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1997**Title: Interaction of Light and Ethylene on Stem Gravitropism****P.I.: Dr. Marcia A. Harrison****Grant #: NAGW 3859****Date: October, 6, 1996 Final Report**

The major objective of this study was to evaluate light-regulated ethylene production during gravitropic bending in etiolated pea stems. Previous investigations indicated that ethylene production increases after gravistimulation and is associated with the later (counter-reactive) phase of bending. Additionally, changes in the counter-reaction and locus of curvature during gravitropism are greatly influenced by red light and ethylene production. Ethylene production may be regulated by the levels of available precursor (1-aminocyclopropane-1-carboxylic acid, ACC) via its synthesis, conjugation to malonyl-ACC or glutamyl-ACC, or oxidation to ethylene. I examined the regulation of ethylene production by quantifying ACC and conjugated ACC levels in gravistimulated pea stems. I also measured changes in protein and enzyme activity associated with gravitropic curvature by electrophoretic and spectrophotometric techniques. An image analysis system was used to visualize and quantify enzymatic activity and transcriptional products in gravistimulated and red-light treated etiolated pea stem tissues.

Analysis of ACC, and Conjugate ACC levels during gravitropic bending in green-pretreated and red-irradiated etiolated pea stems

Red-light pretreatment (18 hours prior to experimentation) of etiolated seedlings, results in a shift in the locus and overall kinetics of upward bending. My previous research indicated that light-induced reduction of ethylene production is at least partly responsible for the altered growth pattern observed along the stem. Comparison of the ACC levels in etiolated and red-pretreated pea segments before gravistimulation revealed that both treatments had approximately the same amount of ACC. After gravitropism both treatments showed an increase in ACC level. By 120 min, the red-treated level increased to $0.0371 \text{ nmol g}^{-1} \text{ ACC}$ and the etiolated to $0.0249 \text{ nmol g}^{-1} \text{ ACC}$. An appreciable increase in ACC level in red-treated stems is seen beginning at 90 min ($P = 0.02$) and continuing through 120 min gravistimulation ($P = 0.004$). The level of conjugated ACC was higher ($P = 0.032$) in etiolated seedlings compared to red-treated. However, after gravistimulation no significant difference is apparent between the two treatments. These data imply that etiolated seedlings exhibit an overall decrease in conjugated ACC content during gravistimulation.

Since ACC increases during the later phase of gravitropic curvature in red-pretreated stems, the differences in ACC and conjugated ACC levels were investigated in the upper and lower halves of the red-treated pea segments. Although ACC levels increase during the later phase of curvature in red-treated stems, there was not difference between the ACC levels in upper and lower stem halves. However, there was a greater level of conjugated ACC accumulation in the lower than in the upper, but the difference was not significant ($P = 0.15$) at 60 min gravistimulation. A significant accumulation of conjugated ACC is seen in the upper compared with the lower stem tissue at 120 min ($P = 0.04$).

Overall, it was concluded that ethylene biosynthesis is altered throughout the stem during gravitropism. Increased ACC in the stem is consistent with the reports of stimulated ethylene biosynthesis during the later phases (90 to 120 min after stimulation) of stem gravitropic curvature.

Conjugations of ACC also appears to be regulated after gravistimulation and the rate of conjugation is different in the upper compared with lower side of the curving stem. Ultimately, regulation of ACC level affects the overall ethylene production of the stem. Thus, ethylene's effect on the growth rate during the later phase of bending may be due to altered response of the growing cells to the hormone. This is consistent with hormone models that propose that response to a hormone is dependent on the target (enzyme/gene) of the cellular transduction system. In our case, ethylene may cause the migration of curvature or net slowing of growth in the counter-reaction primarily in the rapidly growing cells of the lower side of the stem. The slowly growing cells of the upper surface may respond differently or to a lesser effect. Thus, the target of ethylene action during gravistimulation or after red-light irradiation (e.g., cell wall enzymes, phosphorylated proteins) is the focus of subsequent and future research.

