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Mechanisms of Establishing a Light Gradient for First Positive Phototropism in Zea mays (L.)

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Carol Joyce Piening

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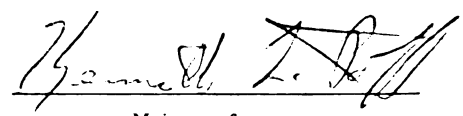

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MECHANISMS OF ESTABLISHING A LIGHT GRADIENT FOR FIRST POSITIVE PHOTOTROPISM IN *ZEA MAYS* (L.)

By

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ABSTRACT

MECHANISMS OF ESTABLISHING A LIGHT GRADIENT FOR FIRST POSITIVE PHOTOTROPISM IN ZEA MAYS (L.)

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Carol Joyce Piening

Pigment distribution in individual seedlings of SAN 9789 treated, control, and four "carotenoidless" mutant lines was mapped in coleoptilar, shoot, mesocotyl, and root tissue. Total attenuation of light, as well as absorption, was measured spectrophotometrically. Longitudinal transmission of three wavelengths (632, 514, and 450 nm) of light was quantified.

Mechanisms of establishing a light gradient to detect a dose of light in the first positive range were evaluated. It is concluded that first and second positive phototropism share a number of characteristics. Carotenoids play the role of screening pigments for both responses. Attenuation by absorption and scatter sets up the gradient of light necessary for both first and second positive phototropic responses.

To Ruth Piening and Isabelle Lynch

I could while away the hours
Conferrin' with the flowers
Consultin' with the rain...
E.Y. Harburg

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REVIEW OF LITERATURE

Early experiments

Phototropism is a mystery on any level beyond a descriptive one. It is well established that a plant responds to a unilateral dose of light by bending toward or away from the light source. However, the mechanisms for detecting light direction, for transducing the stimulus into differential growth, and the primary photoreceptor all remain unknown.

Charles Darwin is often credited with the first methodical observations of phototropism. Power of Movement in Plants, published in 1881, first reported the importance of the tip to the phototropic response of a coleoptile. Darwin was also first to observe the transmission of a physiological effect of light, noting that the response to a unilateral stimulus appeared at the tip and migrated to the base of the coleoptile. Darwin was convinced that phototropism was simply a modified form of circumnutation, light causing the plant to "modify its movement for a time in a beneficial manner."

Another early explanation of the mechanism of phototropism is the "light growth hypothesis", which, in its

simplest form, states that light itself is the stimulus which works on the plant and that the light gradient across a unilaterally illuminated shoot could explain its differential growth (Blaauw, A.H.,1914). Blaauw's work shows that in the case of Helianthus globosus, an increase in dose results in a decrease in growth on the more brightly lighted side of the plant. Blaauw's light growth hypothesis has since been qualified to state that it may be important only in second positive phototropism (Firn and Digby, 1980).

The Cholodony-Went hypothesis states that tropic curvatures, whether they are in response to light or to gravity, are mediated by the establishment of lateral auxin gradients across the stimulated organ. Went and Thimann (1937) noted that the discovery and subsequent study of auxins was a result of work on phototropism.

DuBuy and Nuernbergk (1934) were first to summarize the body of data which show that plants respond differently to different doses of light. At low doses of high intensity and short duration, plants show positive (first positive, system I, or type a) curvature; at higher doses, a negative (first negative, system II, or type b) curvature, and at still higher doses, (low intensity and long duration) another positive (second positive, system III, or type c) response.

The influence of the coleoptile tip on growth, the asymmetric distribution of auxin in a unilaterally stimulated coleoptile, the shape of the dose-response curve,

and the action spectrum showing maximum phototropic response to blue light outline the basic studies on the phenomenon of phototropism. In order to understand phototropism, the mechanism of detecting a light gradient, the photoreceptor pigment and the transduction pathway must all be known. This thesis examines the first step in phototropism, that is, the mechanism by which a unilateral light stimulus is detected.

Action spectra

Action spectra have been used in many systems to help identify photoreceptor pigments. An action spectrum is generated by irradiating a sample with light of different wavelengths and measuring the relative response to each wavelength. Assuming that only light which is absorbed can cause a reaction, the wavelengths of light which are absorbed most efficiently will elicit the greatest response. Within certain limits, the action spectrum should parallel the absorption spectrum of the photoreceptor pigment.

Shropshire (1972) discussed the limitations of action spectra as a tool for identifying photoreceptor pigments. An example of a problem in the case of the blue light photoreceptor is presented by screening pigments. A high concentration of "foreign" pigment molecules can alter the response of the plant by absorbing actinic light before it reaches the photoreceptor. Such screening pigments change the action spectrum, and make parallels to the absorption spectrum less obvious.

There are two families of pigments, flavins and carotenoids, which are the most likely candidates as photoreceptors for phototropism. Both absorb light maximally in the 400 - 500 nm range. Carotenoids extracted in water show a triple peak between 400 - 500 nm like that seen in action spectra. Riboflavin in water does not show this fine structure, but when extracted in ethanol and kept at $77^{o}K$, the triple peak appears (Sun et al., 1972, cited in

Dennison, 1979). Both pigment families absorb in the 280 - 300 nm region. Flavins, but not carotenoids, absorb in the 340 - 400 nm region, showing a peak similar to that which appears in the action spectra of Phycomyces and monocots.

However, an identification of the blue light photoreceptor cannot be made based solely on a correlation between action and absorption spectra. Action spectra can be affected by factors other than screening pigments, including the concentration and distribution of the photoreceptor and the quantum efficiency with which the light is absorbed. In addition, the absorption spectrum of a given pigment can be altered by a number of other factors. In an extract, solvent and temperature can shift absorption peaks and change peak heights. Location within a cell or association with a cell component or with another molecule contribute to the absorption spectrum.

Action spectra are based on dose response curves and are valid only over a limited dosage range. To generate dose-response curves, it is necessary to assume that reciprocity holds; that response depends only on the total dose of light given, and is independent of the duration or intensity of irradiation. For phototropism by plant seedlings, this is true for only a limited range of doses, the region known as first positive phototropism. For first negative and second positive responses, the duration of stimulus becomes increasingly important (Blaauw and Blaauw-Jansen, 1970a). Approximate action spectra for

systems where reciprocity does not hold have been made, but must be interpreted cautiously.

Other approaches have been used to distinguish between flavins and carotenoids as photoreceptors for phototropism. Presti et al. (1977) found evidence against carotenoids as the photoreceptor when working with carotenoidless mutants of Phycomyces. The strain, blocked at each of six steps of biosynthesis between phytoene and β-carotene, had less than 4×10^{-5} of the wild-type carotene concentration, yet had a normal phototropic response.

Vierstra and Poff (1981b) treated corn seedlings with SAN 9789, an inhibitor of carotenoid biosynthesis. They found that seedlings lacking 98% of their carotenoids showed about 60% of their normal second positive phototropic response. Vierstra and Poff (1981b) concluded that the "bulk" carotenoids eliminated by SAN 9789 acted as screening pigments and increased the light gradient for second positive phototropism, rather than acting as photoreceptor pigments. However, they could not rule out a carotenoid photoreceptor pigment entirely. Similar experiments with flavinless mutants or tissue treated with inhibitors of flavin synthesis cannot be performed, since the absence of flavins would be expected to be lethal.

Several experiments using inhibitors of action rather than of synthesis of pigments have been performed. Schmidt et al. (1977) surveyed several compounds to find a specific inhibitor of phototropism. They found that chloride (KCl)

inhibits neither phototropism nor geotropism, while cyanide (KCN) inhibits both. Iodide (KI), azide (NaN_3), and phenylacetic acid (PAA) inhibit phototropism specifically. The authors suggested that iodide, azide, and PAA inhibited a flavin photoreceptor directly, but did not rule out the possibility that metabolic processes may have been inhibited.

Vierstra and Poff (1981a) showed that concentrations of PAA necessary to cause specific inhibition of phototropism are lower than those which affect geotropism. Strong auxins such as IAA inhibited photo- and geo- tropism equally. PAA's inhibitory effect is probably at the photoreceptor pigment, rather than as a result of its auxin-like activity.

Vierstra et al. (1981) found that xenon specifically quenched the triplet excited state of flavin molecules, but did not inhibit phototropism. KI is an effective inhibitor, but quenches both triplet and singlet excited states of flavin molecules. Azide and PAA, both specific inhibitors, quench only the singlet state. Thus, if flavin is the photoreceptor pigment, the triplet excited state is probably not the state through which the photochemistry of phototropism proceeds. Bridges and Wilkins (1971) looked at another series of compounds including mannitol, carbowax 1540 and KCl. All were effective in inhibiting phototropism and geotropism, contrary to the results of Schmidt et al. (1977) for KCl. Glycerol was not an effective inhibitor.

The authors concluded that the mechanism for these compounds is an osmotic inhibition of growth.

Blue-light-induced changes at the chemical level have been examined in an attempt to identify the photoreceptor pigment. Song (1980) summarized the molecular changes which may occur in flavinoids and carotenoids upon irradiation with blue light. In a more physiological system, Poff and Butler (1974) noted blue-light-induced absorbance changes in mycelial mats of Phycomyces, in Dictyostelium discoideum slugs and in cell-free extracts derived from D. discoideum. Muñoz and Butler (1975) found similar reversible light induced absorbance changes in mycelia of Neurospora crassa. The absorbance changes were due to the photoreduction of a b-type cytochrome and were apparent only in partially oxidized tissue. Action spectra showed that a flavin mediated the reduction of the cytochrome. Bleaching the flavin resulted in an irreversible light-induced absorbance change and a loss of the reversible cytochrome absorbance changes. From this latter result, Muñoz and Butler (1975) proposed that the photoreceptor is a flavin, and that the first step in the transduction pathway is the flavin-induced reduction of cytochrome. Brain et al. (1977) found similar light induced absorbance changes in membrane fractions of Neurospora and corn. Other workers (see Briggs and Iino, 1984) have further localized the cytochrome to the peripheral plasma membrane.

The identity of the blue light photoreceptor remains unknown. Spectral evidence points to a flavin or a carotenoid. Experiments with inhibitors favor a flavin photoreceptor, but carotenoids have not been decisively eliminated.

Dose-response curves

Dose-response curves for most phenomena follow a simple pattern of increasing response with increasing stimulus, up to the level of saturation. This is not true of phototropism to blue light. Instead, for oats and corn, there are three distinct regions to the dose-response curve. Response to doses just above threshold, usually given in short flashes of high intensity, are called first positive curvature. In this region, the response increases with dose and is dose dependent. Within limits, the Bunsen-Roscoe law of reciprocity holds, i.e., the same dose given with varying duration and intensity elicits the same response. At a dose in the range of 50 - 100 pE x cm^{-2} (Zimmerman and Briggs, 1963a, Blaauw and Blaauw-Jansen, 1970a, Iino et al., 1984), the first positive curvature peaks, and at higher doses the response decreases with increasing stimulus. In oats, negative curvature can be induced. This region is known as first negative, and is also dose-dependent. At still higher doses, the response is increasingly dependent on exposure time rather than dose. The curvature in this region is termed second positive phototropism (Dennison, 1979).

Zimmerman and Briggs (1963a) generated dose response curves for three different intensities of actinic light. They found a lack of reciprocity in the second positive photoresponse. Everett and Thimann (1968) obtained similar results. Blaauw and Blaauw-Jansen (1970a) mapped the curvature response for doses from threshold through second

positive, at several combinations of intensity and time. For the second positive response, they also found that curvature depended mostly on exposure time, although the steepness of the slope was dependent on intensity. The failure of reciprocity in the second positive response makes it impossible to generate true action spectra or the dose-response curves on which they are based. Relative quantum efficiency of different wavelengths of light cannot be evaluated if response is dependent on exposure rather than dose.

Thimann and Curry (1960) separated Avena curvature into two morphologically different responses. First positive stimuli trigger a tip response, a curvature which starts near the apex and migrates basally along the coleoptile. Second positive doses produce a base response, where curvature is distributed along the entire length of the coleoptile. To explain the different responses, they proposed that two different mechanisms for phototropism exist, perhaps based on two different photoreceptor pigments. The distinction between a tip response and a base response has since been ascribed to uncontrolled red light effects (Blaauw and Blaauw-Jansen, 1970b), but the possibility of the involvement of more than one pigment or mechanism remains.

Recent work has shown that light of wavelengths longer than 500 nm can also induce a phototropic response. Iino et al. (1984) have described phytochrome-mediated

phototropism in response to 667 nm light. Mandoli and Briggs (1981) have documented growth changes in response to green "safe" light. Steinitz (personal communication) has shown that Arabidopsis seedlings respond phototropically to light up to 550 nm.

Even in systems where unilateral blue light is used as an actinic light, there exists evidence that more than one pigment is involved. As discussed previously, it appears that both a flavinoid photoreceptor and a carotenoid screening pigment are necessary for normal second positive phototropism in corn. The role of screening pigments in first positive phototropism will be discussed in the present work. For many dicots, only tissues which have greened to some extent are phototropically responsive, raising the possibility that chlorophyll acts as a screen. Screening pigments would increase the light gradient across the tissue, and would also alter the spectral quality of the light received by the photoreceptor pigment itself.

A second line of evidence supporting the involvement of more than one pigment in phototropism has been demonstrated by preirradiating the plants with red light. Asomaning and Galston (1961) found that preirradiation with red or blue light increased the carotenoid and flavin content of oats and barley. Several workers have noticed an effect of red light on subsequent phototropism (see Briggs 1963b for a review of early work). Zimmerman and Briggs (1963a) have shown that oat coleoptiles preirradiated with red light show

a less sensitive first positive and a more sensitive second positive phototropic response. The first negative response disappears entirely. Blaauw and Blaauw-Jansen (1970a) summarized the effects of red preirradiation on their system. Briggs (1963a) found that red light effects disappeared after about three hours. Chon and Briggs (1966) showed that the action spectrum for red light preirradiation resembled that for other phytochrome mediated responses, but Briggs and Chon (1966) found no simple correlation between physiological response and the photochemical state of phytochrome. They proposed that only a small fraction of the total phytochrome present was active. Kang and Burg (1974) found that red light enhanced phototropism of etiolated peas, and proposed that lateral auxin transport was affected. Franssen and Bruinsma (1981) proposed that both phytochrome and a blue-light photoreceptor are involved in phototropic responses of Helianthus, based on a red - far red reversible inhibition of phototropism in preirradiated plants. Thus, it appears that phytochrome may be involved in phototropism, but whether it has some effect on the perception of light direction, on auxin distribution, or at some other point in the transduction pathway remains unknown. Given the wide variety of experimental techniques, it is difficult to draw any general rules about the effect of red light.

Blaauw and Blaauw-Jansen (1970b) proposed that second positive phototropism has a "tonic" and a "tropic"

component. They showed that a unilateral dose of light can be replaced by the same dose of light in which a proportion of the dose is given from above. Depending on the length of the vertical exposure and the interval between vertical and unilateral stimuli, the same or greater curvature can be reached. They proposed that one light dose affects the tonic condition of the plant, and requires a lag time in which to work. A separate dose determines the actual curvature. Thus, first and second positive curvature would both show reciprocity, except that the second positive response takes longer to appear because the coleoptile is desensitized by the amount of light to which it is exposed.

The similarity in action spectra for a wide variety of organisms has led to the hypothesis that there is one primary blue light photoreceptor pigment (see Dennison, 1979). Briggs and Iino (1983) raised the possibility that there is more than one blue light photoreceptor pigment, though the difference may be as slight as a flavin in different environments. Their arguments for more than one photoreceptor pigment are based mostly on differences in action spectra for a number of blue light triggered responses in a variety of organisms.

Thus, it appears that more than one pigment is involved in phototropism, whether the second pigment be a screen, receptor pigment for a different phenomenon, or an auxillary pigment which primes the response of the photoreceptor pigment.

Transduction pathway

Early observers of phototropism noticed that light-induced curvature appears to start at the tip of a unilaterally irradiated coleoptile and progresses, with time, down the axis (Darwin, 1881). This observation led to the postulate that some diffusible substance manufactured at the tip of a plant was necessary for phototropism to occur. Experiments with coleoptiles which had their tips removed, a block of gelatin inserted, and tips replaced confirmed that a diffusible substance was necessary for phototropism (Boysen Jensen, cited in Went and Thimann, 1937). Went later found evidence that an asymmetric distribution of the growth substance was responsible for the curvature of the shoot. The Cholodony-Went hypothesis states that an auxin gradient through a shoot or a root is responsible for the differential growth which leads to curvature (Went and Thimann, 1937). Went and Thimann (1937) list four possible ways of distributing auxin to lead to differential growth: increased transmission of a growth promoter on the dark side of a unilaterally irradiated plant, decreased transmission of a promoter on the light side, increased transmission of an inhibitor on the light side, or decreased transmission of an inhibitor on the dark side. Cholodony and Went (Went and Thimann, 1937), working separately, established that the first two possibilities above occur.

Galston (1959) pointed out that, if auxin were responsible for phototropic curvature, there must be a

4000-fold amplification step somewhere in the transduction pathway. He based this figure on an estimate of the number of quanta incident in the near side of a unilaterally illuminated coleoptile and the approximate concentration of auxin, extrapolated from experiments using the Avena curvature test. He discussed three possible mechanisms by which this amplification could occur: auxin destruction, redistribution based on electrophoretic migration or light-induced alteration of auxin synthesis.

A series of experiments using split coleoptiles gave rise to the idea that perhaps auxin transport, rather than synthesis or destruction, is necessary for a gradient to be established. Briggs (1963a) found that corn coleoptiles exposed to light and those kept in darkness gave the same amount of auxin as measured by the Avena curvature test. Coleoptiles were slit longitudinally except for the top 0.5 mm, and exposed to unilateral light. Briggs found more auxin in receiver blocks from the shaded side of the partially split coleoptile tip than in the blocks from the lighted side. Receiver blocks from the bases of totally split tips showed no difference in auxin content, whether from the lighted or the shaded side.

Radiolabelled auxin enabled workers to measure the amount of auxin transported more accurately than the Avena curvature test allowed. Experiments using radiolabelled IAA in agar blocks also showed that lateral auxin transport occurred in response to gravity (reviewed in Wilkins, 1977).

Further studies using asymmetrically applied auxin were made by Gardner et al. (1974). In this work, a micropipette was used to apply high specific activity auxin to one side of the tip of intact or excised corn or oat shoots. In many, but not all, experiments performed, a correlation between auxin redistribution and curvature was found. Unilateral illumination with a first positive dose of blue light caused significant lateral movement of auxin away from the lighted side of both corn and oat coleoptiles. Second positive doses, however, caused lateral movement of auxin in corn but not in oats. In intact Avena seedlings, inhibition of basipetal auxin transport by second positive dosages was also found. Even though lateral gradients of auxin were found to be induced by unilateral light, under certain conditions curvature toward a first positive dose of blue light occurred, but no auxin gradient was seen.

Elliott and Shen-Miller (1976) found a correlation between phototropism and photoinhibition of growth, based on dose response curves and action spectra. They proposed that the growth inhibition is a result of photoinhibition of basipetal auxin transport. Blaauw (1915) had previously set forth the light growth hypothesis, predicting that light was responsible for phototropic curvature by causing inhibition of growth.

Studies of the growth rate of seedlings have been employed in an attempt to determine the role of auxin in phototropism, but results and conclusions of different

workers are contradictory. Franssen et al. (1981), using marker beads and sucessive photographs of a phototropically responding Avena, Lepidium, or Cucumis shoot, found that growth stopped on the illuminated side, and that no statistically significant increase in growth occurred on the shaded side. The authors argued against auxin mediation of the observed patterns of growth. They proposed that if a high concentration of auxin were present in the responding tissue, a large drop in concentration would be necessary on the lighted side in order for growth to stop. If concentrations were low, a small increase should lead to increased growth on the shaded side. An intermediate auxin concentration should lead to a coordinated change in the growth rates of both sides. As an alternative theory to explain their data, they accepted Blaauw's light growth hypothesis, that is, that light brings about a direct inhibition of growth in the lighted side of the seedling.

Franssen et al. (1982) continued to examine the applicability of the Cholodony-Went hypothesis by studying the curvature of intact, black-capped, and decapitated Avena coleoptiles. They found that the apex is not essential to the perception of a unilateral light stimulus, but that subapical regions responded independently to unilateral light. Initial growth responses are not different in intact and black-capped coleoptiles, and even decapitated coleoptiles show curvature after a lag. Franssen et al.

(1982) concluded that the role of the apex has been overemphasized in explanations of phototropism.

Firn and Digby (1980) provided a review of mechanisms of phototropism and geotropism with an emphasis on the "behavior" of the plant during curvature. They criticized the Cholodony-Went hypothesis on four counts: 1) the role of the apex is overemphasized, 2) phototropism has been known to occur in the absence of auxin gradients, 3) the magnitude of auxin redistribution, when it does occur, has not been shown to be great enough to account for the changes in growth rate observed, and 4) the lag time before phototropism begins is often shorter than would be necessary for the establishment of an auxin gradient. They advocated consideration of Blaauw's light growth hypothesis for an explanation of second positive phototropism.

Iino and Briggs (1984) examined relative growth rates on shaded and lighted sides of phototropically stimulated corn coleoptiles. They performed their experiments under red light in order to stabilize any contribution by phytochrome mediated processes. Under these conditions, they found that first positive curvature was the result of increased growth on the shaded side and decreased growth on the illuminated side of the coleoptile. They correlated this lateral redistribution of growth with lateral redistribution of auxin.

Working on the assumption that auxin indeed is responsible for phototropic curvature, Galston and Hand

(1949) found that riboflavin could sensitize the photooxidation of IAA, as well as the oxidation of other compounds. This, along with the presence of riboflavin in coleoptiles and the similarity of the riboflavin absorbtion spectrum with the action spectrum of coleoptiles, first led to consideration of flavin, rather than carotenoid, as the photoreceptor pigment for phototropism.

Evidence about the nature of the transduction pathway for phototropism remains largely circumstantial. Further work, including the mechanism for Galston's (1959) hypothesized "amplification step" is necessary before the immediate cause of phototropic curvature is known.

INTRODUCTION

The first step in responding to a directional stimulus is to detect the direction from which it comes. For plants responding to light, this entails establishing a gradient of light across the organ doing the sensing. Once the gradient has been created, the organism can measure it either spatially (at one moment in time) or temporally (by comparing the intensity of light on a photoreceptor at two different moments) (Feinleib, 1980).

Three potential mechanisms for establishing a spatial gradient of light are refraction, absorption, and scatter. This thesis will examine the relative contribution of these mechanisms to the detection of a light gradient for first positive phototropism in corn (Zea mays L.).

Refraction was proposed as a mechanism for establishing a gradient of light in Phycomyces by Blaauw in 1915. A cylindrical sporangiophore in air has a refractive index of 1.38, independent of cell diameter (Castle, 1933a). The sporangiophore thus refracts light, bringing it to a focus within the cell on the side distal to the light source, thus acting as a lens. Phycomyces shows a transient increase in

growth rate when given a brighter light than that to which it is adapted, and the sporangiophore twists as it grows. These two characteristics of Phycomyces' growth combine so that a unilaterally irradiated sporangiophore grows toward the light source, but away from the most brightly illuminated area within the sporangiophore (Dennison, 1979).

