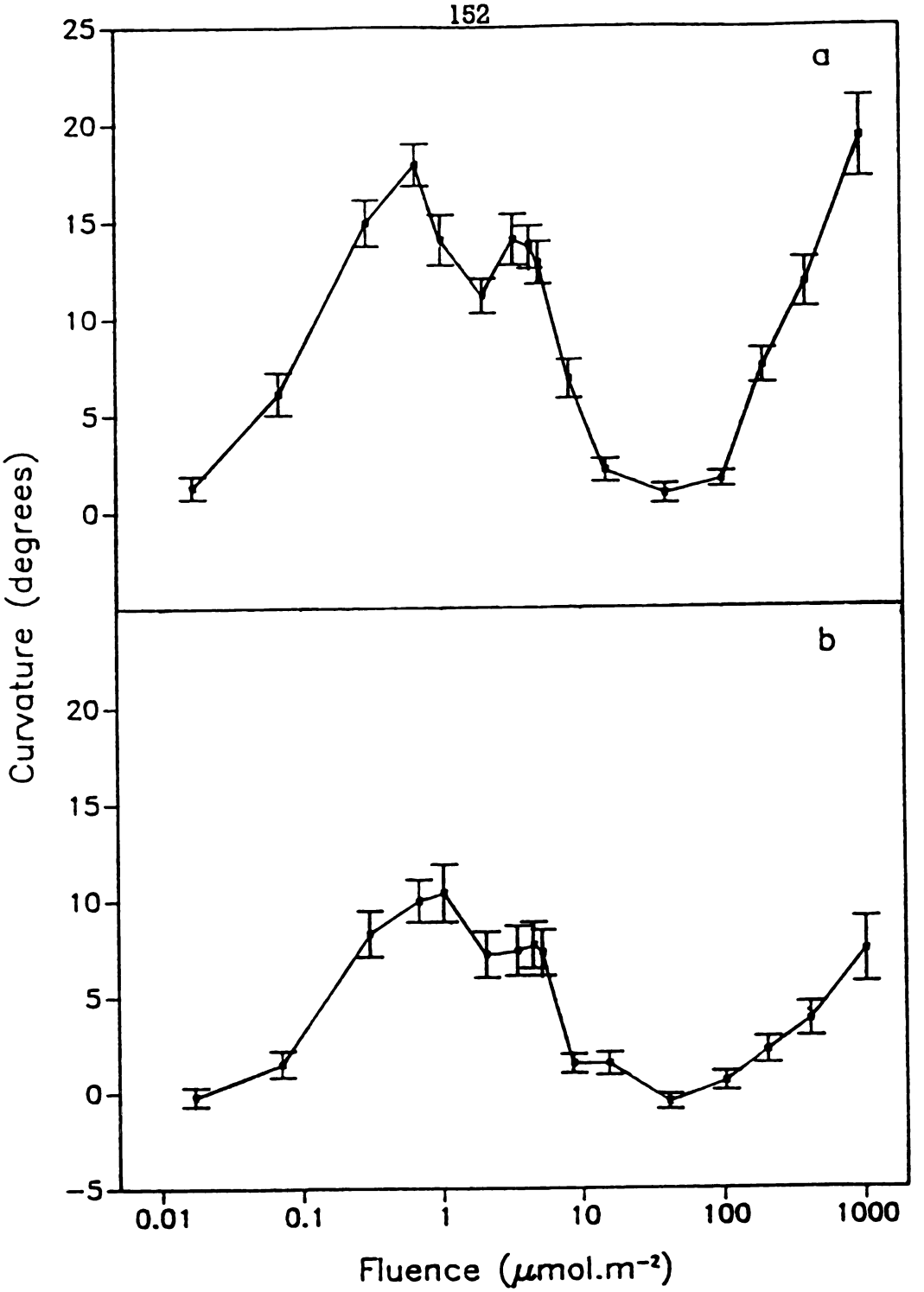

Figure 4. Fluence-response relationship for induction of phototropism of RL pre-irradiated: normally pigmented-(a) and pale-(b) seedlings; n>60; vertical bars represent ±1 SE.