Several experiments have been performed which manipulate the lens and alter Phycomyces' phototropism. They are reviewed in Bergman et al. (1969). Briefly, Buder immersed Phycomyces sporangiophores in mineral oil with a refractive index much higher than that of the cell, and found negative phototropism. Shropshire (reviewed in Bergman et al., 1969) modified this approach by using media of intermediate refractive indices, and found a medium which rendered the sporangiophores phototropically neutral. He placed a cylindrical lens in front of a sporangiophore at the proper distance to turn the sporangiophore into a diverging lens, and found negative phototropism. However, he found that sporangiophores also avoided the cylinder in the dark, though to a lesser degree. This "avoidance response" complicates the question of the existence of a lens as a focusing mechanism, as do objections raised that sporangiophores immersed in liquids of high refractive index are subjected to changes in oxygen levels.

Dictyostelium discoideum may use a lens as a mechanism for establishing a gradient. When a lens is at work, the organism which shows positive phototropism or phototaxis

turns away from the most brightly lit portion of itself. Francis (1964) illuminated only one side of migrating Dictyostelium pseudoplasmodia, and found that they would indeed turn away from the most brightly illuminated region. Bonner and Whitfield (1965) found that slime molds immersed in a liquid of high refractive index showed a negative photo response. Further support for the lens effect in Dictyostelium comes from an experiment in which an artificial screening pigment, neutral red, was incorporated into Dictyostelium pseudoplasmodia (Häder and Burkhart, 1983). The artificial screening pigment increased the amount of unilateral light absorbed or scattered by the slug, shifting the balance of light intensities in the proximal and distal halves such that the proximal half was more brightly illuminated. The slug, in turning away from the more brightly lit side, showed negative phototaxis.

Coleoptiles are nearly cylindrical, light sensitive organs. The primary leaf inside the coleoptile is dense enough to eliminate the possibility of a lens effect along most of its length. If a focusing advantage exists in coleoptilar tissue, it would be at the extreme apex, above the primary leaf. Lange (1928) showed that the greatest phototropic sensitivity resides in the most apical 500 microns of an Avena shoot. Some sensitivity is present in lower portions as well (Franssen et al., 1982).

Converging and diverging beams of light have been directed at Avena coleoptiles, which then show positive and

negative phototropism, respectively (Shropshire, cited in Dennison 1979). Avena has also been the subject of immersion experiments, and has shown negative phototropism when immersed in a liquid of high refractive index. Ziegler (1950) observed inversion of phototropism in Phycomyces sporangiophores, liverwort rhizoids, dicot seedlings and Avena coleoptiles under paraffin oil. Others have observed negative phototropism in Avena coleoptiles immersed in liquids of a high refractive index. There is some question whether this reversal is due to a focusing effect at the organ or cellular level, or to changes in the cell wall structure caused by penetration of oil (Dennison, 1979).

Below the extreme apex of a coleoptile, where both coleoptilar and primary leaf tissue are present, the most likely mechanisms for establishing a gradient of light are absorption and scatter. Attenuation through absorption can take two forms. For self screening, the photoreceptor pigment is present in high enough concentration that it can establish a gradient of light itself. For foreign screening, the photoreceptor pigment is less concentrated; a second pigment acts as a screen, absorbing light before it reaches the photoreceptor, thus altering the amount and possibly the spectral quality of the light received by the photoreceptor.

Bünning et al. (1953) found a decrease in phototropism when the primary leaf was removed from a coleoptile. Replacing the primary leaf with a dye solution restored

phototropic capability. Meyer (1969) attempted to increase the absorption gradient across an Avena coleoptile by infiltrating the coleoptiles with neutral red, but did not see a significant change in first positive phototropism. She took advantage of the natural asymmetry in pigmentation and pathlength which arises from the elliptical cross-section of a coleoptile, and found a slight increase in phototropic response when the plants were illuminated along the long axis. She saw a greater response when the apical 500 microns of the coleoptile were illuminated than when the next lower increment of 500 microns received the unilateral dose, which agrees with earlier observations that the greatest phototropic sensitivity resides in the tip of a coleoptile.

Vierstra and Poff (1981) have examined the role of carotenoids in second positive phototropism of corn seedlings. Since seedlings treated with SAN 9789 retained about 60% of a normal second positive phototropic response, but had only 2% of their normal carotenoid concentration, Vierstra and Poff (1981) concluded that carotenoids acted as screening pigments.

Attenuation of light by scattering must also play a role in establishing a gradient across an organ. Seyfried and Fukshansky (1983) have developed a model for calculating light gradients in scattering, layered tissue. Based on the morphology, reflective and absorptive properties of Cucurbita pepo cotyledons, they performed a series of

mathematical manipulations which allowed them to approximate the scatter attenuation of different wavelengths of light through the tissue.

Seyfried and Schafer (1983) measured reflectance and transmittance of green, etiolated, and SAN 9789 treated Cucurbita cotyledons (two to seven days old). They calculated absorption and scattering coefficients for the tissues at the different ages and treatments. Developmental changes which occurred between the second and seventh days were reflected in changes in the optical properties of the plants. Seyfried and Schafer (1983) discussed the effect the changes in optics might have on light absorption by phytochrome.

Parsons et al. (1984) attributed most of a plant's phototropic capability to scatter. They have measured the percent transmission of red and blue light through different thicknesses of tissues. Seyfried and Fukshansky (1983) pointed out that this method does not account for changes in light intensity due to backscatter or specular reflection, but Parsons et al. (1984) assumed that these changes would probably enhance the gradient. Their data showed that light is attenuated quickly with increasing tissue thickness. They found that the light intensity at the shaded side is only 2% of that at the lighted side. The attenuation of light with tissue thickness is about the same for both etiolated and de-etiolated hypocotyls of Vicia and Helianthus hypocotyls. Treatment with SAN 6706 to inhibit

carotenoid synthesis in Avena and Helianthus did not alter the light gradient appreciably. Parsons et al. (1984) altered the scattering properties of plant tissue by infiltration with cedarwood oil, which makes the tissue more optically homogeneous and much more transparent. They illustrated how the path of a normal and an oblique ray of light diverges through five cell layers. From these data, Parsons et al. (1984) concluded that absorption is relatively unimportant to light attenuation across a coleoptile.

Poff (1983) pointed out that neither absorption nor scatter alone would allow an organism to detect a light stimulus. By definition, some light must be absorbed for a light-dependent response to occur. Unless all wavelengths of incident light are absorbed by the photoreceptor, some light will be scattered by the molecule itself. In addition, organisms are inherently light-scattering objects. Subcellular components, changes in tissue composition, and changes in index of refraction as a light ray travels through cytoplasm, cell wall, and vacuole could all contribute to attenuation by scatter. Thus, in any biological system, scatter and absorption will both contribute to the gradient of light.

The relative contributions of a lens effect, scatter, and absorption differ among organisms. It is also possible that within an organism, different mechanisms could predominate in first positive, first negative, and second

positive phototropism. The contributions of a lens effect, scatter, and absorption by screening pigments to the gradient of light for first positive phototropism will be considered in this thesis. Chapter 1 contains a spectrophotometric analysis of pigment distribution in individual control, mutant, and SAN 9789 treated seedlings. Chapter 2 examines the contribution of bulk carotenoids to first positive phototropism. Chapter 3 considers the contribution of scatter to the light gradient of intact seedlings, both axially and longitudinally. Results are summarized and recommendations for future work given.

CHAPTER 1

Distribution of Pigments in Individual Corn Seedlings

Introduction

Most *in vivo* absorption spectra have been obtained using tissue pooled from many plants (see Vierstra and Poff 1981b for example). Pooled measurements can be used to estimate an average spectrum for a population, but there are difficulties in localizing pigments or in gauging the variation among seedlings. All spectra in this thesis were obtained from individual seedlings. In this chapter, spectra of different tissues from individual SAN 9789 treated, mutant and control seedlings are presented in order to illustrate relative pigment distributions. In later chapters, the relationships among pigmentation, other optical qualities, and phototropism will be examined.

Methods

The instrument used to generate these absorption spectra is a modified Cary 14 single beam spectrophotometer with double monochromator. The spectrophotometer was on line with a Hewlett-Packard 21MX minicomputer, a system similar to that described by Davis *et al.* (1973). Light from the monochromator fell on a seedling which was taped in

place over a square hole about 0.5 mm x 0.5 mm. Except where noted, the corn seedling was oriented so that the measuring beam passed through the short cross-sectional axis of the coleoptile, between the vascular bundles. A large portion of the light which passed through the sample was collected by an EMI 9659 QA photomultiplier tube with a 5 cm diameter cathode 3 cm from the sample. The current from the photomultiplier was converted to a voltage of -log i, digitized, and stored by the computer. The resulting spectra consisted of absorbance measurements as a function of wavelength at 0.4 nm intervals. The spectra could be manipulated by the computer and output directly for figures, using an x-y plotter (Hewlett Packard 7047A).

Hybrid corn seeds (Zea mays hybrid MS WFg x Bear 38, Custom Farm Seed Research, Decatur, IL.) were soaked overnight in distilled water or in a solution containing a known amount of SAN 9789. SAN 9789, trade name Norflurazon, is an inhibitor of carotenoid biosynthesis and is available as an 80% wettable powder from Sandoz, Inc., Crop Protection (San Diego, CA). In preliminary experiments, it was found that SAN 9789 treated seedlings which were sown in soil produced a substantial amount of pigment, but those sown on Kimpak germinating paper or in vermiculite did not. For subsequent experiments, seeds were sown, embryos up, on Kimpak or in coarse vermiculite which had been soaked with the same solution in which the seeds had been imbibed. Boxes containing seeds sown on Kimpak were covered with

plastic wrap and placed in dark chambers where they received no light before use (dark grown), or in chambers where they received one hour of red light per night (red grown). Red light was supplied by a General Electric red fluorescent bulb. Intensity was 22 $\mu W \times cm^{-2}$ at plant height, as measured with a Kettering radiometer (model number 7720). The spectral distribution of this red light is shown in Figure 2.1. Plants grown in red light were straighter and shorter than those grown in darkness. All plants were grown at 24^{o} +/- 2^{o} C, and the humidity was maintained at 80 - 100%. The seeds were started to imbibe on day zero, planted on day one, and used on days 4-9.

Mutant seeds for initial testing and for seed multiplication were the gift of Dr. D.S. Robertson, Iowa State University, Ames, Iowa. The methods for and results of seed multiplication are detailed in Appendix 1. The mutants were from four class I "carotenoidless" lines, with single mutations at either the lw1, lw2, w7748, or clp allele. The phenotype of class I mutants is a seed with a white or pale yellow endosperm and a seedling lacking carotenoids. Wild type seeds are yellow, and give normally pigmented seedlings. Seeds from the four mutant lines were imbibed overnight in distilled water and planted, embryos up, in 8-dram vials filled with coarse vermiculite soaked with distilled water.

The contribution of scatter to light attenuation was approximated by drawing a slanted baseline with the angle of

the slant determined by using the red region of the spectrum where little light is absorbed. Attenuation above this line is assumed to be due to absorption. The height of peaks above the slanted baseline is used as a basis for comparing absorption by different tissues.

Results and discussion

Spectra of seedlings grown in darkness, with one hour of red light per night, or in white fluorescent light all have absorption maxima at about 415 nm and 470 nm (see Figure 1.1). Red- and dark-grown seedlings show an additional peak at about 435 nm. Blue-light absorbing pigments known to be present in corn include β-carotene, zeaxanthin, and lutein (Fong et al., 1982). Seedlings from all three light treatments have a smaller peak in the red. In dark grown corn, the peak is around 640 nm, and at 660 nm in red and light grown seedlings. These peaks are probably due to protochlorophyll and chlorophyll, respectively (Shibata, 1957). The 660 nm peak is most pronounced in white light grown tissue.

Figure 1.2 shows a series of absorption spectra taken through the seedling at the points indicated. Using the 470 - 480 nm peak as a basis for comparison, and assuming that other peaks do not contribute to absorption at 470 nm, the excised coleoptile (curve A) shows an absorbance of 0.05 A. Five millimeters from the tip (curve D), the absorbance is 0.3 A, and in the mesocotyl, 0.17 A. Coleoptile and mesocotyl tissue are less heavily pigmented

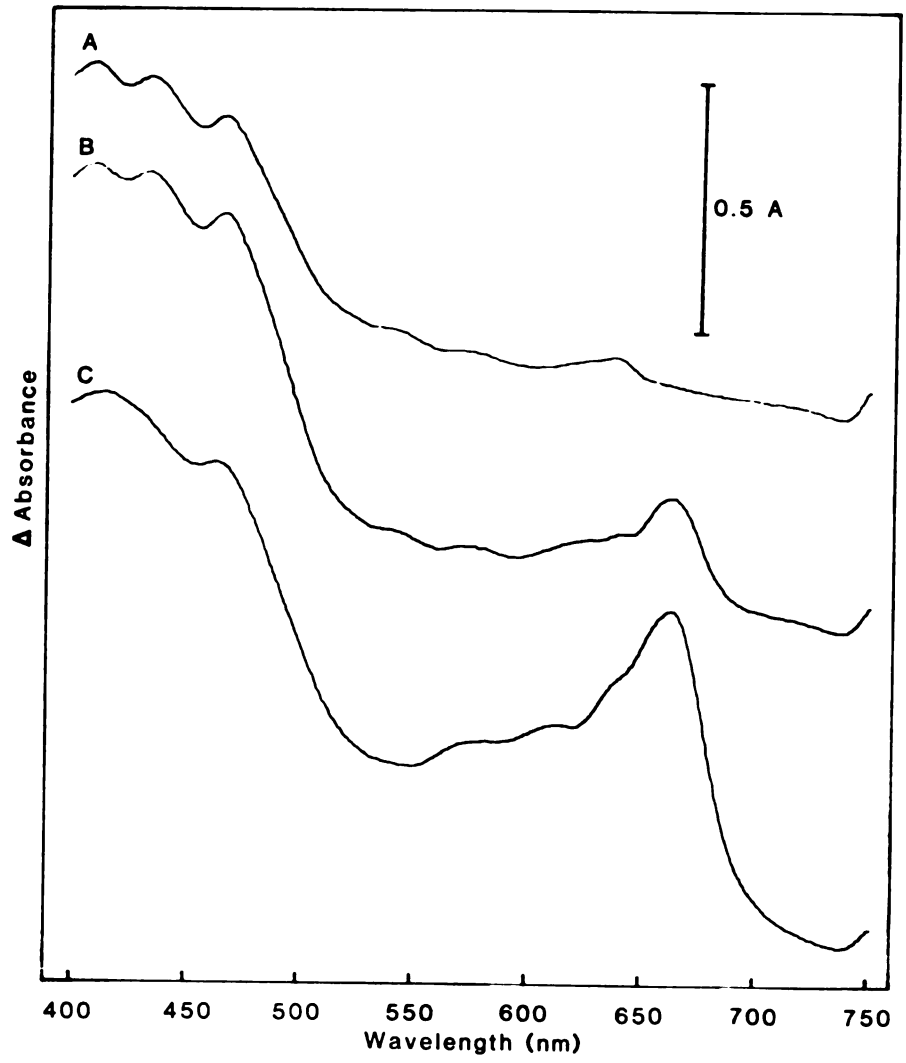

Figure 1.1. Absorption spectra of corn seedlings, taken through coleoptile and primary leaf, 0.5 cm from tip. A) Dark grown seedling. Peaks occur at about 415 nm, 435 nm, 470 nm, and 640 nm. B) Seedling received one hour of red light per night. Peaks occur at about 415 nm, 435 nm, 470 nm, and 660 nm. C) Seedling was grown under fluorescent lights. Peaks occur at about 415 nm, 470 nm, and 660 nm. Curves are offset along the y axis for clarity in all figures.

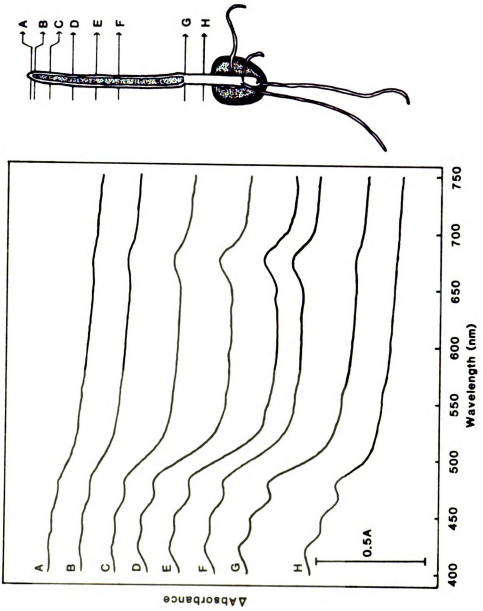

Figure 1.2. Spectra taken through the short cross-sectional axis of a red grown seedling, perpendicular to the vascular bundles. A) extreme tip, coleoptile tissue alone. B) 1.5 mm from tip. C) 3 mm from tip. D) 5 mm from tip. E) 7 mm from tip. F) 9 mm from tip. G) 14 mm from tip, on node. H) 17 mm from tip, in mesocotyl.

than the primary leaf. In intact shoots, the orientation of the plant makes little difference in the absorption spectrum, since the primary leaf is so heavily pigmented compared to the coleoptile. However, in an excised coleoptile, a slight increase in absorption is seen (Figure 1.3) when the spectrum is taken along the long cross-sectional axis, through the vascular bundles. This may be a result of the higher concentration of carotenoids around the vascular bundles (Galston, 1959). Scatter intensification over the longer pathlength may also add to the difference.

Absorption spectra of seedlings grown with different concentrations of SAN 9789 were measured (Figure 1.4). Vierstra and Poff (1981b) reported that a solution of 1×10^{-4} M SAN 9789 inhibited carotenoid production 98 - 99%. For experiments presented in this thesis, a concentration of 2×10^{-4} M SAN 9789 was the standard concentration for inhibiting carotenoid synthesis. In higher concentrations of SAN 9789 the plants were crooked and grew slowly and at lower concentrations more carotenoids were synthesized. At 2×10^{-4} M SAN 9789, many of the plants had short primary leaves, so that the coleoptile tips were hollow. Absorption spectra for the intact coleoptile, mesocotyl, excised coleoptile, and root of a seedling grown with 2×10^{-4} M SAN 9789 were generated (Figure 1.5). Based on a comparison with absorption by a control shoot (Figure

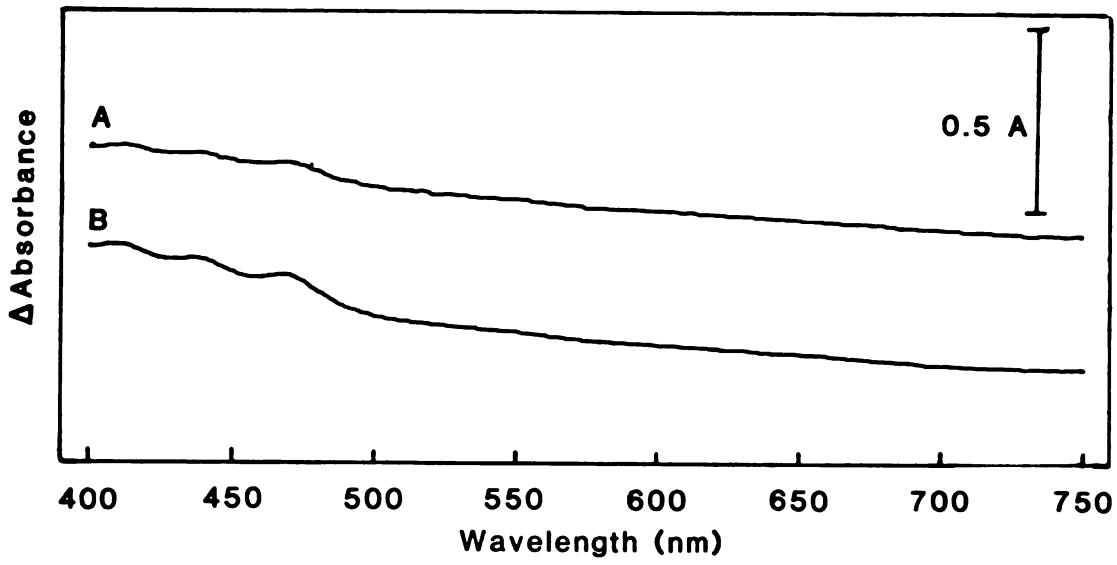

Figure 1.3. Six day old excised coleoptile, cross-sectional spectra. A) Spectrum taken along short axis, perpendicular to vascular bundles. B) Spectrum taken along long axis, through vascular bundles.

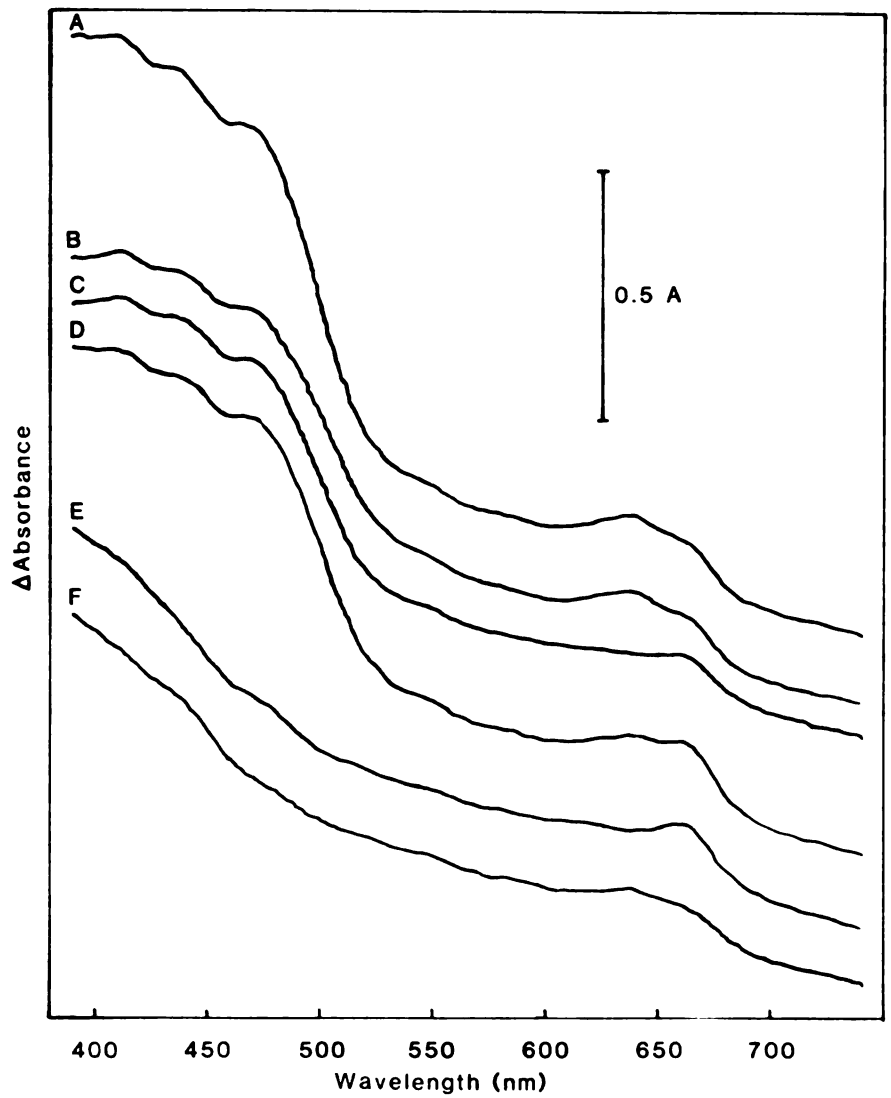

Figure 1.4. Seedlings treated with different concentrations of SAN 9789. Spectra taken 5 mm from tip of shoot. A) Control. (B-F) SAN 9789 treated. B) 4×10^{-7}M. C) 4×10^{-6}M. D) 4×10^{-5} M. E) 4×10^{-4}M. F) 4×10^{-3}M.

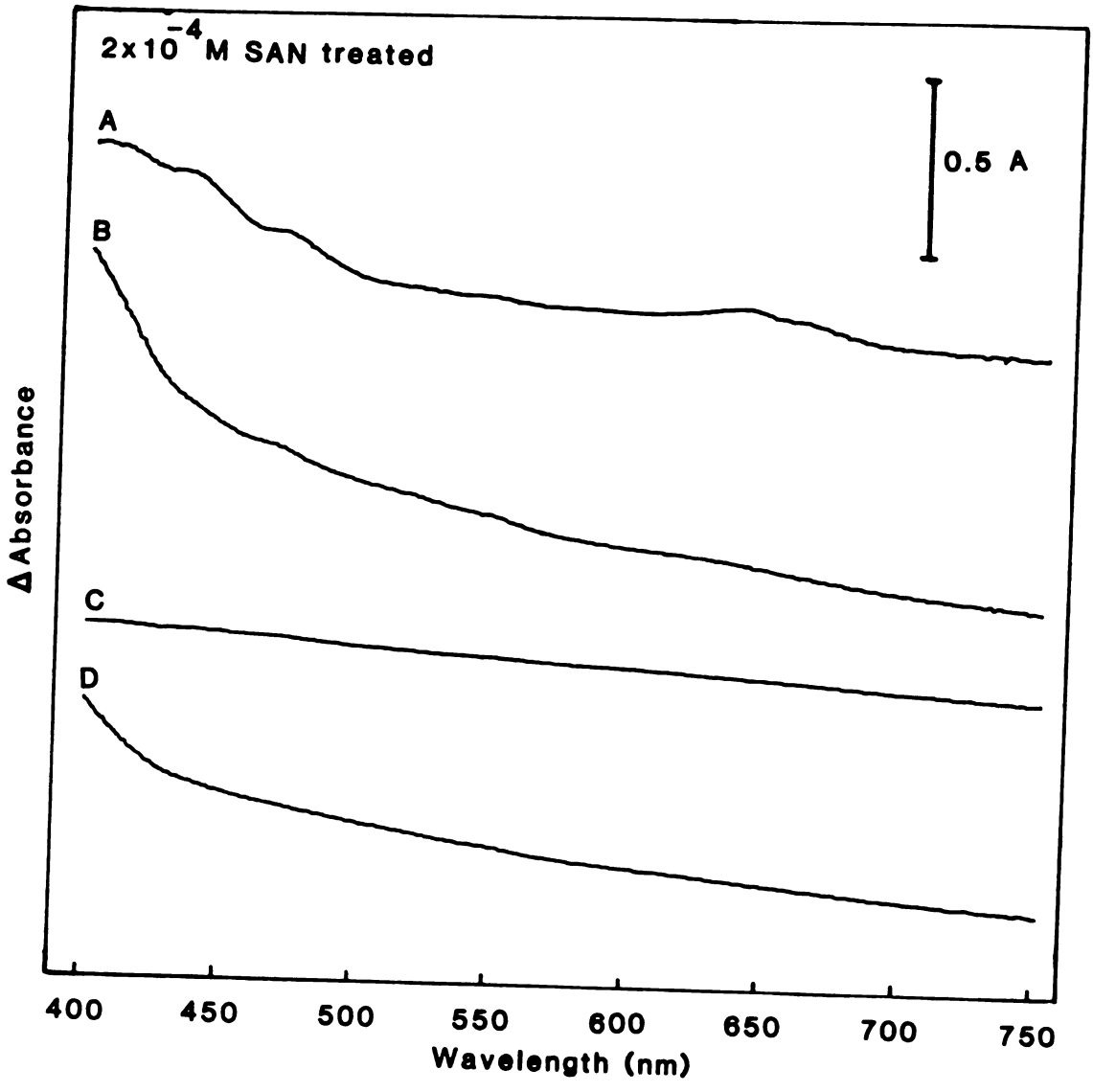

Figure 1.5. 2 x 10^{-4}M SAN 9789 treated, red grown seedling. A) shoot 5 mm from tip. B) mesocotyl. C) excised coleoptile. D) root.

1.6), it appears that carotenoid synthesis in the shoot is inhibited by about 80%.

There is some variation among individual corn seedlings grown under the same conditions. Figures 1.6 and 1.7 illustrate the extent of the variation. For the control seedlings, the difference in absorption at 480 nm is about 0.06 A, for 2×10^{-4} M SAN 9789 treated, 0.02 A. In each case the variation is about 10%. Based on observations made in the course of other experiments, the pigmentation of SAN 9789 treated seedlings may be more variable than indicated in Figure 1.7. It was noted that SAN 9789 treated seedlings appeared to accumulate pigment as they aged, so for subsequent experiments, seedlings were used when they were as young as possible.

The four carotenoidless mutant lines used are characterized by seeds which have pale endosperm and which give rise to albino seedlings. The endosperm of pale seeds was usually distinctly lighter than that of yellow seeds within the same genotype, but occasionally the distinction between pale and yellow endosperm was difficult to make. In these cases, spectra of individual dry seeds were used to differentiate between the two. Spectra were generated using a method similar to that used for seedlings, but with a "pinhole" slightly smaller than the seed. The seed was held in place with modeling clay. Although the broad, flat-topped curves observed for yellow seeds (Figure 1.8) are characteristic when the measuring system is saturated,

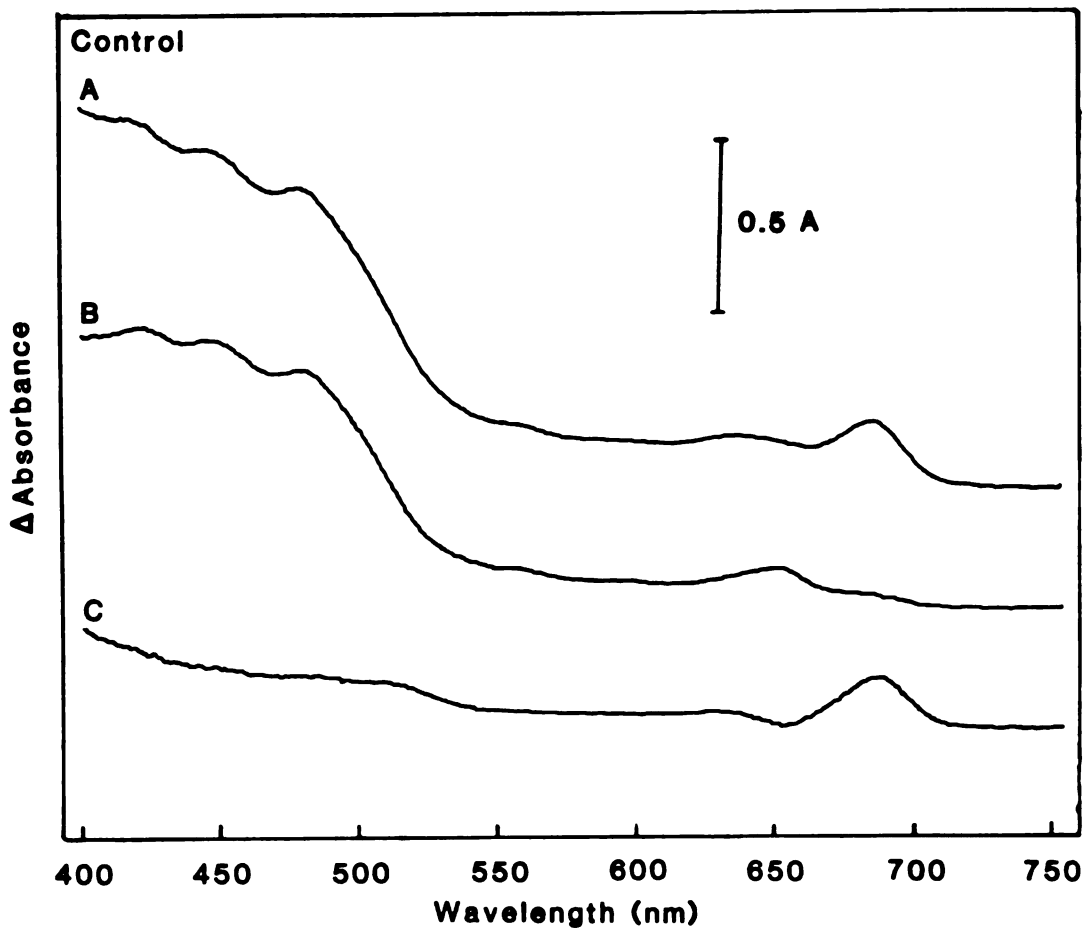

Figure 1.6. Two control seedlings grown in the same conditions under red light were selected randomly to illustrate the variation among individuals. A) and B) are absorption spectra taken 5 mm from the tip. C) is the difference spectrum (A-B).

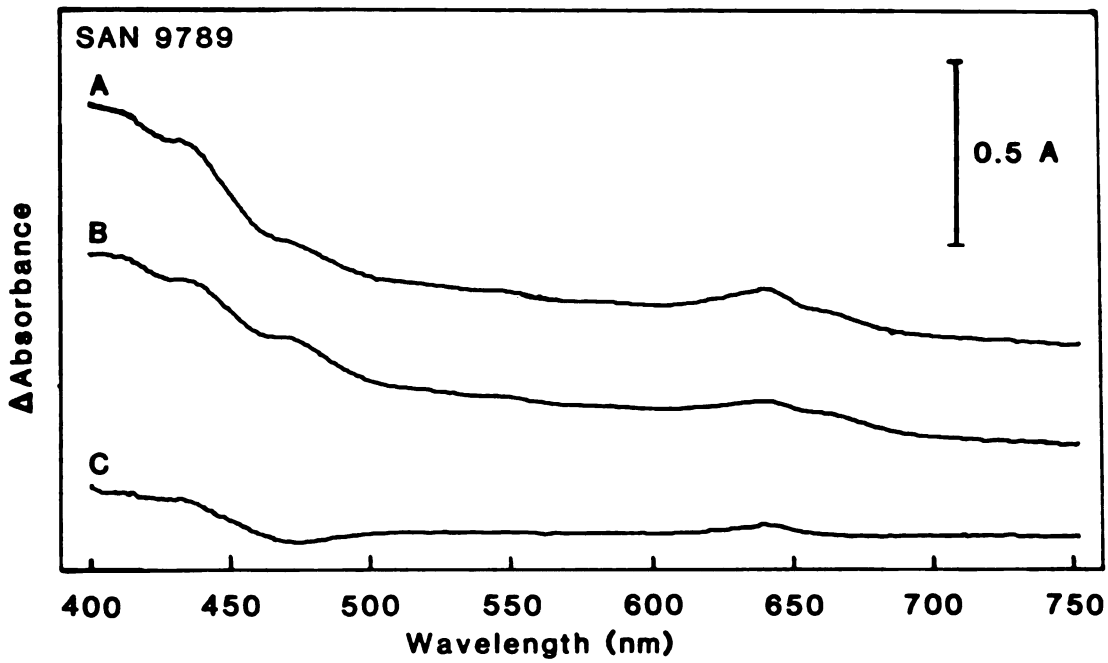

Figure 1.7. As with Figure 1.6, but seedlings were treated with 2 x 10^{-4}M SAN 9789. A) and B) are spectra taken 5 mm from the tip of two randomly selected individuals. C) is the difference spectrum (A-B).

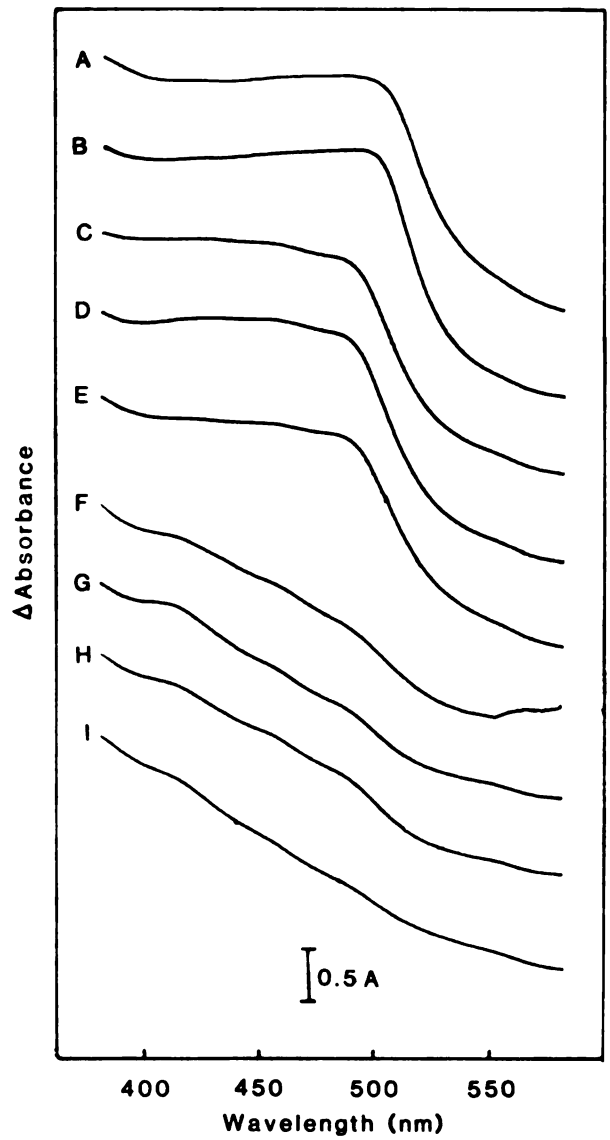

Figure 1.8. Spectra of dry seeds. A) hybrid control. B) lw1 yellow. C) lw2 yellow. D) w7748 yellow. E) clp yellow. F) lw1 pale. G) lw2 pale. H) w7748 pale. I) clp pale.

such saturation did not occur. In a highly scattering sample, wavelengths of light which would normally not be absorbed will be attenuated, making absorption peaks less distinct. This may explain the shape of the absorption spectra of seeds.

Spectra from intact coleoptiles, excised coleoptiles and mesocotyls were measured for pale and yellow seedlings of all four mutant lines. Results are shown in Figures 1.9 - 1.12. In each of these figures, curves A, B, and C represent the absorption spectrum of intact coleoptile, mesocotyl, and excised coleoptile of a yellow seedling, respectively. Curves D, E, and F are spectra for similar tissues of a pale seedling. Comparisons of absorption at the 470 - 480 nm peak based on these spectra show that for mutant line lw1, the carotenoid content in the pale seedling is reduced about 72% from that of the wild type. For lw2, the reduction is 67%, for w7748, 61% , and for clp, 53%. Variation among individual seedlings is also about 10% for the mutant lines. Absorption spectra for hybrid corn and for wild type seedlings of the four mutant lines are qualitatively similar. Wild type seedlings may be more heavily pigmented than hybrid control seedlings. Pale mutant seedlings show a more distinct set of peaks in the blue region of the spectrum than do seedlings treated with 2×10^{-4}M SAN 9789. The mutants' pigment content relative to their respective wild type is not reduced as much as 2×10^{-4}M SAN 9789 treated seedlings relative to controls.

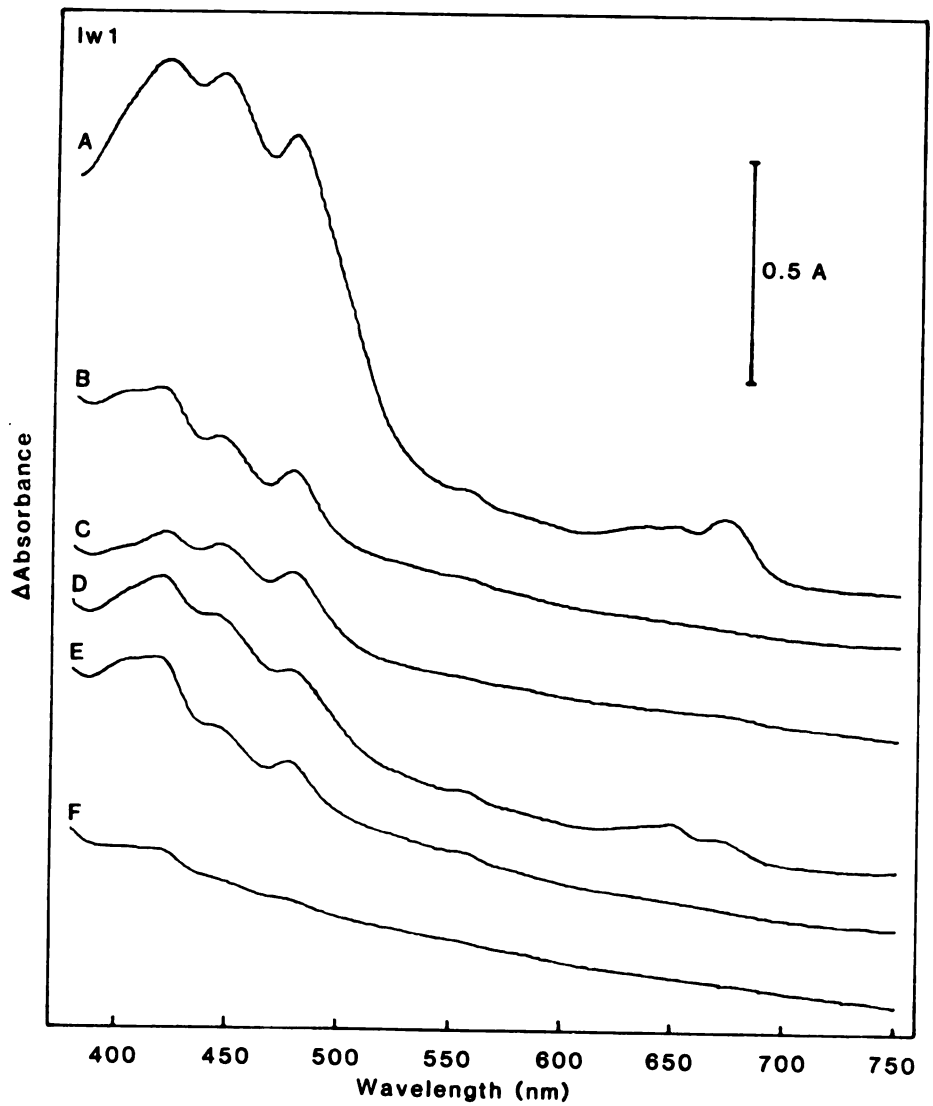

Figure 1.9. Absorption spectra of different tissues of lw1 seedlings. (A-C) wild type. A) intact shoot 5 mm from tip. B) mesocotyl. C) excised coleoptile. (D-F) mutant. D) intact shoot 5 mm from tip. E) mesocotyl. F) excised coleoptile.

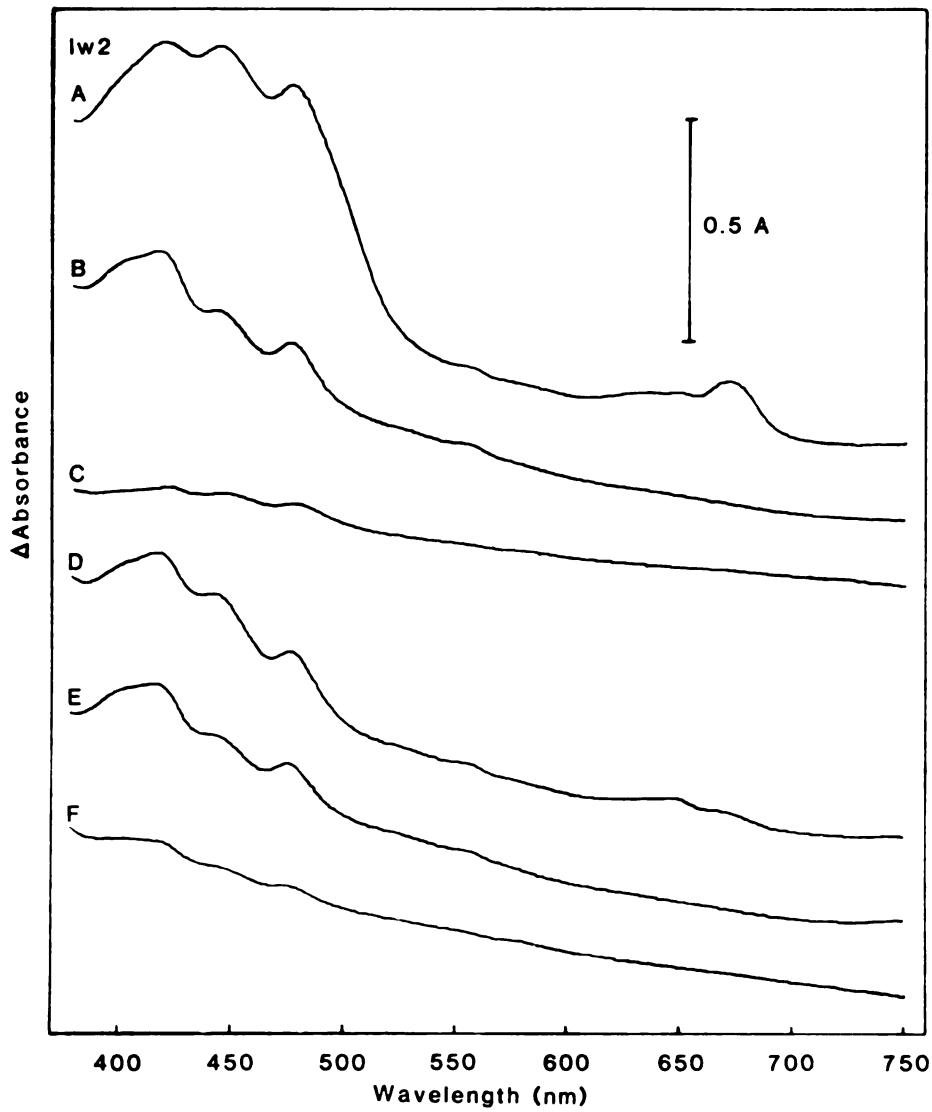

Figure 1.10. Absorption spectra of different tissues of lw2 seedlings. (A-C) wild type. A) intact shoot 5 mm from tip. B) mesocotyl. C) excised coleoptile. (D-F) mutant. D) intact shoot 5 mm from tip. E) mesocotyl. F) excised coleoptile.