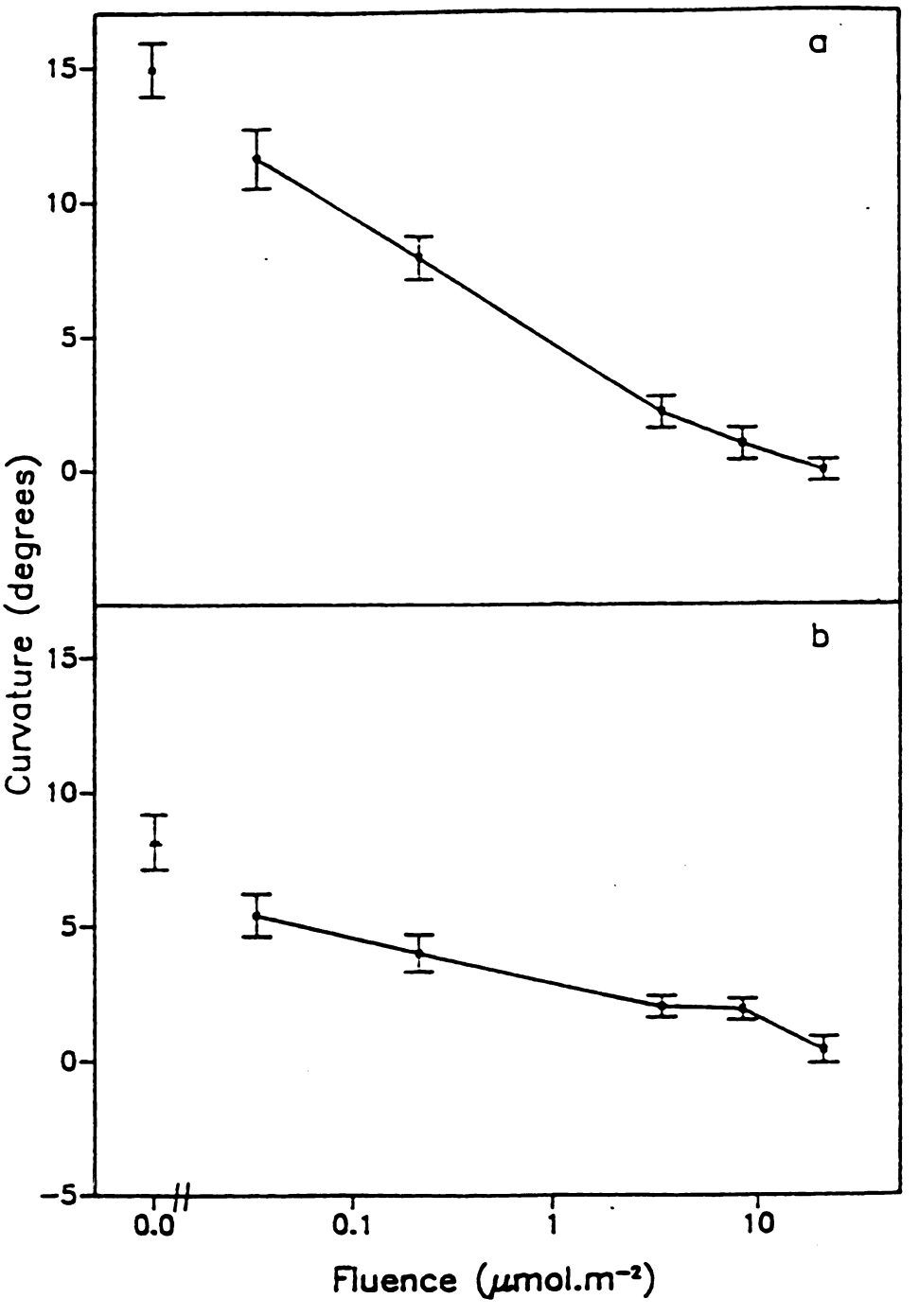

Figure 5. Fluence-response relationship for induction of phototropism following the BL pulse from above; normally pigmented-(a) and pale-(b) seedlings; n>60; vertical bars represent ±1 SE.

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