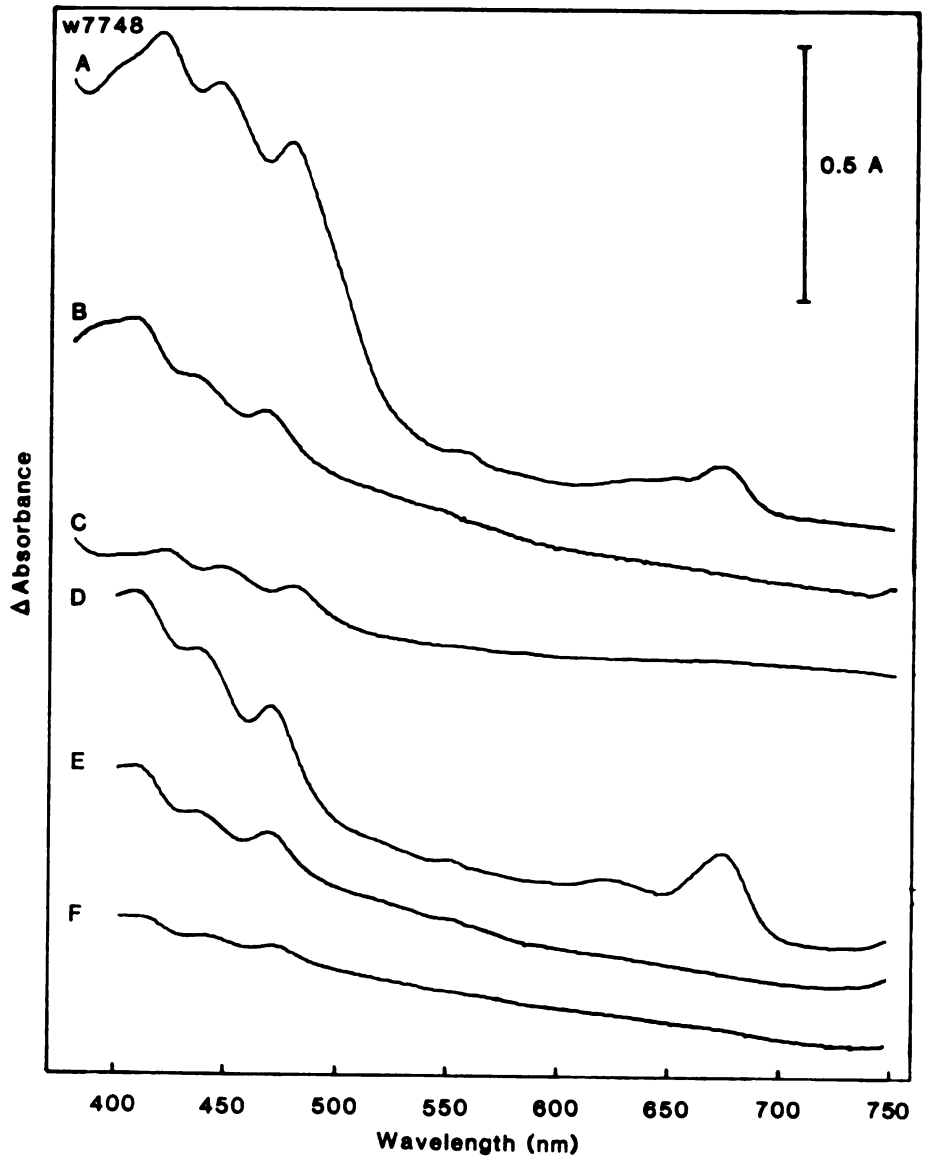

Figure 1.11. Absorption spectra of different tissues of w7748 seedlings. (A-C) wild type. A) intact shoot 5 mm from tip. B) mesocotyl. C) excised coleoptile. (D-F) mutant. D) intact shoot 5 mm from tip. E) mesocotyl. F) excised coleoptile.

Figure 1.12. Absorption spectra of different tissues of clp seedlings. (A-C) wild type. A) intact shoot 5 mm from tip. B) mesocotyl. C) excised coleoptile. (D-F) mutant. D) intact shoot 5 mm from tip. E) mesocotyl. F) excised coleoptile.

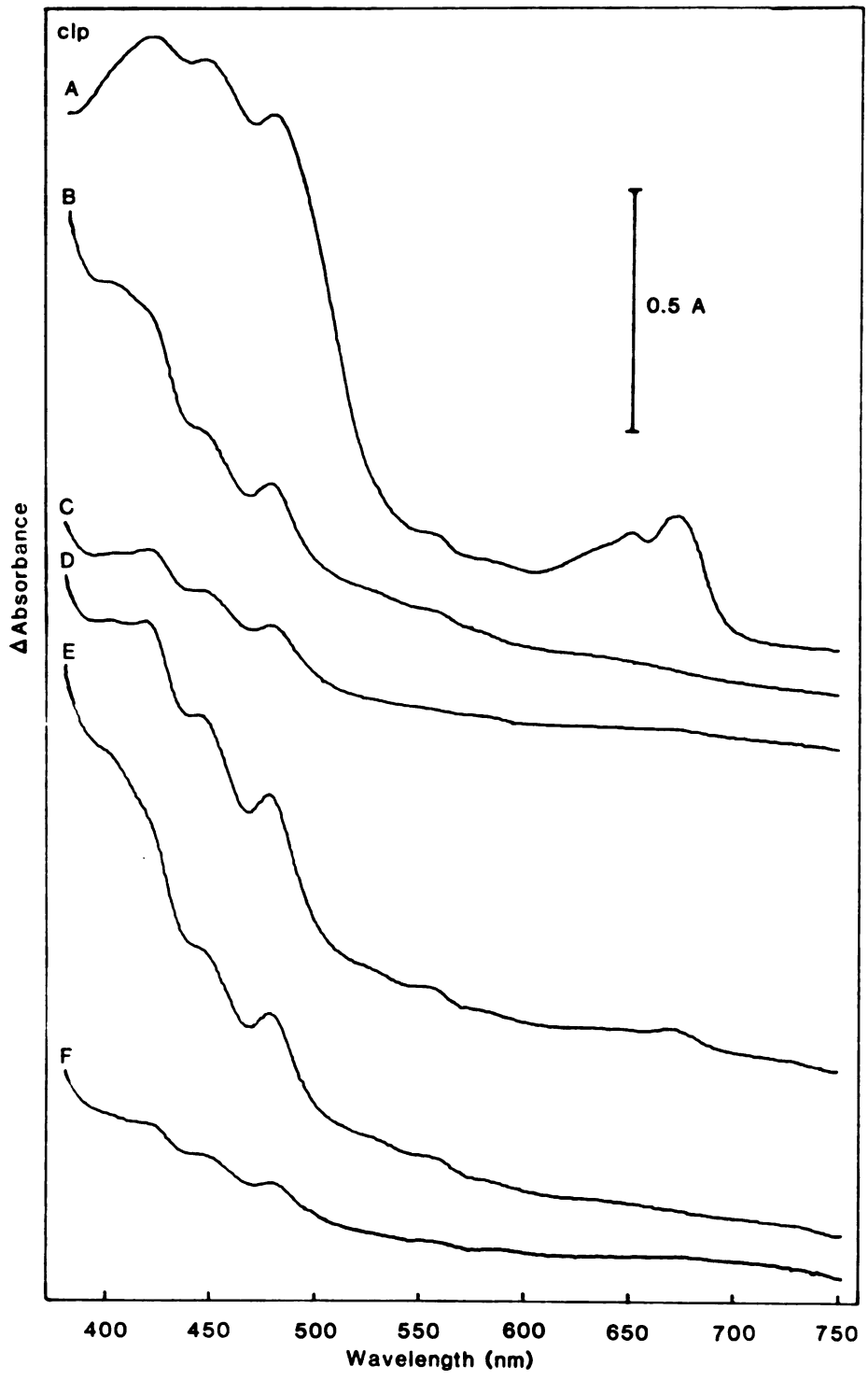
clp
A
B
C
D
E
F
0.5 A
ΔAbsorbance
400
450
500
550
600
650
700
750
Wavelength (nm)

Figure 1.12

Summary

Bulk pigments have been mapped in a number of seedlings from different genetic lines and growth regimes. Two sets of seedlings with reduced pigments, one generated by treatment with SAN 9789, the other a group of mutants with similar phenotypes, have been characterized. These two groups of seedlings with reduced pigments will be used to assess the role of carotenoids in first positive phototropism.

The technique presented in this chapter could be used to separate individual seedlings with pigmentation differences too subtle to be culled visually. Changes in pigmentation with time or light regime could be monitored in an individual seedling, since sample seedlings are left intact. Pigments present in specific areas of the plant can be mapped more accurately than is possible with pooled material. For these reasons, the ability to generate absorption spectra from individual seedlings will be useful in further studies of phototropism.

CHAPTER 2

The Role of Carotenoids in First Positive Phototropism of Corn Seedlings

Introduction

The complex way in which monocots respond to increasing doses of unilateral blue light (light impinging on the plant from one direction, normal to the longitudinal axis) has led to several hypotheses about the mechanism used to sense the light. The possibility that more than one pigment or response system is involved in phototropism has been discussed. Poff (1983) suggested that, at an even more fundamental level, there may be more than one mechanism for establishing the light gradient necessary for detecting a unilateral light stimulus. He proposed a lens and a mechanism of screening by absorption and scatter as two alternate possibilities. Vierstra and Poff (1981b) showed that absorption by screening pigments is important for second positive phototropism. Removal of screening pigments by treatment with an inhibitor of carotenoid synthesis decreased phototropic response 40% without affecting geotropism. Bandurski and Galston (1951) reported that mutant albino corn seedlings with no detectable carotenoids showed 50% of the wild type's first positive phototropic response.

In this chapter, the role of carotenoids in first positive phototropism will be examined. The phototropic response of inhibitor treated or mutant seedlings with reduced carotenoid content will be assessed. Geotropism and growth rates are used to verify that reduced carotenoid content affects only the phototropic response.

Methods

Hybrid corn seeds (*Zea mays* hybrid MS WFg x Bear 38, Custom Farm Seed Research, Decatur, IL) were soaked overnight in distilled water or in a solution of 2×10^{-4}M or 2×10^{-5}M SAN 9789. Seeds were planted, embryos up, about 5 cm deep in 10 cm x 20 cm x 8 cm boxes filled with vermiculite which had been saturated with water or a solution of SAN 9789. Seeds were planted in diagonal rows, so that seedlings would not shade each other. Twenty seeds were planted per box. Before exposure to actinic light, ten straight seedlings with coleoptiles still intact were selected for use. The rest of the seedlings were snapped off at the soil line. For experiments with mutant seedlings, seeds were planted individually, 5 cm deep in 8 dram vials containing wet vermiculite. Planting in vials allowed a maximum number of a limited supply of seedlings to be used.

Of the several mutants known (see Robertson, 1975, for review), two lines were used to assess further the contribution of carotenoids to first positive phototropism. Mutants with carotenoidless lw1 and lw2 alleles (pale

seedlings) and the counterpart wild types (yellow seedlings) were used to generate dose response curves and were compared to control and SAN 9789 treated hybrid seedlings. A limited number of mutant seeds was available (see Appendix 1), so only three light doses in addition to a dark control were evaluated. The three doses chosen correspond to the peak of first positive response for hybrid corn, a point in first negative, and a point in second positive. Three experiments were run.

All plants were grown for five days in 90 - 100% relative humidity at 24^{o} +/- 2^{o} C. They received one hour of red light per night (intensity 22 $\mu W \times cm^{-2}$, spectral distribution as shown in Figure 2.1). A flashlight covered with a piece of green plexiglas and an interference filter (560 nm peak, 10 nm half-band) was used as a "safe" worklight.

To measure phototropic and geotropic responses, seedlings were photographed before any stimulus was given, and again three hours after commencement of the stimulus. Kodak high speed infrared 2481 film was used to take the photographs. Light for photography was provided by a flash covered with a Kodak 87C Wratten filter with a cutoff at 800 nm. Plants did not respond in any discernable manner to the infrared light used for photography. During the interval between photographs, when the response to a stimulus was developing, plants were kept in the dark, humid growth chamber. Curvatures were measured with a protractor

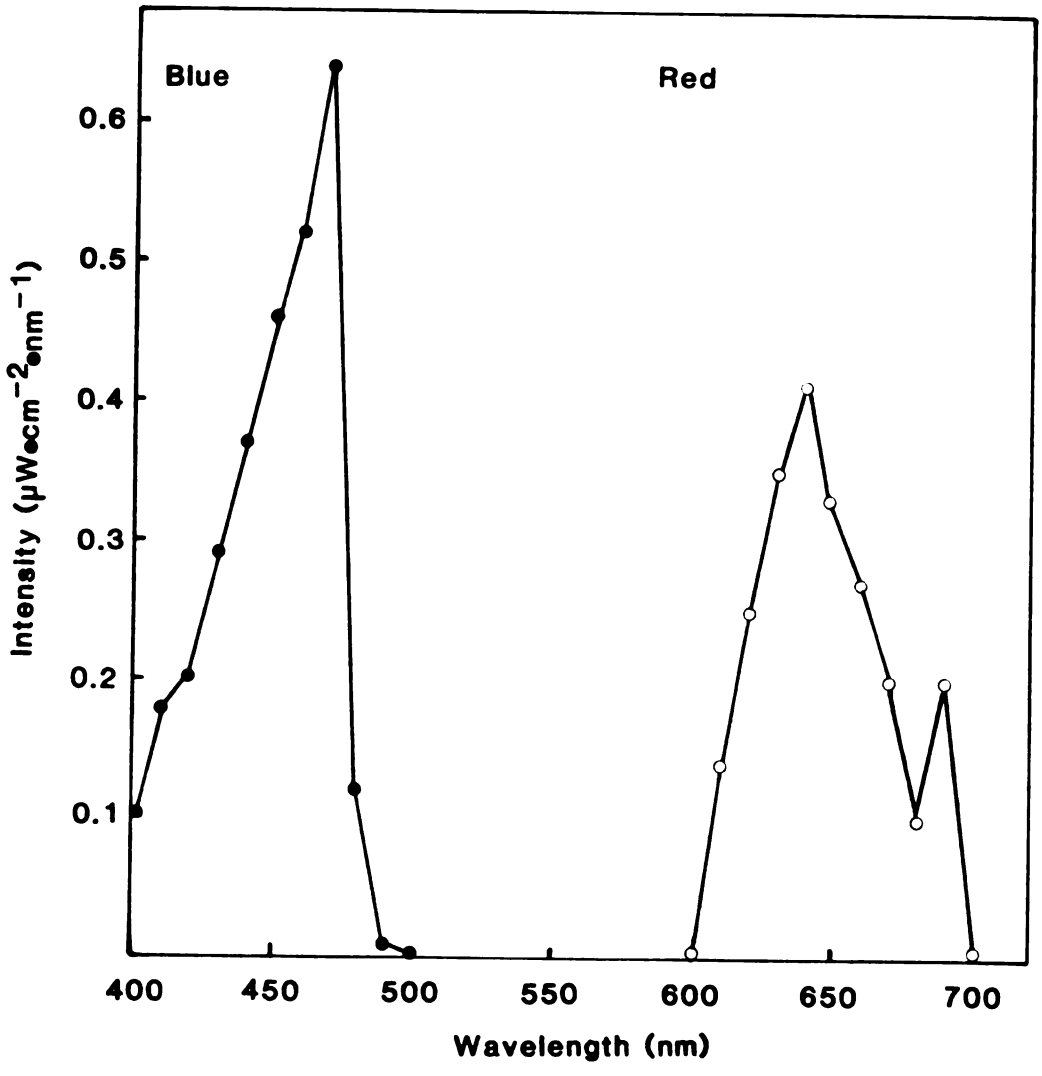

Figure 2.1. Spectral distribution of the blue light used as phototropic stimulus and red light used to promote coleoptile elongation and inhibit mesocotyl elongation.

from the projected image of the negative. Mean curvature before stimulus was subtracted from mean curvature after stimulus and a three hour development time to give net curvature.

The actinic light used to generate the dose response curves was provided by a Beseler Slide King II projector. Light passed through a DT Blaauw broad band blue filter and 3.5 cm of 5% (w/v) copper sulfate, giving a peak at 470 nm (spectral distribution is shown in Figure 2.1). For all exposure times shorter than three hours, light intensity was 12 $\mu W \times cm^{-2}$. Dose was varied by changing exposure time from 0.1 to 180 seconds, using a shutter and timing unit (Uniblitz). When a stimulus for second positive curvature was needed, the light intensity was cut to 0.9 $\mu W \times cm^{-2}$ with neutral density filters, and exposure time was increased to three hours.

For all dose response curves measured, seedlings were oriented randomly with respect to their transverse axes. The straightest seedlings were selected, or seedlings were oriented so the actinic light was normal to the straightest longitudinal axis.

Results and discussion

Meyer (1969) reported a slight increase in the curvature of Avena coleoptiles when they were oriented so that an actinic beam of light passed along the long transverse axis. Recently, Iino and Briggs (1984) have taken care to orient plants so the long transverse axis was

parallel to the direction of irradiation. In the present experiments, a comparison of mean curvature for plants illuminated along the long and the short transverse axes was made. Means were compared by a two-tailed Student's t test (see Table 2.1). No significant difference in curvature was found at any dosage tested. For subsequent experiments, no attempt was made to orient seedlings with respect to their transverse axes.

In order to choose an appropriate time interval after stimulus at which curvature could be evaluated, it was necessary to know the rate at which curvature occurred. A dose of light to elicit maximal first positive response was given to control and 2×10^{-4}M SAN 9789 treated seedlings. The seedlings were photographed at 30 minute intervals for three hours, and curvature of individual seedlings followed (see Figure 2.2). For both control and SAN 9789 treated seedlings, curvature began about 60 minutes after the stimulus was given. SAN 9789 treated plants reached a peak response by three hours, but control plants had not. Geotropic straightening does not begin until after five hours or more (data not shown).

In order to assess the role of carotenoids in first positive phototropism, two sets of experiments were performed using plants with reduced carotenoid content. In the first set of experiments, dose response curves for seedlings treated with SAN 9789 were compared to those for control (water treated) seedlings. SAN 9789 has a number of

Table 2.1. Mean curvature of seedlings irradiated along long and short transverse axes. Different doses were evaluated on different days, so comparisons between different doses should not be made. Methods given in text. SE: standard error of the mean. Mean curvature in degrees.

log(dose) ($Wxcm^{-2}$ x t)	df	mean curvature (SE) short axis	long axis	t
-5.3	24	9.3 (2.1)	9.5 (1.5)	0.08
-5.0	26	5.8 (1.8)	6.6 (2.6)	0.24
-4.5	26	8.3 (1.5)	9.7 (2.4)	0.49
-4.5	21	15.3 (2.5)	12.5 (2.7)	0.76
-4.5	14	11.4 (3.3)	6.4 (3.5)	1.03
-3.3	18	5.6 (2.2)	1.9 (2.3)	1.20
-.75	10	25.0 (4.8)	22.3 (2.6)	0.49
-.69	17	2.3 (1.4)	1.1 (1.1)	0.65

Figure 2.2. Time course of bending. Left: 2 x 10^{-4}M SAN 9789 treated seedlings. Right: control seedlings. All seedlings were given two seconds of 12 μW x cm^{-2} blue light to elicit a nearly maximal first positive response. After three hours, SAN 9789 treated seedlings have reached a maximal response, where control plants may be capable of some further curvature.

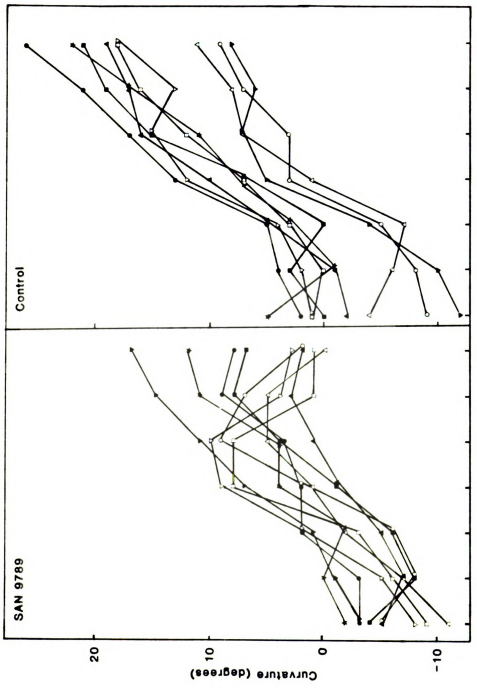
SAN 9789
Control
Curvature (degrees)
20
10
0
-10
0
30
60
90
120
150
180
Time after flash (min)

Figure 2.2

effects aside from inhibiting carotenoid synthesis. In plant tissue with no carotenoids, photobleaching occurs, and neither chlorophyll nor chloroplast proteins accumulate (Quarrie and Lister, 1984). Ribosomal RNA (Bartels and Watson, 1978, Quarrie and Lister, 1984) and abscisic acid (Quarrie and Lister, 1984, Henson, 1984) also do not accumulate in light grown SAN 9789 treated tissue. SAN 9789 and related compounds alter the fatty acid composition of galactolipids (St. John, 1976). How these alterations in plant metabolism affect phototropism is not known, but these non-specific effects of SAN 9789 make it necessary to run controls to show that the effect on phototropism is due to reduced carotenoid concentration, and not some more generalized physiological factor.

If SAN 9789 treatment affects phototropism only by reducing carotenoid content, then one would expect a correlation between carotenoid content and response. Unfortunately, the range of effective SAN 9789 concentrations is limited. At concentrations of 10^{-3}M or greater, seedlings are generally crooked and unhealthy. At concentrations of 10^{-6}M or less, carotenoid content and response to a peak first positive stimulus are indistinguishable from untreated seedlings. Within the concentration range where the major effect of SAN 9789 appeared to be on carotenoid content, two dose response curves were generated. The first, comparing control seedling curvature to that of seedlings treated with

2×10^{-4}M SAN 9789 (Figure 2.3) showed that an approximately 90% reduction in carotenoid content (Figure 1.4) corresponded to a 39% reduction in the peak of the first positive response (Table 2.9). Treatment with 2×10^{-5}M SAN 9789 resulted in a 31% reduction in peak first positive response (Figure 2.4). The results for reduction in carotenoid content agree qualitatively with those of Vierstra and Poff (1981b), but a lower concentration of SAN 9789 appears to reduce curvature more effectively in the present work. The shape of the dose response curves at both concentrations is similar, with a peak response occurring at a log (dose) of -5.0. This dose corresponds to an intensity of 12 $\mu W \times cm^{-2}$ and time of 0.7 seconds. Analyses of variance for the two sets of experiments show that at both concentrations of SAN 9789, differences in response for control and SAN 9789-treated seedlings are highly significant (0.01 level) (Tables 2.2 and 2.3). Both the dose of light to which a seedling had been exposed and the herbicide treatment affected its response. In addition, at the higher SAN 9789 concentration, there was an interactive effect between light dose and herbicide treatment significant at the 0.05 level.

Both control and SAN 9789 treated seedlings which had been kept in darkness (except for infrared flash and safelight) were used as a measure of undirected movement by the seedlings. Since they received no directional stimulus, only the absolute value of their net curvature is

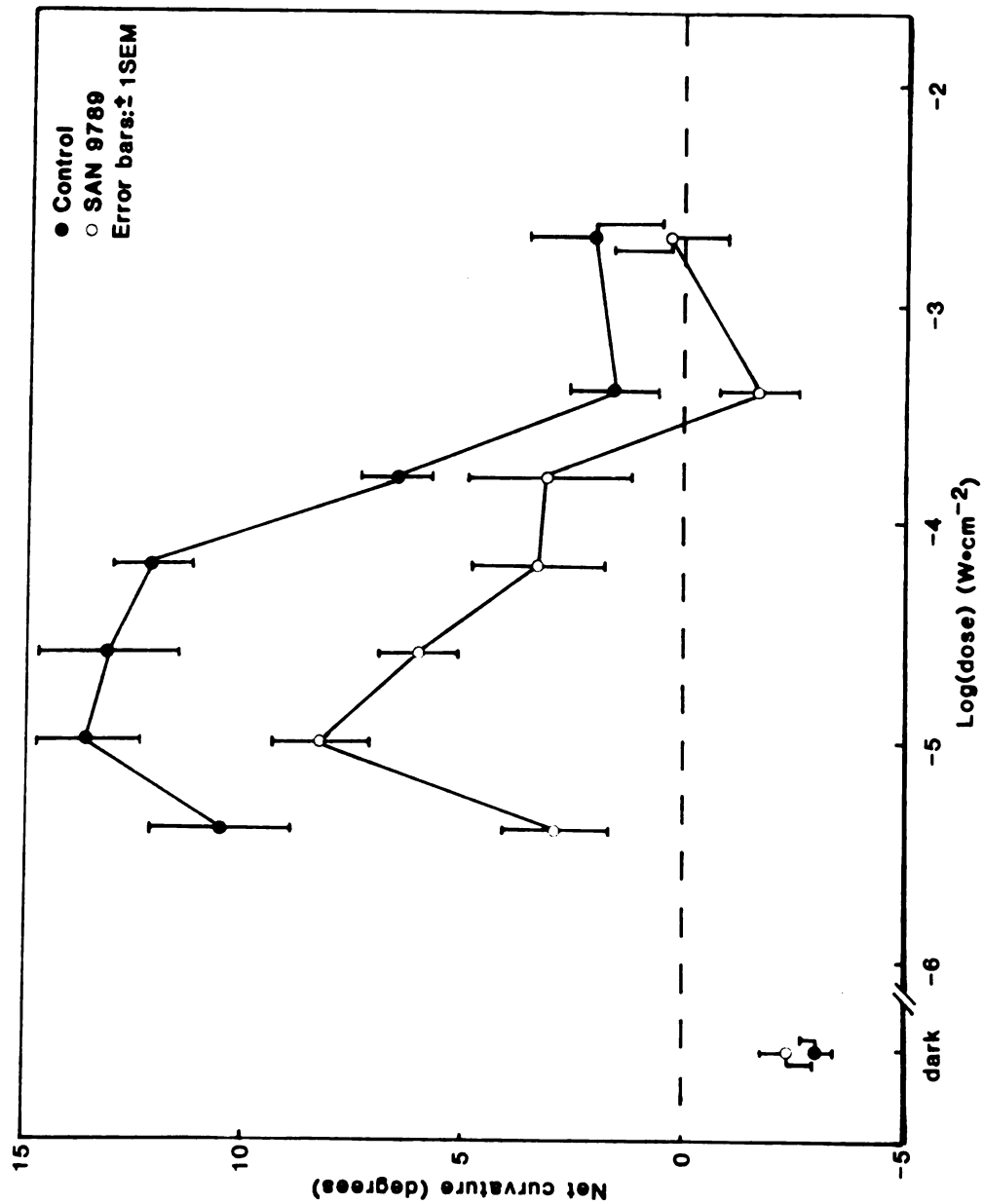

Figure 2.3. Dose response curves for control and 2 x 10^{-4}M SAN 9789 treated seedlings. Experimental details in Methods. Each point represents the mean of six experiments, and each experiment used 9 or 10 seedlings per point. Standard error for data pooled from six experiments was +/-1.2^{o}.

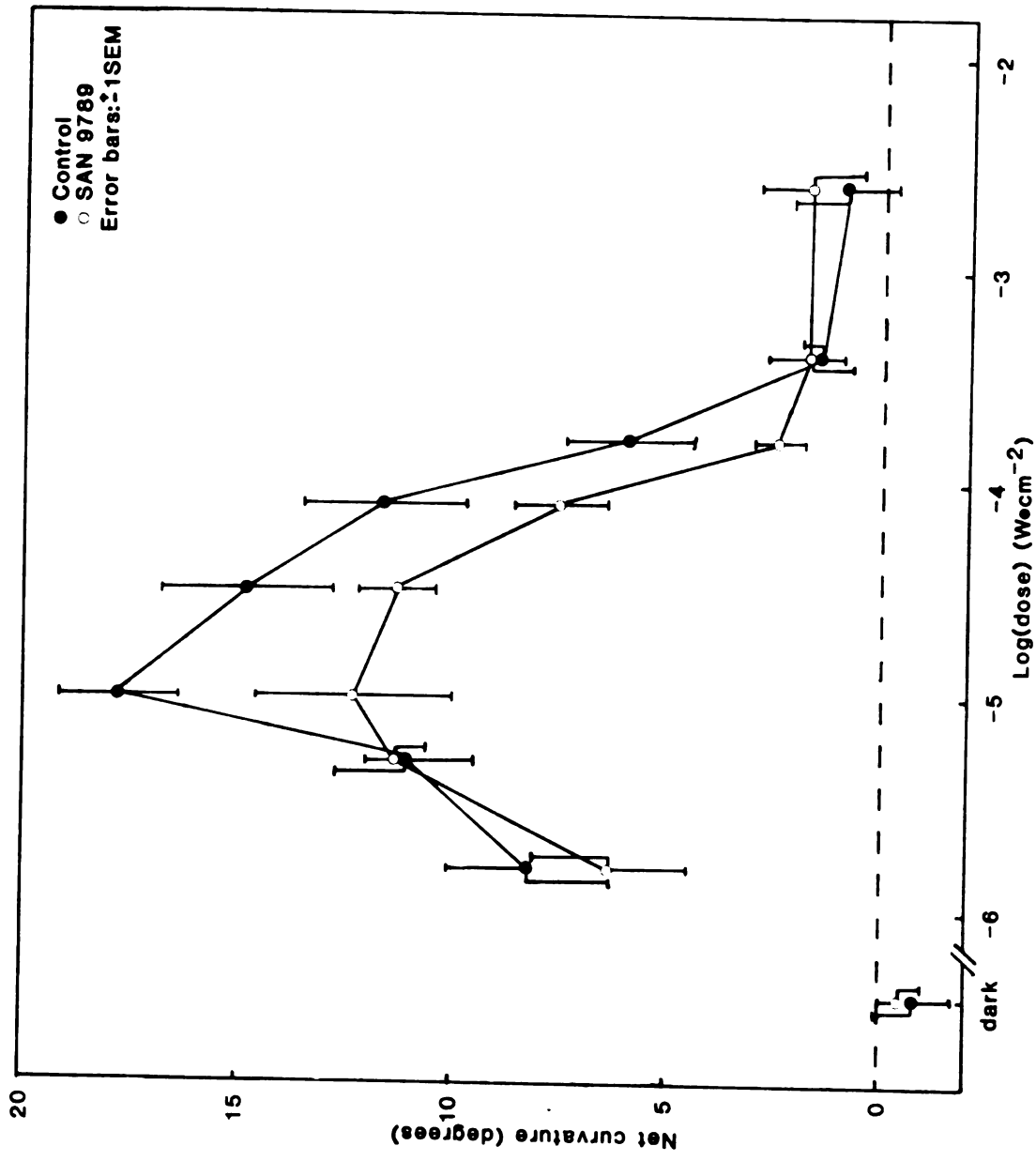

Figure 2.4. Dose response curves for control and 2 x 10^{-5}M SAN 9789 treated seedlings. Methods and replications as for Figure 2.3. Standard error for data pooled from six experiments was +/-1.4^{o}.

Table 2.2. Analysis of variance for dose response curves of control and 2×10^{-4} M SAN 9789 treated seedlings. Data shown in Figure 2.3.

source	df	F
treatments	15	21.25**
doses	7	34.60**
SAN 9789	1	58.90**
dose x SAN 9789	7	2.53*

*, ** significant at 0.05 and 0.01 levels.

Table 2.3. Analysis of variance for dose response curves of control and 2×10^{-5} M SAN 9789 treated seedlings. Data shown in Figure 2.4.

source	df	F
treatment	17	15.80**
doses	8	31.19**
SAN 9789	1	7.48**
dose x SAN 9789	8	1.45

** significant at the 0.01 level.

meaningful. Up to 3^{o} of curvature (Figure 2.3) was attributed to random movement by the plant.

Geotropism serves as a second control for the specificity of SAN 9789. Since phototropism and geotropism both involve a unilateral redistribution of growth across the plant, it is likely that they share some common steps in the transduction pathway. Detecting the two different stimuli involves different mechanisms, but the changes in growth rate necessary to bring about curvature should be the same for both responses. If phototropism is affected by an inhibitor to a greater degree than is geotropism, then the inhibitor must work at a point not held in common between the two responses. In preliminary experiments, it was found that SAN 9789 treated seedlings required high humidity for expression of a full geotropic response. Appendix 2 presents data showing the geotropic responses of control and SAN 9789 treated seedlings at 60% and 100% humidity. Thereafter, all geotropic experiments, like phototropic experiments, were carried out in 100% relative humidity.

Once the humidity requirement was met, SAN 9789 treated seedlings responded as strongly to a geotropic stimulus as control seedlings (Table 2.4). Using a Student's t test, no significant differences in geotropic curvature for seedlings given different SAN 9789 treatments were found.

A final estimate of non-specific effects of SAN 9789 was made by measuring the growth rate for control and SAN 9789 treated seedlings. It was observed that seedlings treated

with 2 x 10^{-4}M SAN 9789 grew more slowly than control plants over the course of the five days before they were used. However, when the growth increments of control, 2 x 10^{-5}M, and 2 x 10^{-4}M SAN 9789 treated seedlings which had received no actinic irradiation were measured over a three hour period, no differences in growth rate were observed (Table 2.5). If phototropism and geotropism are the result of a lateral redistribution of growth (Iino and Briggs, 1984) and if this distribution of growth remains constant despite changes in growth rate, then a plant which shows a reduced growth rate could still be capable of normal tropic curvature.

From these data, it appears that SAN 9789 inhibits first positive phototropism without affecting geotropism, as long as high humidity is maintained. An 80% reduction in carotenoid content reduced the phototropic response only 39%, leading to the conclusion that carotenoids contribute as screening pigments to the gradient of light for first positive phototropism.

Another approach to reducing the carotenoids in a corn seedling which avoids the problems inherent in using an inhibitor is to introduce a mutant allele which gives a "carotenoidless" plant. For mutant seedlings, as with control and SAN 9789 treated seedlings, a first positive response is separated from a second positive response by an indifferent or negative response (Figure 2.5).

Table 2.4a. Geotropism vs SAN 9789 concentration in 100% relative humidity. Each experiment included 9-11 individuals. Curvature measured in degrees. SE: standard error of the mean.

treatment	# of experiments	mean curvature	SE
control (water)	9	53.44	1.6
2×10^{-5}M SAN 9789	5	55.1	6.2
2×10^{-4}M SAN 9789	10	59.4	2.0

Table 2.4b. Values of Student's t for indicated comparisons

comparison	t
control vs 2×10^{-5}M SAN 9789	0.55
control vs 2×10^{-4}M SAN 9789	0.85
2×10^{-4}M vs 2×10^{-5}M SAN 9789	1.79

Table 2.5. Growth rates of control and SAN 9789 treated seedlings over a three hour interval. SE: standard error of the mean.

treatment	growth increment	SE
control	0.15	0.02
2×10^{-5}M SAN 9789	0.14	0.02
2×10^{-4}M SAN 9789	0.19	0.02

A statistical analysis (Table 2.6) shows that the difference in curvature between normally pigmented seedlings and pale or SAN 9789 treated seedlings is significant. By analysis of variance (Table 2.6a), most of the difference between the dose response curves shown in Figure 2.5 could be attributed to an effect of dose. However, the "corn type" (whether control or 2 x 10^{-4}M SAN 9789 treated hybrid, yellow or pale mutant) made a small contribution, significant at the 0.1 level. Since it was the effect of "corn type" that was considered most interesting, a further analysis of the differences between dose response curves due to corn type was made using orthogonal contrasts (Table 2.6b). This showed that the difference in phototropic responsiveness (significant at the 0.5 level) is between fully pigmented seedlings and seedlings with reduced carotenoid content. At the first positive dose tested, the response of pale mutant seedlings was reduced 62% from that of the wild type (Table 2.9).

Geotropism and growth rate were used as controls for mutant seedlings as well. Because of the geometry of the holders for individual vials used in these experiments, geotropic and phototropic stimuli were given in the same plane. An analysis of variance (Table 2.7) shows that the previous light dose has an effect on the subsequent geotropic curvature. This is probably because seedlings which responded strongly to a phototropic stimulus started responding to a geotropic stimulus from a negative

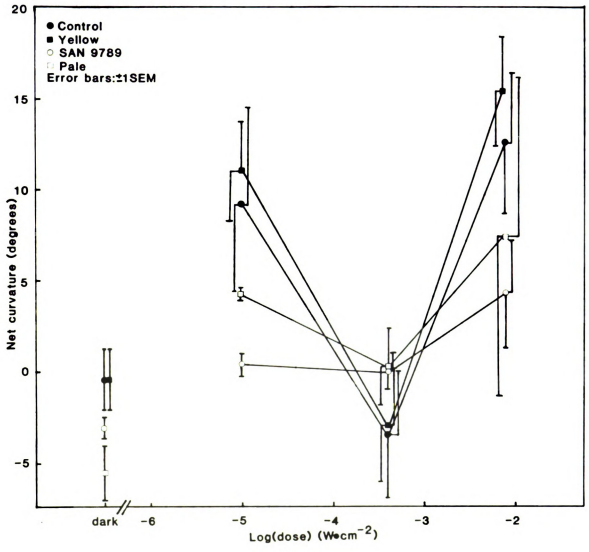

Figure 2.5. Dose response curves for control and SAN 9789 treated hybrid seedlings, yellow and pale mutant seedlings. Experimental details in Methods. Each point represents the mean of three experiments. Each experiment used 7 or 8 seedlings per light point, 5 or 6 seedlings per dark control. Standard error for data pooled from three experiments was +/-3.06°.

Table 2.6a. Analysis of variance for dose response curves of wild type and pale mutant seedlings. Data in Figure 2.5.

source	df	F
treatments	15	4.20**
doses	3	14.93**
corn type	3	2.57+
dose x corn type	9	1.17

+, ** significant at 0.1, 0.01 levels.

Table 2.6b. Orthogonal contrasts for corn type.

source	df	F
control and yellow vs SAN and pale	1	7.06*
control vs yellow	1	0.36
SAN vs pale	1	0.28

* significant at 0.05 level

Table 2.7. Analysis of variance for geotropism of yellow and pale mutant seedlings.

source	df	F
treatments	7	4.80**
doses	3	8.42**
pigmentation	1	1.60
dose x pigmentation	3	2.24

** significant at 0.01 level.

Table 2.8. Growth rates of mutant seedlings. SE: standard error of the mean.

phenotype	growth increment	SE
lw1 pale	0.1	0
lw2 pale	0.19	0.03
lw2 yellow	0.06	0.02

Table 2.9. Summary of correlations among carotenoid concentration, phototropic curvature, and geotropic curvature. Carotenoid content is based on absorption at 480 nm of randomly selected individuals (Figures 1.5, 1.9, and 1.10), phototropic responses on dose response curves (Figures 2.5, 2.4, and 2.5), and geotropic responses on geotropic controls used for dose response curves. For pale mutants, wild type seedlings served as control, for SAN 9789 treated seedlings, hybrid seedlings treated with water were used. Responses are expressed in terms of percentage of control response.

corn type	carotenoid content	phototropic response	geotropic response
lw1 and lw2	30 [1]	38	107
2×10^{-5} M SAN 9789	ND	69	103
2×10^{-4} M SAN 9789	13	61	111

[1] mean of two individuals. ND: no data.

curvature. More importantly, the analysis of variance also shows that pale and yellow seedlings did not differ in their geotropic responses.

Growth rates from one experiment (Table 2.8) indicate that pale mutants may elongate more quickly than wild type seedlings. Thus, carotenoidless mutants appear to be capable of normal growth and geotropic curvature, but like SAN 9789 treated seedlings show a reduced response to doses of light which stimulate first and second positive phototropism.

Summary

Nonspecific effects in plant tissue treated with SAN 9789 which may alter phototropic capability. It is also possible that pleiotropic effects in the mutants could affect phototropism. One method to estimate the effects of SAN 9789 in systems other than carotenoid synthesis would be to treat pale mutant seedlings with SAN 9789. Since pale seedlings are low in carotenoids and carotenoid-derived metabolic products, further reduction in phototropic response could be attributed to nonspecific SAN effects.

Further comparisons between pale mutants and SAN 9789 treated seedlings might help to define effects not specifically related to carotenoid concentration which alter phototropism. The mutant's response to gravity in 60% humidity and the kinetics of bending for mutant seedlings are data which would allow further comparison of the metabolism of SAN 9789 treated and pale seedlings.

Carotenoids appear to act as screening pigments in first positive phototropism, a role suggested by Vierstra and Poff (1981b) in second positive phototropism. A decrease in carotenoid content correlates with a decrease in phototropic response, but does not affect geotropism (Table 2.9).

CHAPTER 3

Mechanisms of Establishing a Light Gradient: Lens, Longitudinal Light Transmission, and Scatter

Introduction : Lens

If a cylinder is sufficiently transparent, and has an index of refraction sufficiently different from the surrounding medium, it can act as a lens. The refractive index relative to the surrounding medium will determine whether the cylinder will cause unilateral light to converge or diverge. However, the amount of absorption and scatter will determine how quickly light is attenuated. In a Phycomyces sporangiophore in air, light is focused on the side of the sporangiophore away from the light source. The attenuation across the sporangiophore is sufficiently small that the absolute intensity in the distal half is larger than in the proximal half. Thus, there is a "focusing advantage" within the sporangiophore, and a "lens effect" exists (Dennison, 1979). This "lens effect" is the mechanism which Phycomyces sporangiophores use to determine the direction of a unilateral light stimulus (see Introduction). Poff (1983) and others have suggested that a

focusing advantage may also exist in the nearly-cylindrical shoot tips of monocots.

The shoot of a corn plant consists of mesocotyl, node, and tightly rolled primary leaves encased in the coleoptile. The primary leaves are dense enough to eliminate the possibility of a lens effect along much of the length of the shoot. In the apical 0.5 to 1 mm of the shoot, only coleoptile tissue is present. If a lens contributes to detection of light direction in a corn seedling, it would be here, where the tissue is most transparent.

The tip of a coleoptile is a cone with an elliptical base and an off-center apex. The two vascular bundles converge at the tip, and approximately intersect the long axis of the ellipse. Because of the different path length and tissue composition along the long and short transverse axes of the shoot, measurements of light intensity, like spectra generated in previous chapters, were made along the short axis of the ellipse, perpendicular to the vascular bundles.

Methods

Using a modified Cary 14 spectrophotometer with a picoammeter attached to the photomultiplier tube, currents from the phototube representing initial intensity (I_0) and final intensity (I_2) were measured. I_1 is the intensity at the border between the front half and the back half of the cylindrical "coleoptile". The difference between I_0 and I_2 is due to both absorption and scatter.

Results and discussion

A number of simplifying assumptions make it possible to evaluate the possibility of a lens effect in corn coleoptiles in a manner similar to Castle's treatment of Phycomyces sporangiophores (Castle, 1933b). Castle (1933a) measured a refractive index of 1.38 for Phycomyces. Assuming a circular cross-section, he found that the total path length of focused light in the rear half of a sporangiophore was 1.26 times greater than the path length in the front half. These values, and a set of similar assumptions, are used here to evaluate corn seedlings.

1. Rather than an ellipse, the cross-section of a model "coleoptile" is assumed to be a circle. Ten coleoptiles measured just above the primary leaves averaged 1.01 mm along the long axis, 0.78 mm along the short axis, so the diameter of the model coleoptile is taken to be 0.9 mm.

2. Effects of cell boundaries are ignored: it is assumed that the coleoptile refracts light as a single cell.

3. Index of refraction is taken to be 1.38, as for Phycomyces.

4. The ratio of path length in the back half to the front half is taken to be 1.26, as for Phycomyces.

5. All light incident on the coleoptile is assumed to enter the shoot. Reflection and scatter at the surface are ignored.

These assumptions favor the existence of a lens effect.

The following equations were used to calculate the

intensity midway through the coleoptile (I_1) using measured values of I_0 and I_2.

$$I_1 = I_0 e^{-\alpha l_1} \qquad \text{(a)}$$

$$I_2 = I_1 e^{-\alpha l_2} \qquad \text{(b)}$$

where α= absorption coefficient, l_1 = path length in front half of coleoptile, l_2 = path length in back half of coleoptile = $1.26 l_1$

Substituting into equation (b),

$$I_2 = I_0 (e^{-\alpha l_1})(e^{-1.26\alpha l_1})$$

$$1.75 \times 10^{-6} \text{ amps} = 1.13 \times 10^{-5} \text{ amps } (e^{-2.26\alpha l_1})$$

$$0.55 = e^{-2.26\alpha l_1}$$

$$0.825 = \alpha l_1$$

Substituting into equation (a),

$$I_1 = I_0 e^{-0.825}$$

$$\ln \frac{I_1}{I_o} = -0.825$$

$$\ln I_1 - \ln (1.13 \times 10^{-5} \text{ amps}) = -0.825$$

$$\ln I_1 = -12.2$$

$$I_1 = 5.03 \times 10^{-6} \text{ amps}$$

Then, absorption in front half = $I_0 - I_1$

$$= 1.13 \times 10^{-5} - 5.03 \times 10^{-6} = 6.27 \times 10^{-6} \text{ amps}$$

Absorption in back half = $I_1 - I_2$

$$= 5.03 \times 10^{-6} - 1.75 \times 10^{-6} = 3.28 \times 10^{-6} \text{ amps}$$

$$\frac{\text{absorption in back half}}{\text{absorption in front half}} = 0.52$$

The absorption coefficient can be estimated from the incident and final light intensities: $\log \frac{I_0}{I_2} = OD$

$$\log \frac{1.13 \times 10^{-5} \text{ amps}}{1.75 \times 10^{-5} \text{ amps}} = 0.8 \text{ OD over } 0.9 \text{ mm or}$$

$\alpha = 8.9$ for a standard pathlength of 1 cm.

Castle (1933b) generated a curve which shows the dependence of relative absorption in the front and back halves of a cell on α, and stated that similar curves can be generated for cells of different diameters, larger cells giving curves with steeper slopes. Castle's (1933b) figure is adapted in Figure 3.1. The curve from his data, using Phycomyces sporangiophores with a diameter of 0.08 mm, is shown by the solid line. The corresponding curve for a model coleoptile 0.9 mm in diameter is shown by the dashed line. The critical value of α for a pathlength of 0.9 mm is 2.6, or an optical density of 0.23 for a standard pathlength of 1 cm. Therefore, for a cylinder 0.9 mm in diameter, α must be less than 2.6, and the ratio of absorption in the back half to absorption in the front half must be greater than 1.0 for a focusing advantage to exist. With values of $\alpha = 8.9$ and the ratio of absorption in the back half to absorption in the front half = 0.52, there can be no lens effect.

Introduction : Longitudinal light transmission

Light gradients exist longitudinally, as well as transversely, along a unilaterally irradiated coleoptile.

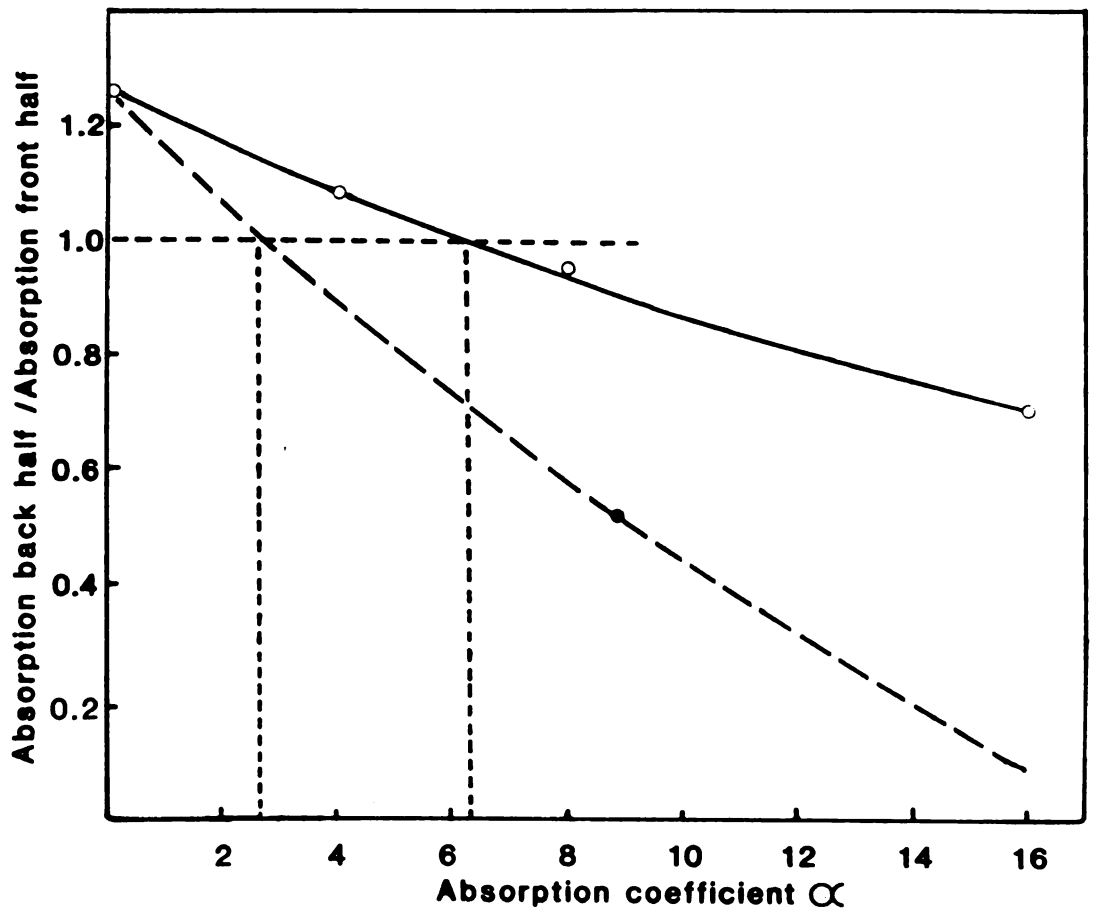

Figure 3.1. Critical values for absorption coefficient (α) for a Phycomyces sporangiophore (solid line, open circles) and a model corn coleoptile (dashed line, solid circles). Adapted from Castle (1933b).

Mandoli and Briggs (1982b) have shown that plant tissues are capable of axial light transmission over 20-25 mm, and attribute this transmission to internal reflection, or "piping", of the light. Scatter must also have an effect on the longitudinal distribution of light. Light rays will scatter off particles and/or when moving through cell components of different refractive indices. Both scattered and piped light are attenuated log-linearly with distance. Unlike randomly scattered light, piped light is transmitted coherently through the tissue (Mandoli and Briggs, 1982b). Mandoli and Briggs proposed that light transmitted axially could trigger photomorphogenesis (1982a) or phototropism (1982b) by exciting pigments at a location distant from the area directly illuminated.

The axial transmission of light is wavelength dependent. Scatter and absorption would tend to attenuate short wavelengths of light more quickly than longer wavelengths. In addition, Mandoli and Briggs (1984) attributed some attenuation to less effective "piping" of blue light. Their results are qualitative, and trace relative transmission of light from 400 - 750 nm through several plant tissues. The experiments presented here are an attempt to quantify the amount of light transmitted at selected wavelengths.

Methods

Corn seeds were grown on Kimpak as described in Chapter 1. Plants from six to ten days old were used.

Seedlings at least 2.5 cm long, with primary leaves extending the entire length of the coleoptile, were chosen for use. A segment of a coleoptile plus primary leaf 2.5 cm long was placed in a holder, with 1 cm of tissue extending above the holder. The coleoptile was held in place with black plasticine, which also prevented light leaks between the plant tissue and the holder (see Figure 3.2). The light sources used were a helium - neon laser with an output at 632 nm, or the 514 nm and 458 nm lines of an argon laser. The different bands of light from the argon laser were separated by a prism. Each wavelength of light was passed through a slit and an interference filter of appropriate wavelength (Baird atomic, 630, 510, or 460 nm peak, 10 nm half-band) to eliminate stray light of other wavelengths. The intensities of the green and red bands were adjusted with neutral density filters (Inconel) so that the incident intensity of the three wavelengths used was nearly equal. The beam of light was then passed through a lens and brought to a focus on the tip of the coleoptile, and normal to its longitudinal axis. The bottom of the coleoptile was abutted against an optical fiber, which led to a photomultiplier tube. Current from the photomultiplier was measured with a picoammeter and subsequently converted to units of intensity. Incident light intensity was measured with a Kettering radiometer (model 7720).

When light was incident on the tip of the coleoptile, the top 1 - 1.5 cm "glowed" from light scattered from the

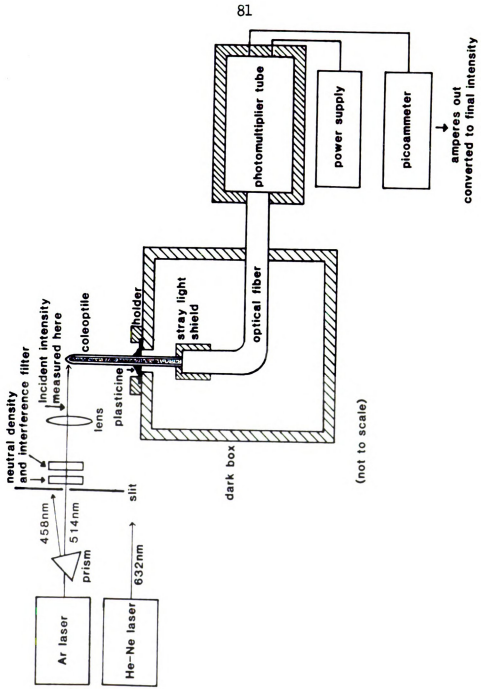

Figure 3.2. Apparatus used for measuring axial light transmission through corn coleoptile segments. For details, see text.

sides of the tissue. To prevent this scattered light as well as any stray light from striking the photomultiplier tube, the bottom of the coleoptile was inserted into a hole in a cap on top of the optical fiber. Each coleoptile was exposed to 632 nm, 510 nm, and 460 nm beams of light. Measurements were taken from ten control and ten SAN 9789 treated plants for each of the five ages used.

The photomultiplier tube was run at 600 V. At this voltage, dark current on the phototube was 9×10^{-11}A to 1.2×10^{-10}A. Dark current was subtracted from measurements of transmitted light before converting the measurements of current (A) to units of intensity (μW). The system could measure intensities over eight orders of magnitude before dark current became limiting.

The ratio of (light in / light out) when converted to log terms gives the optical density (A) of the sample. In this experiment, transmission losses include losses at the surface, so are an overestimate of the actual density of the tissue.

To measure the attenuation of transmitted light as a function of distance, coleoptiles were placed in the same holder apparatus and irradiated at the tip with 632 nm light. Transmitted light was measured, a segment of known length was cut from the coleoptile, and transmitted light was measured again. Three to six sequential measurements were made from each plant.

Results and discussion

Light incident on a coleoptile can be reflected, absorbed, or scattered. Some of the scattered component will be transmitted axially, as well as in other directions through the tissue (see Figure 3.3). The proportion of the light which is transmitted axially has been reported to depend strongly on the incident angle of light, with different tissues and plants showing different acceptance angles (Mandoli and Briggs, 1982b). Preliminary experiments with the present system did not show this angular dependence (Figure 3.4), so the incident angle used in subsequent experiments was always 0^{o}, normal to the longitudinal axis, as with actinic light for phototropic experiments.

Dark current varied over the several days necessary to collect data, so it is presented as a shaded area, 7.6 to 8.0 orders of magnitude less than incident intensity (Figure 3.5). A representative dark current of 1.2×10^{-10}A can be expressed using conversion constants as 0.17 pW x cm^{-2} (9×10^{-5}pE x cm^{-2} x sec^{-1}) for 632 nm light, 0.14 pW x cm^{-2} (6×10^{-5}pE x cm^{-2} x sec^{-1}) for 514 nm light, or 0.22 pW x cm^{-2} (8.3×10^{-5}pE x cm^{-2} x sec^{-1}) for 460 nm light. Thus, the lower limit of sensitivity of the system is on the order of 0.5 pW x cm^{-2} or 1×10^{-4}pE x cm^{-2} x sec^{-1}, two orders of magnitude more sensitive than the light levels reported by Mandoli and Briggs (1981) to be necessary to evoke a threshold "very low irradiance response" in Avena.

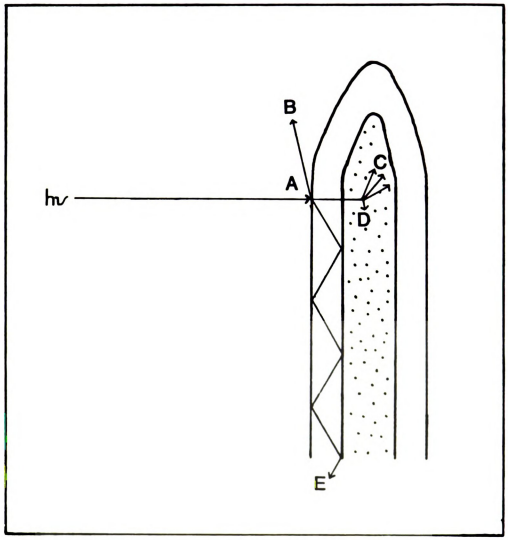

Figure 3.3. Light incident on a coleoptile (A) can follow a number of different paths. Some (B) is reflected or scattered at the surface, and never enters the tissue. Of the light which enters the tissue, some is scattered randomly (C), some is absorbed (D), and some may be scattered axially, or "piped" (E).

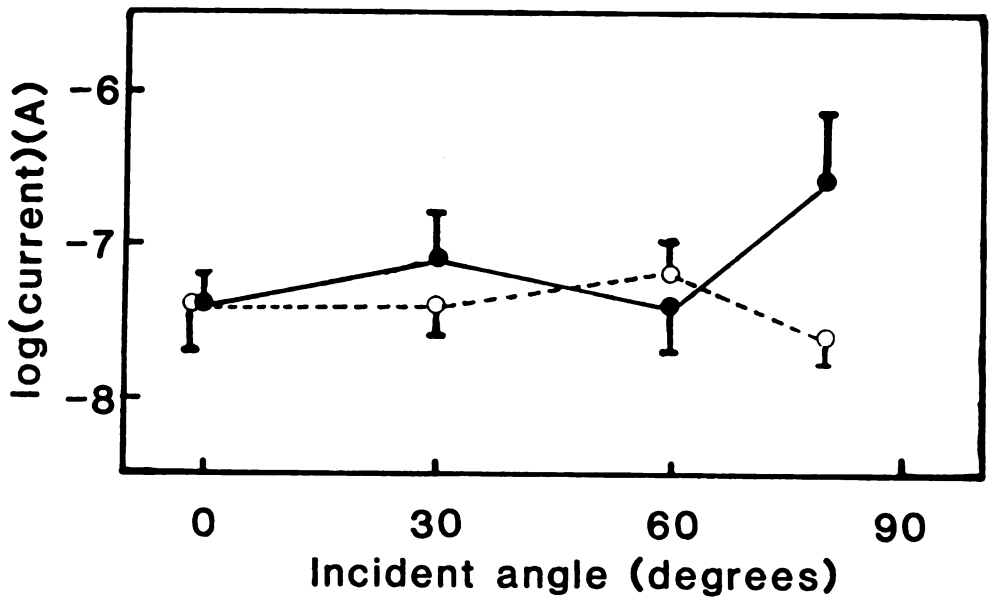

Figure 3.4. The apex of a coleoptile segment was illuminated with red light at the indicated incident angle (degrees from normal). No angle dependence was found. Closed circles: control tissue. Open circles: 2×10^{-4}M SAN 9789 treated tissue. Error bars: standard error of the mean.

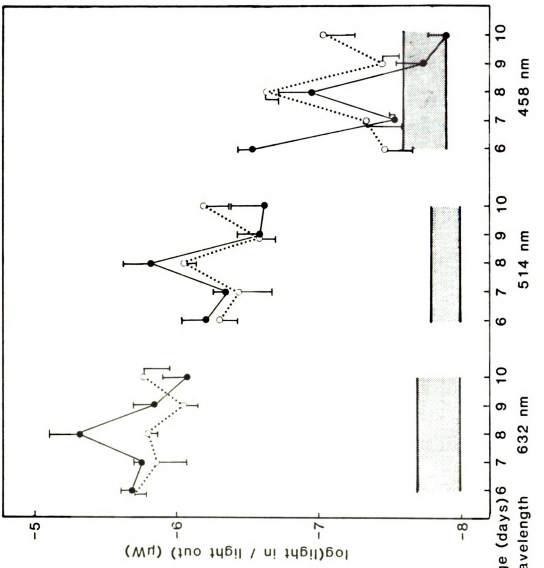

Figure 3.5. Longitudinal light transmission. Transmission of three wavelengths of light was measured through 2.5 cm segments of corn seedlings of indicated ages. Control seedlings (●——●), 2 x 10^{-4}M SAN 9789 treated seedlings (○·····○). Shaded areas represent the range of dark current over the several days needed to collect data. Error bars: standard error of the mean.

There is some age-dependence to the longitudinal transmission of light, though the dependence is not direct. Differences attributed to age may be due to mean cell length, the degree to which tissues were hydrated, or some other factor not directly measured.

More interesting is the relative transmission of red, green, and blue light through 2.5 cm of tissue. Acceptance losses should be equal for all three wavelengths of light, so a comparison based on wavelength shows the relative efficiency with which each wavelength is "piped". Red light is transmitted most efficiently, but over 2.5 cm is attenuated 5.3 to 6.1 orders of magnitude (Figure 3.5). Over the same length of tissue, green light is attenuated 5.8 to 6.6 orders of magnitude, and blue light between 6.5 and 7.9 orders of magnitude. The final blue light intensity is very low, approaching the limit of measurement. It seems unlikely that such extremely low intensities of transmitted blue light would affect a plant's phototropic response when a much stronger stimulus is present elsewhere along the shoot. Red light, on the other hand, may be transmitted strongly enough to have some physiological effect; Parks and Poff (personal communication) find that 0.4 W x cm^{-2} red light incident at one point in a corn shoot can cause conversion of phytochrome at a location at least 1.4 cm away.

Absorption and/or scatter are potential mechanisms to explain the additional attenuation of blue light over red.

To test the possibility that absorption by carotenoids affected the longitudinal transmission of blue light, transmission by seedlings treated with 2×10^{-4}M SAN 9789 was also measured. No significant difference between SAN 9789 treated and control tissue was found (Figure 3.5, Table 3.1). Measurements of the transmission of green light were included as a second method to try to separate attenuation by absorption from scatter. Green light should be absorbed very little, so if absorption were the source of the attenuation, transmission of green light should resemble that of red. On the other hand, green light should be scattered similarly to blue light. A statistical analysis using orthogonal contrasts showed that the transmission of red, blue, and green light are all different from one another. Transmission of green light is intermediate to that of red and blue, so that it is not particularly useful in distinguishing between scatter and absorption as mechanisms of attenuation. The similarity of light transmission in control and SAN 9789-treated tissue eliminates absorption as a source of the additional attenuation of blue light, leaving scatter as an alternative.

An analysis of variance (Table 3.1) shows that the wavelength of light and the age of the tissue both have a highly significant effect on the longitudinal transmission of light. Interactive effects of light x age, also highly significant, may be due to the tendency of SAN treated

Table 3.1. Analysis of variance of data for longitudinally transmitted light. Data shown in Figure 3.5.

source	df	F
treatments	29	18.07**
light	2	208.65**
age	4	13.34**
SAN 9789	1	1.05
light x age	8	0.827
light x SAN 9789	2	1.285
age x SAN 9789	4	5.787**
light x age x SAN 9789	8	2.49*

*, ** significant at 0.05 and 0.01 levels, respectively.

seedlings to accumulate pigments as they age. Third order interactions (light x age x SAN 9789) are statistically significant, but difficult to explain.

In order to estimate transmission losses with distance, sequential measurements were made from a number of individual seedlings. The tip of a seedling of known length was irradiated with 632 nm light, and transmission was measured. A segment was sliced from the bottom of the seedling, and transmission was measured again. Since acceptance losses are the same for all measurements, the slope of the line plotted for attenuation with distance gives an accurate measure of the density of the tissue. The mean slope for control seedlings (Figure 3.6) is -0.74 A, for 2 x 10^{-4}M SAN 9789 treated seedlings, -0.82 A (Figure 3.7).

Mandoli and Briggs (1982b) have measured light transmission from the proximal and distal sides of a unilaterally irradiated coleoptile, and find a higher intensity on the distal side, when irradiating along the short axis. They suggested that, due to the shape of the coleoptile tip, unilateral light was closer to the acceptance angle of the cells on the distal side, such that they intercept and transmit more light. However, Vogelman and Haupt (in press), using a fiber optic to probe the distribution of blue light across a corn coleoptile found that light intensity was greatest on the proximal side of the plant for all angles measured.

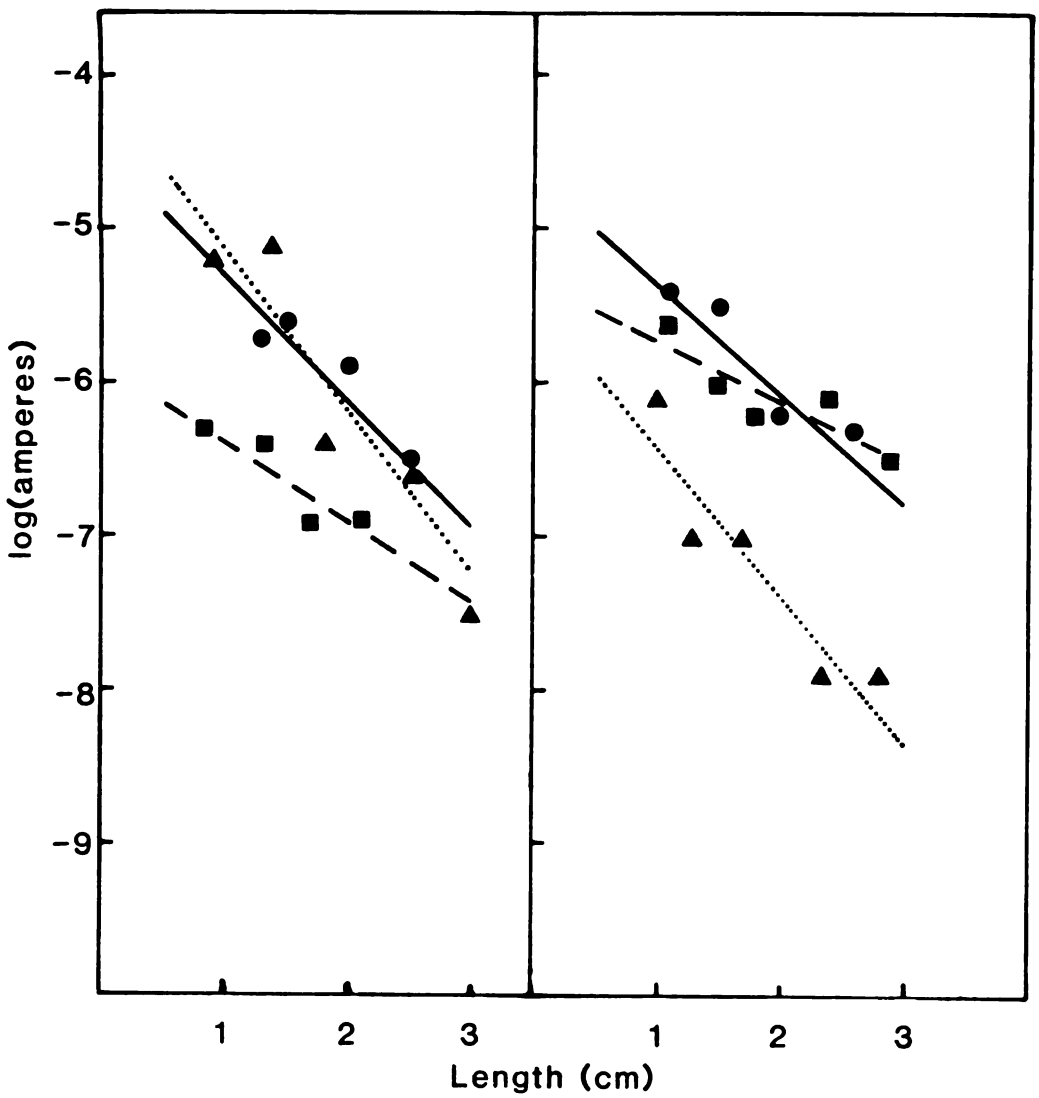

Figure 3.6. Longitudinal transmission of red light as a function of tissue length. Control seedlings. Transmitted light was measured in terms of current from a photomultiplier tube. Each set of symbols represents measurements taken from one individual, with a best fit line through each set of data. Data separated along x axis for clarity. Mean slope -0.74.

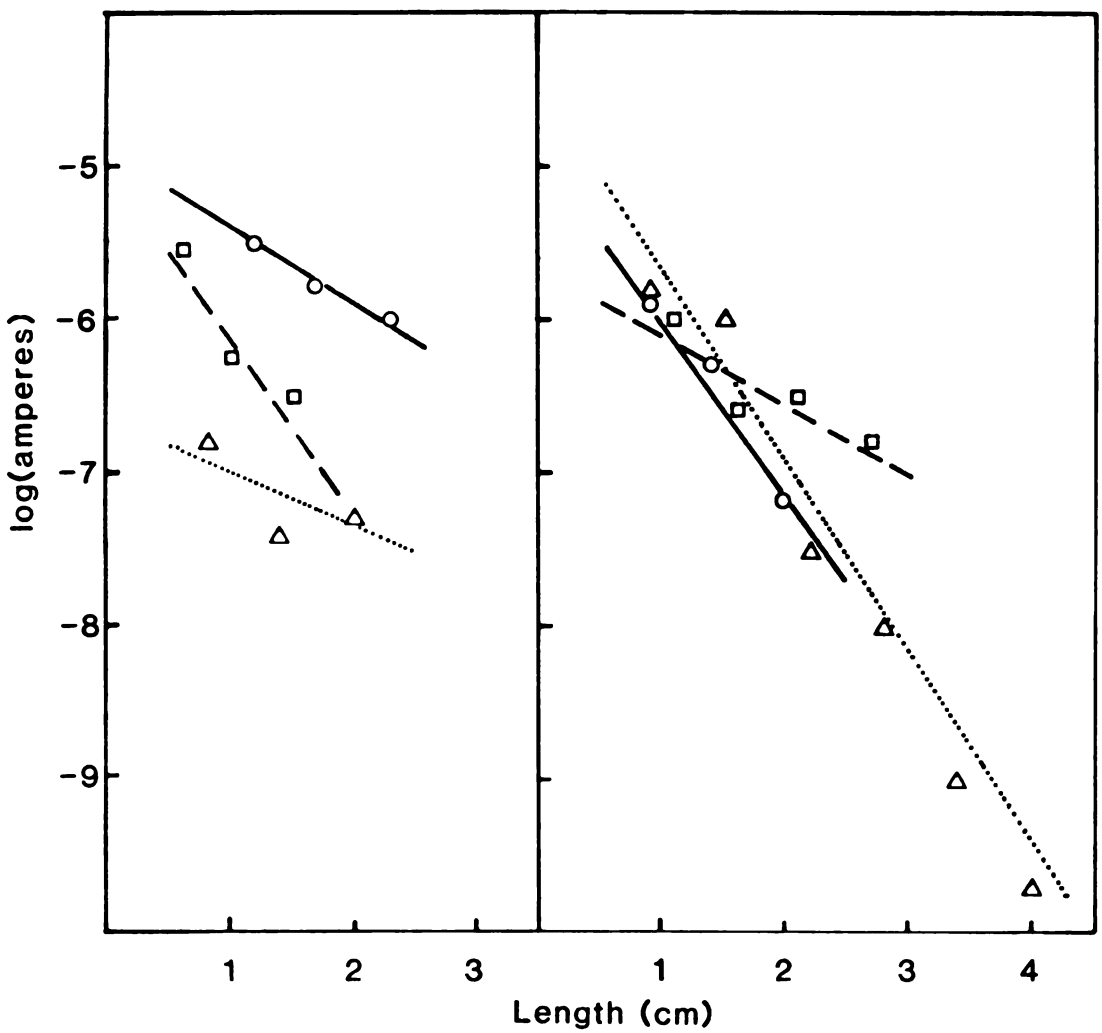

Figure 3.7. Longitudinal transmission of red light as a function of tissue length. 2 x 10^{-4}M SAN 9789 treated seedlings. Transmitted light was measured in terms of current from a photomultiplier tube. Each set of symbols represents measurements taken from one individual, with a best fit line through each set of data. Data separated along x axis for clarity. Mean slope -0.82.

If "piping" contributes to the light gradient for phototropism, it seems likely that it will be because of an effect on the way light is intercepted by the plant, rather than because of transmission over a distance. Blue light is attenuated quickly, so probably does not contribute much to a gradient of light distant from the point of incidence.

Introduction : Scatter

Manipulation of the scattering properties of a plant affect the plant in other physiologically important ways. Parsons et al. (1984) have estimated the contribution of scattered light to perception of direction by measuring attenuation through different thicknesses of tissue. They have reduced scatter, and thus the gradient of light across a shoot, by infiltrating tissue with oil of high refractive index.

Seyfried and Fukshansky (1983) have developed a method for calculating light gradients in layered tissue. They point out that measuring light inside an object, although necessary, interferes with the optical properties being measured. Their model allows estimates of light gradients to be made unobtrusively. Using a similar method, Seyfried and Schaefer (1983) monitored changes in reflectance and transmittance of Cucurbita cotyledons giving special attention to scattering properties of the tissue.

In a corn coleoptile, attenuation due to scatter comes not only from light scattered off molecules and other cellular components, but also from refractive changes as

each ray of light moves from cell to cell. A model of the attenuation of light due to scatter by refraction is presented by Parsons et al. (1984). They argued that most of the light gradient in a coleoptile was due to refraction and that absorption played a relatively minor role.

Methods

Measurements of light attenuation due to scatter and absorption in different tissues of corn seedlings were made with a modified Cary 14 spectrophotometer. Spectra were generated as in Chapter 1. In addition, measurements of current were taken every 10 nm from a picoammeter connected to the photomultiplier tube. Similar measurements were made without any sample in place for the system response. The ratio of system / sample gives a measure of total attenuation by the sample.

Results and Discussion

Total attenuation (absorption plus scatter) can be measured directly for various tissues of a seedling. When plotted as a ratio of system response to sample vs wavelength, data corresponding to absorption spectra are generated (Figure 3.8 - 3.12). The location along the y-axis is a function of absolute attenuation. Although absorption of shoot and mesocotyl tissue differs, total attenuation by these two tissues is quite similar in both dark and red-grown, control and SAN 9789-treated tissue. Variations in absorption in seedlings grown under different conditions are apparent, but there also appears to be a

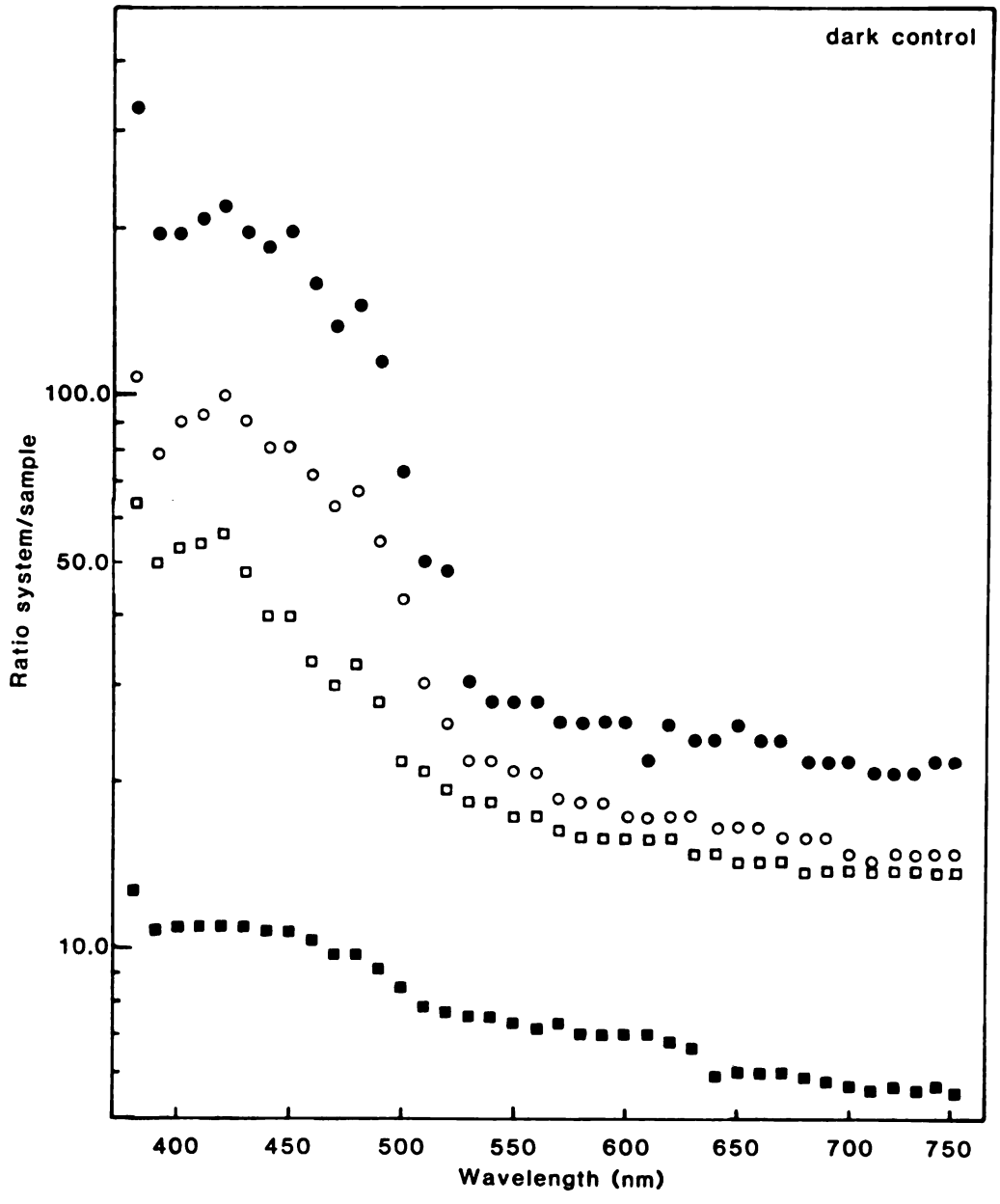

Figure 3.8. Total attenuation of light (absorption plus scatter) by various tissues of a completely etiolated control seedling. (●) Tip of seedling through coleoptile and primary leaf. (○) Base of seedling through coleoptile and primary leaf. (□) Mesocotyl. (■) Excised coleoptile. Measurements of current on the photomultiplier tube were used to calculate the ratio of system / sample.

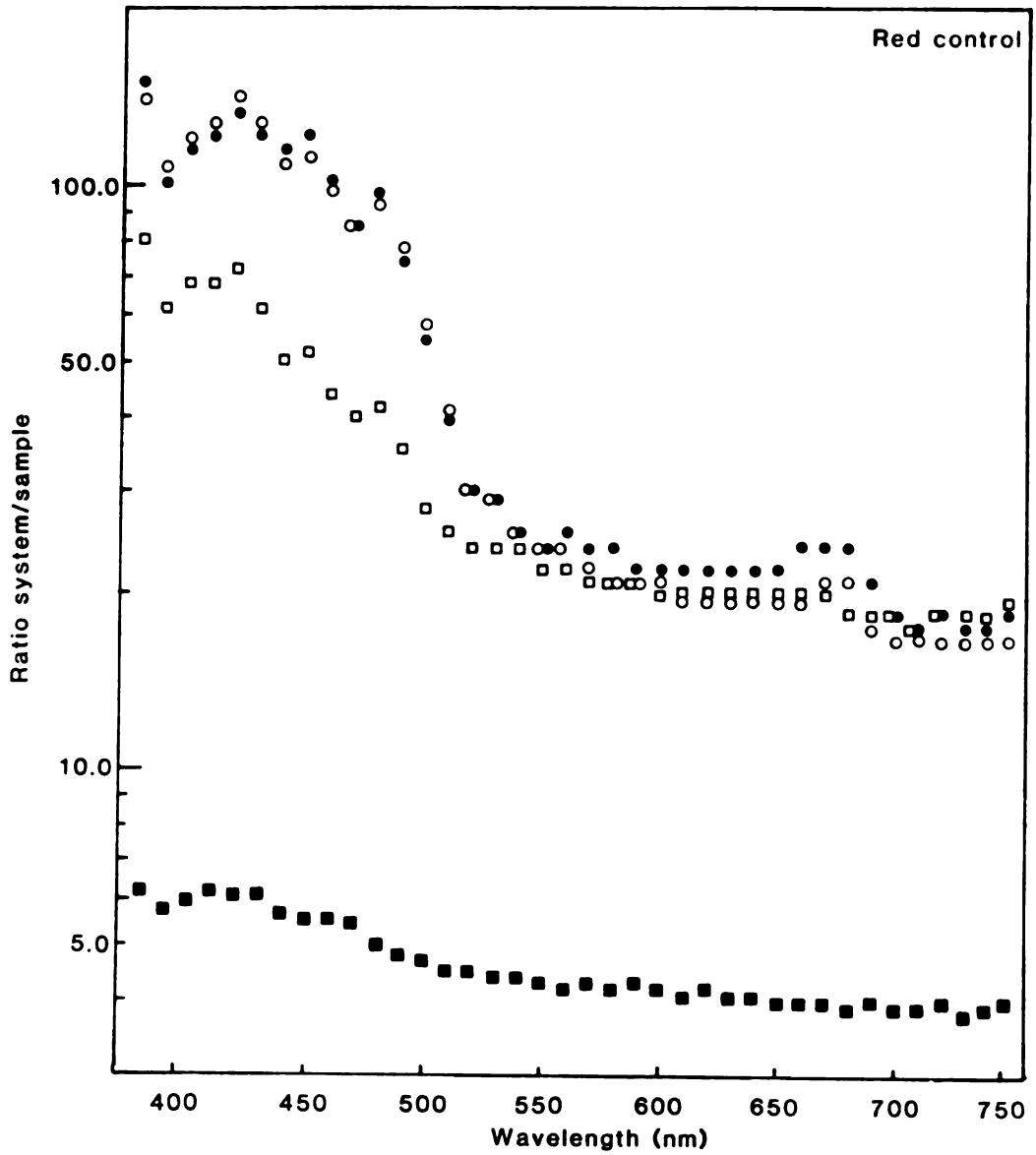

Figure 3.9. Total attenuation of light (absorption plus scatter) by various tissues of a control seedling grown with one hour of red light per night. (●) Tip of seedling through coleoptile and primary leaf. (○) Base of seedling through coleoptile and primary leaf. (□) Mesocotyl. (■) Excised coleoptile. Measurements of current on the photomultiplier tube were used to calculate the ratio of system / sample.

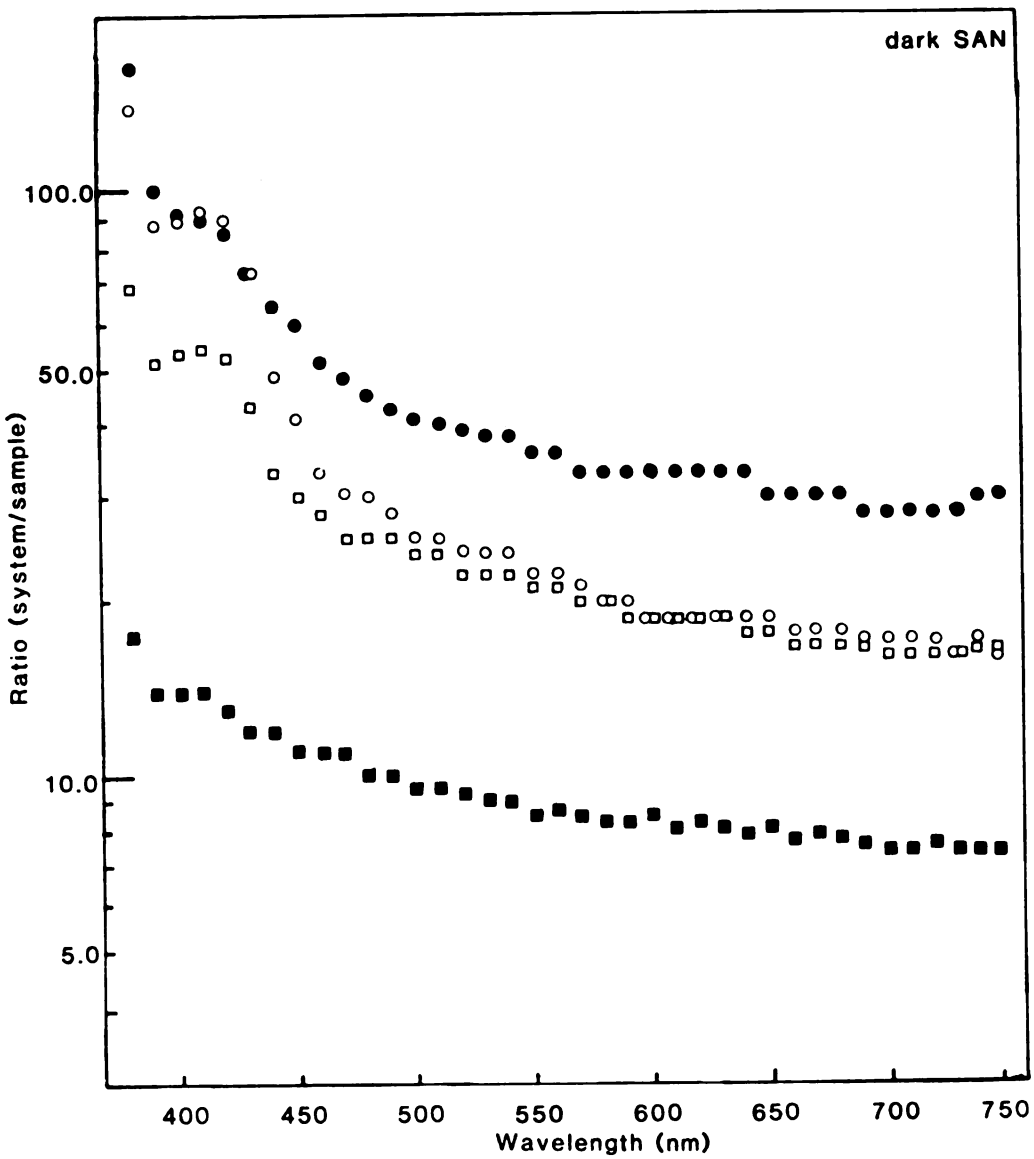

Figure 3.10. Total attenuation of light (absorption plus scatter) by various tissues of a 2 x 10^{-4}M SAN 9789 treated, dark grown seedling. (●) Tip of seedling through coleoptile and primary leaf. (○) Base of seedling through coleoptile and primary leaf. (□) Mesocotyl. (■) Excised coleoptile. Measurements of current on the photomultiplier tube were used to calculate the ratio of system / sample.

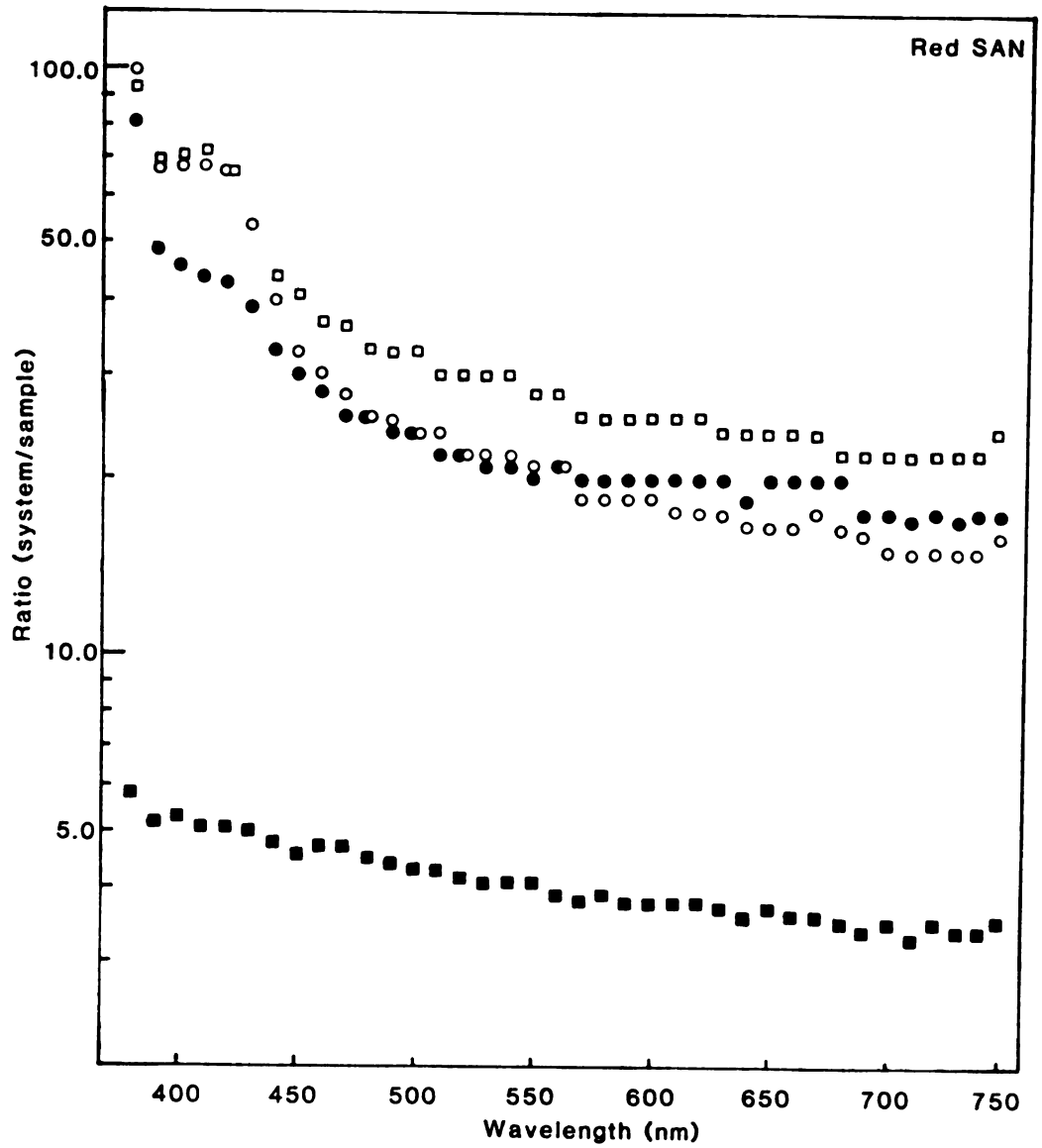

Figure 3.11. Total attenuation of light (absorption plus scatter) by various tissues of a 2 x 10^{-4}M SAN 9789 treated seedling grown with one hour of red light per night. (●) Tip of seedling through coleoptile and primary leaf. (○) Base of seedling through coleoptile and primary leaf. (□) Mesocotyl. (■) Excised coleoptile. Measurements of current on the photomultiplier tube were used to calculate the ratio of system / sample.

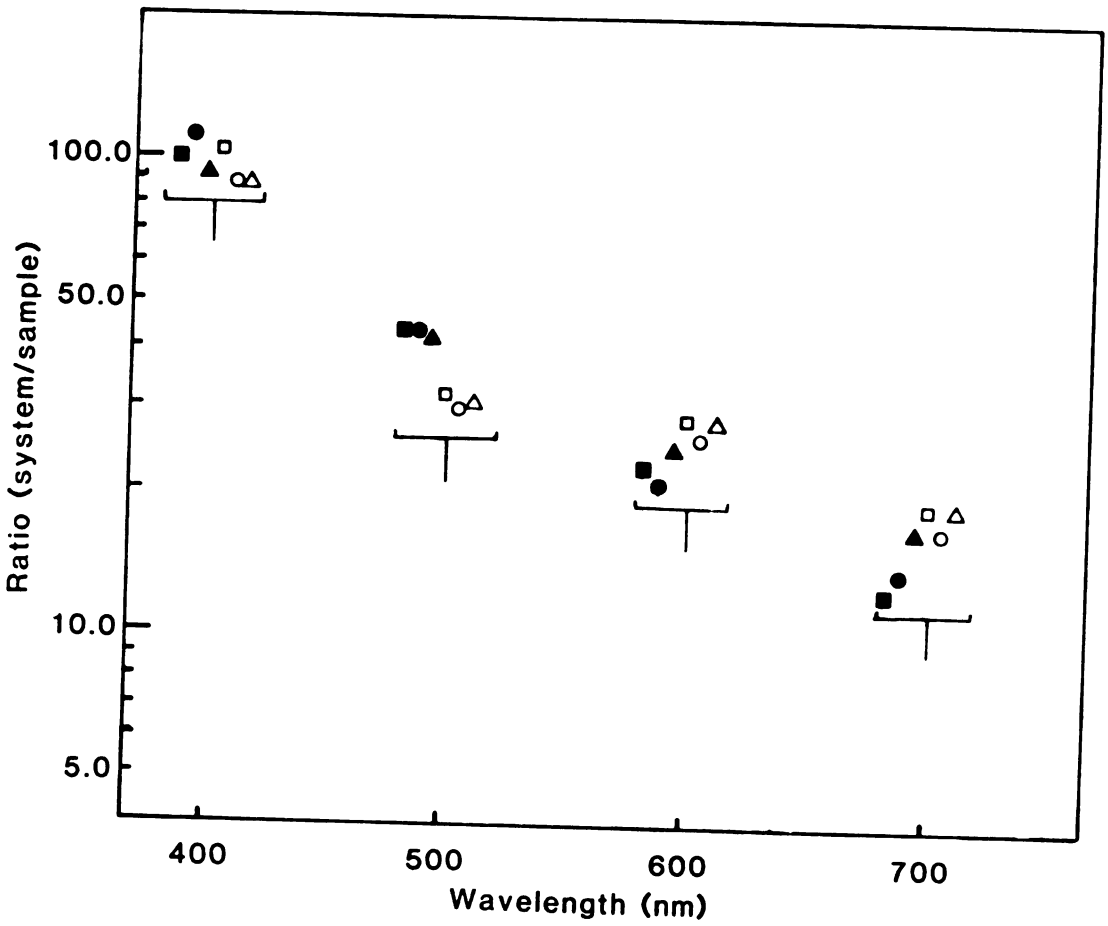

Figure 3.12. Total attenuation of light (absorption plus scatter) by shoot tips of control, SAN 9789 treated, yellow and pale mutant seedlings at selected wavelengths. (■)Control. (●)lw1 yellow. (▲)lw2 yellow. (□) 2 x 10^{-4}M SAN 9789 treated. (○)lw1 pale. (△)lw2 pale.

constant contribution to light attenuation by scatter, independent of growth conditions.

Estimates of the portion of attenuation of light by a corn seedling which is due to absorption can be made from computer-generated difference spectra. By subtraction of a baseline "scatter spectrum" from a tissue spectrum such an estimate can be given. There are three possible ways to generate a baseline (Figure 3.13).

1. A line congruent with the part of the spectrum which shows no peaks could be drawn and extended through wavelengths where absorption occurs (Figure 3.13A). Attenuation above this baseline is attributed to absorption, below, to scatter. This method overestimates the amount of absorption in the blue, since the baseline has a constant slope. In reality, shorter wavelengths of light scatter more than longer wavelengths, giving a curved baseline rather than a straight line. Using this method, the seedling represented in Figure 3.13A has an apparent absorption at 470 nm of 0.52 A.

2. The scatter ramp can be approximated by generating a baseline spectrum using a highly-scattering, non-absorbing substance such as layered Kimwipes (Figure 3.13B). This gives a curve of the appropriate shape, but it is difficult to choose the proper thickness of Kimwipes which will most accurately approximate the amount of scatter seen in plant tissue. Apparent absorption at 470 nm is 0.39 A.

3. Plant tissue can be used to approximate scatter, if it does not absorb blue light. Mesocotyl tissue, excised coleoptiles, carotenoidless mutants, or SAN 9789 treated tissue, of a thickness similar to the pigmented sample, can be used (Figure 3.13 C, D, and E, respectively). This method would tend to underestimate the contribution of absorption, since such tissue is not completely pigmentless. Residual pigments (carotenoids, flavins, or cytochromes) which are present in the tissue used for the baseline are subtracted from the sample. The tissue chosen for the baseline changes the shape of the absorption spectrum. At 470 nm, apparent absorption using mesocotyl tissue as a baseline gives an an absorption of 0.32 A, coleoptile tissue 0.45 A, and SAN 9789 treated tissue 0.41 A. A wild type (yellow) lw2 shoot, using a mutant (pale) shoot as a baseline, has an apparent absorption of 0.29 A (Figure 3.13F).

The actual contribution of absorption to total light attenuation probably falls between the approximations provided by method one and method three above, that is, between 0.3 A and 0.5 A at 470 nm.

With a measurement of total light attenuation and an approximation of the attenuation due to absorption, it is possible to estimate the contribution of scatter to the light gradient. At 450 nm, scatter accounts for about 1.5 A of the attenuation across the shoot (Table 3.2) in both control and SAN 9789 treated tissue. Treatment with

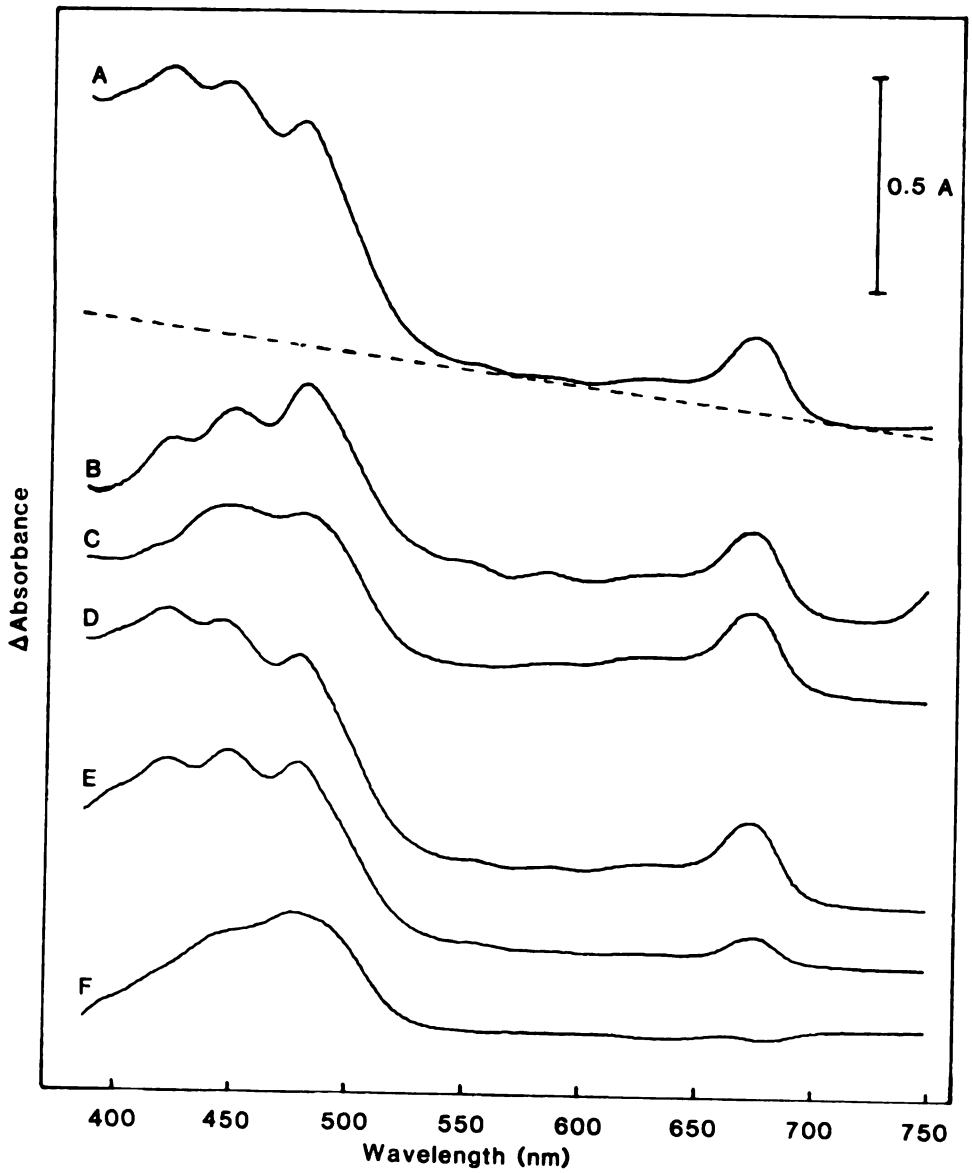

Figure 3.13. Approximating a scatter ramp artificially or with plant tissue low in pigment. A) control shoot plus ramp (dashed line). B) control shoot minus 14 layers of Kimwipes. C) control shoot minus mesocotyl. D) control shoot minus excised coleoptile. E) control shoot minus 2×10^{-4}M SAN 9789 treated shoot. F) lw2 yellow shoot minus lw2 pale shoot.

Table 3.2. Mean optical density at 450 nm of two randomly selected corn seedlings. Shoot tip measurements were taken through both coleoptile and primary leaf tissue. Coleoptile measurements were made on excised coleoptiles. Red grown seedlings received one hour of red light per night. Dark grown seedlings were completely etiolated. Data were obtained from measurements made for Figures 3.8 - 3.11.

tissue	light treatment	herbicide treatment control	herbicide treatment 2×10^{-4} M SAN 9789
shoot tip	red grown	2.1	1.5
shoot tip	dark grown	2.15	1.6
coleoptile	red grown	0.8	0.75
coleoptile	dark grown	0.95	0.95

SAN 9789 does not affect the light scattering properties of the plant. By subtraction of the optical density of an excised coleoptile from an intact shoot, an estimate of the attenuation of the primary leaf can be obtained. In control plants, the primary leaf is 1.3 A, in SAN 9789 treated plants, 0.75 A. Scattering properties of lw1 and lw2 seedlings are comparable to control and SAN 9789 treated hybrid seedlings (Figure 3.12).

Thus, it appears that scatter and absorption in corn seedlings establishes a gradient of light for first positive phototropism. The shoot tip as a whole is not sufficiently transparent to allow a "lens effect". As in second positive phototropism, light incident on a seedling will be attenuated by absorption and scatter, giving a gradient with the highest intensity on the proximal side of the plant.

CONCLUSIONS AND RECOMMENDATIONS

A method has been developed to monitor the light attenuation and pigment distribution in individual seedlings. Using this method, the distribution of bulk pigments in corn seedlings from different genetic lines and from different growth regimes has been mapped. Total light attenuation by various tissues of the seedlings has also been measured. The contributions of absorption and scatter to the gradient of light for first positive phototropism have been assessed.

The possibility that different photoreceptor pigments or different mechanisms for establishing a gradient of light are used in first positive and in second positive phototropism has been raised (Poff, 1983). Evidence presented in this thesis supports the hypothesis that there is no such fundamental difference between first and second positive phototropism (see Chapter 3). Instead, it appears that carotenoids play a similar role in first positive to that in second positive, and that the gradient for detecting a unilateral light stimulus is established in the same way for both responses.

Carotenoids act as screening pigments, rather than photoreceptor pigments, in first positive phototropism. SAN 9789 treated seedlings with an 80 - 90% reduction in carotenoid content show only a 40% reduction in response. The response of carotenoidless mutants is more variable, but data presented here show that albino seedlings with a 70% reduction in carotenoids show a 60% reduction in response. Thus, it seems likely that the photoreceptor pigment remains unaffected by manipulations of the carotenoid content. The reduced response is due to a reduced gradient of light across the coleoptile.

Attenuation is the mechanism used to establish the gradient of light for first positive phototropism. Even the most transparent part of a corn seedling, the apical 0.5 mm of the shoot tip, is too dense to allow refraction to produce a "focusing advantage". Instead, light incident on a seedling is absorbed and scattered as it passes through the tissue. "Piping" effects may modify this distribution.

Absorption by screening pigments contributes 20 - 25% of the total gradient of light across the seedling (approximately 0.5 A out of a total 2.0 - 2.5 A). The remaining 75% is due to scatter. However, based on experiments with SAN 9789 treated seedlings, it appears that the 25% of the gradient established by carotenoid absorption mediates about 40% of the response. This discrepancy raises the possibility that absorption and scatter do not contribute additively to the gradient of light. It is

possible that a threshold level of attenuation, established by scatter, exists. If this is so, it should be possible to find a very low intensity of light which evokes no phototropic response, regardless of the length of time for which it is presented.

Within a plant, the amount of light scattered is intrinsic to the tissue, and is largely a function of changes in refraction as light moves through cytoplasm, vacuoles, and cell walls (Parsons et al., 1984). Changes in pigment content, on the other hand, are a response to light and are controlled by the metabolism of the plant. It is possible that a seedling could use absorption as a method of "fine tuning" to control its response or level of adaptation to light.

Recent work on "light piping" (Mandoli and Briggs 1982a and b, 1984) and on the distribution of light within plant tissue (Seyfried and Fukshansky, 1983, Seyfried and Schafer, 1983, Parsons et al., 1984, Vogelman and Haupt, in press) has led to consideration of light gradients along the long axis of the shoot, as well as transverse light gradients. Data presented here show that light is attenuated more quickly when traveling transversely through shoot tissue than when traveling longitudinally along the seedling. Shoot tissue attenuates red light at a rate of 0.7 - 0.8 A per cm in the longitudinal direction. Blue light is attenuated more quickly. Because blue light is attenuated so quickly, no measurement of longitudinal optical density

in the blue was made. Axially, shoot tissue is approximately 6.5 - 7.5 A per cm in the red, 10 A per cm in the blue. These values are an overestimate since they include light losses at the surface of the tissue. Still, shoot tissue attenuates light up to 10 times more per cm in the transverse than in the longitudinal direction.

The contribution of absorption to total attenuation has been measured in the transverse direction. Longitudinally, it appears that absorption does not contribute to the gradient of light; however, given the variability of the data, a small difference due to absorption could have been missed. Most absorption occurs in the primary leaves, which have been shown to transmit little light longitudinally (Mandoli and Briggs, 1982a). This could account for the apparent lack of contribution of absorption in altering the longitudinal transmission of light. It is also possible that absorption contributes proportionately less to the longitudinal than to the transverse gradient of light.

A normally pigmented control shoot is about 2.0 to 2.5 A in the blue. The range of the first positive response, from threshold to indifferent or first negative, is about two orders of magnitude. If there is a correlation between light attenuation and the breadth of first positive response, then plants which attenuate light differently should show correspondingly different widths of doses for first positive phototropism. Such a correlation could be based on excitement of the photoreceptor pigment or another

component of the transduction chain. As dose increases, the ratio of excited component in the front to that in the back would increase until saturation was reached in the front half. This would correspond to the peak of first positive response, and could be expected to depend in some way on the rate at which incident light is attenuated. As light dose increases beyond the peak, the excited component in the front half would remain saturated, but an increasing portion of the component would be excited in the back half, decreasing the gradient and the response. When all of the component was excited, no phototropic response would be seen. A first step in testing this model would be to look at correlations between the width of the dosage range for first positive phototropism and the total attenuation of light by a variety of plants with different total attenuations. If such a correlation were important, one might expect SAN 9789 treated seedlings to show a narrower peak of first positive response than control seedlings. From data presented here, it appears that SAN 9789 treated and control tissue show the same breadth of response. SAN 9789 treatment alters only the height of the peak. An experiment with phototropically responsive plants which attenuate much more (or much less) light than control or SAN 9789 treated corn is appropriate.

Further work with "carotenoidless" mutant tissue is desirable. A better defined dose response curve and more replications to minimize the effect of variable responses

are needed. Further comparisons between carotenoidless seedlings generated from mutant stock and from SAN 9789 treatment would help to insure that the reduced response of pale seedlings is a result of carotenoid concentration and not some pleiotropic effect.

The difference between transverse and longitudinal transmission of light could be carried out further. A first step would be to measure acceptance losses from the surface of a transversely illuminated seedling, or to eliminate such losses from calculations of attenuation by measuring the density of different thicknesses of tissue (similar to Parsons et al. 1984).

To see whether cell elongation has an effect on longitudinal transmission of light, transmission through dwarf (compact cells), gibberellin treated (elongated cells) and normal tissue could be compared. Such measurements would also help define how much of the difference in longitudinal and transverse light transmission is due to cell geometry.

Changes in pigmentation and possible correlations to phototropic capability could be monitored. Does the pigmentation of a seedling change after exposure to a first or second positive dose of light? Would such changes affect its subsequent phototropic capability? A normal hybrid population of corn seedlings varies both in pigmentation and in phototropic response. How closely linked are these variations? On a simpler level, it has been observed that

completely etiolated dicots do not respond to phototropic stimuli. Monitoring the synthesis of pigment and the appearance of phototropic capability might help to establish how large a gradient of light is necessary for phototropism to occur.

Attenuation due to scatter and absorption is the source of the gradient of light necessary for a plant to detect unilateral light stimuli. This is true for both first and second positive phototropism. Further studies of light gradients, along with other components of the transduction pathway, are necessary for a complete understanding of phototropism.

APPENDICES

APPENDIX 1

Seed Multiplication of "Carotenoidless" Mutants

"Carotenoidless" mutant corn lines lw1, lw2, clp, and w7748 show simple dominance. None of the four lines are allelic (Robertson, personal communication). Phenotypically, double recessive seeds have a white or pale yellow endosperm, and germinate to give white seedlings lacking much of the normal complement of carotenoids. Two-thirds of the yellow seeds from these lines should be heterozygous for the "carotenoidless" allele.

Approximately forty yellow seeds from each of the four lines (generous gift of Dr. D.S. Robertson, Iowa State University, Ames, Iowa) were planted on August 31 - September 1, 1983. Each seed was planted in an 8-inch diameter plastic pot in Sunshine Peat Complete mix (micronutrients added to the peat), and placed in a greenhouse. Daylight was supplemented by 1000 W metal halide or 400 W high pressure sodium lamps. Light intensity in the greenhouse in full sun, but away from any lamps, was 0.06 $W \times cm^{-2} \times sec^{-1}$. Intensity about 30 cm below a metal halide lamp was 0.07 $W \times cm^{-2} \times sec^{-1}$, and 0.08 $W \times cm^{-2} \times sec^{-1}$ below a sodium lamp. Intensities were

measured using a Kettering radiometer (model 7720, Yellow Springs, Ohio). Daylength was 16 hours (6 am to 10 pm). Temperature was maintained at or above 80° F (27° C). Plants were watered daily with half-strength modified Hoagland's solution from a dripline (see Table A1.1). Plants were sprayed periodically with Pirimore to control aphids, and with a mix of Paramex and Pentac to kill spider mites.

The first pollinations were performed November 3, 1983. Tassels which were shedding pollen were covered with a paper bag held in place with a paper clip. The husks surrounding an ear were clipped just above the ear once silks had appeared, and the ear covered with another paper bag. Pollen shaken into tassel bags was poured directly on silks. Pollinations were performed between 0800 and 0930, in order to use the pollen shortly after it had been shed, since pollen remains viable for only 10 - 30 minutes after shedding (Neuffer, 1982). A given ear was pollinated on several successive days, until silks began to dry. Crosses were made over a period of three weeks. Plants were then allowed to dry, and ears were harvested two to three weeks after the end of pollination. Yields are summarized in Table A1.2.

Whenever possible, plants were self-pollinated. However, self-pollination was not always possible, either because a plant was male-sterile, or because the tassel had matured before silks appeared on the ear. If a plant had receptive silks but no pollen, pollen from another plant of

the same line was used for fertilization. For three of the four lines, lw1, lw2, and w7748, production of silk and pollen were synchronous enough for several successive fertilizations to be made. Plants of the fourth line, clp, were very slow to produce ears. To extend the availability of pollen, excess pollen was stored in sealed vials at 4^{o} C and high humidity or in liquid nitrogen. No seed of the clp line was set from either fresh or stored pollen, so it is impossible to say whether storage would have been useful.

Table A1.1. Modified half-strength Hoagland's solution (E. Light, personal communication).

Nutrient	grams per liter stock solution
Solution A (pH 4.5)	
$Ca(NO_3)_2 \cdot 4H_2O$	295.0
NaFe, 13% or EDTA	38.44
Solution B (pH 3.5)	
KH_2PO_4	34.25
KNO_3	126.65
$MgSO_4 \cdot 7H_2O$	126.65
$ZnSO_4 \cdot 7H_2O$	0.056
$MnSO_4 \cdot H_2O$	0.391
$CuSO_4 \cdot 5H_2O$	0.021
H_3BO_3	0.725
$MoO_3 \cdot 2H_2O$	0.005

Both stock solutions A and B were used at 1:500, and the pH of final solution adjusted to 6.0 - 6.4 using about 0.40 ml 1N KOH.

Table A1.2. Yield of greenhouse-grown "carotenoidless" corn

line	total ears	heterozygous ears	number of pale seeds
clp	0	0	0
w7748	4	2	27
lw1	8	3	45
lw2	11	8	134

Only seeds from lw1 and lw2 were used in subsequent experiments.

APPENDIX 2

Geotropism of Corn Seedlings at 60% and 100% Relative Humidity

Humidity is important to a plant's response to phototropic stimuli (Nathansohn and Pringsheim, 1908). SAN 9789 treated plants, which wilt easily, seemed especially sensitive to humidity. Table A2.1 presents the results of a number of experiments which contrasted the response of control, 2 x 10^{-5}M, and 2 x 10^{-4}M SAN 9789 treated plants to 60% and 100% relative humidity. Based on these results, subsequent experiments were performed in 100% relative humidity (RH).

Table A-1 Humidity effects on control and SAN 9789 treated corn seedlings. Means compared using Student's t test.

treatment	mean curvature (degrees) 60% RH	100% RH	df	t
control	39.0	53.6	23	4.74**
2 x 10^{-5}M SAN 9789	35.7	55.1	5	3.65**
2 x 10^{-4}M SAN 9789	22.7	59.4	20	11.10**

*, ** significant at 0.05 and 0.01 levels.

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