Putative red light- and gravity-regulated proteins during gravitropism in etiolated pea stems

Spectrophotometric analysis of peroxidase activity indicated no significant change between and within the light treatments during gravistimulation. The average peroxidase activity remains consistent throughout the time course. Specific activity of peroxidase at time zero through 90 min gravistimulation is greater in the etiolated when compared with the red-treated, but lacks significance. The total amount of protein increased slightly in the etiolated from 0.694 g l⁻¹ at time zero to 0.747 g l⁻¹ at 120 min. The red-treated began at 0.787 g l⁻¹, reached a peak of 0.826 g l⁻¹ at 60 min, and fell to 0.613 g l⁻¹ by 120 min. Overall, the etiolated seedlings exhibited reduced peroxidase specific activity over time, whereas, the red-treated stem segment activity increased during gravistimulation. Peroxidase activity in the etiolated upper tissue is slightly less than the lower tissue during 60 and 120 min gravistimulation. Overall, the etiolated upper tissues exhibit increased specific activity in relation to the lower tissue and the red-treated reveal an asymmetric distribution at 120 min favoring the lower side.

Regulation of isozyme expression or changes during gravistimulation was also monitored using polyacrylamide gels and isoelectric focusing. Extracts from the etiolated and red-treated samples at times 0, 30, 60, 90, and 120 min were separated and stained for peroxidase activity. Seven isozymes were found at pI's of approximately 3.5, 4.5, 8.3, 9.0, 9.5, 10.0, and 10.5. After gravistimulation, the intensity of isozymes at pI values of 8.3 and 9.5 from red-treated seedlings was much greater than those from etiolated seedlings. IEF analysis revealed a peroxidase isozyme present at pI 3.5 in the red-treated tissue which are not readily visible until 90 min in the etiolated tissue. Other research in my lab indicates that this isozyme is a soluble cell wall peroxidase isozyme.

Invertase activity in extracts from etiolated tissue was slightly higher compared with the samples from red-treated tissue. Overall, a decrease in invertase specific activity is observed in the etiolated tissue during gravistimulation, while the red-treated remain fairly steady throughout the time course.

As an alternative means of studying tissue localization changes in ethylene biosynthesis and enzyme activity, I established protocols for evaluating gravistimulation- or red light-induced changes in transcriptional (mRNA levels of ACC synthase) and post translational (peroxidase and invertase activity) products in stem tissue using microscopy and computer imaging.

Printing of tissue sections onto nitrocellulose paper were performed for peroxidase and invertase activity using colorimetric enzyme/substrate reactions and immunoblotting. Tissue printing provides resolution of 5-10 μm lacks the resolution of histological techniques but is rapid and allows many replicates to be obtained. Peroxidase activity is found throughout the pea stem tissue with a high level of activity in the vascular bundles. There was no observable change in tissue localization during gravitropic curvature. Cell wall peroxidase activity is light regulated and research in my lab indicates stimulation of cell wall peroxidase activity during gravitropism in pea stems. Invertase increases in the lower side of the gravistimulated oat pulvinus and my current research indicates a similar relationship in pea stems. Also, we have successfully detected horseradish peroxidase (HRP) activity in tissue prints using anti-HRP as a primary antibody and an alkaline-phosphatase conjugated secondary antibody visualization system. HRP is localized in the cortex and absent in the vascular tissue.

To address my original questions concerning the regulation of ethylene biosynthesis within gravistimulated stems, I designed and obtained a DNA probe for ACC synthase transcripts from the Marshall University Medical School DNA Sequencing Center. The probe is biotinylated and analysis is colorimetric. Currently, RNA and mRNA extractions and analyses are on-going.

In conclusion, red light pretreatment of etiolated pea seedlings caused ACC accumulation during the later phases of gravitropic curvature. Conjugation of ACC may be regulated on the lower compared with upper sides of the horizontally placed stems in red-pretreated stems. Enzyme analyses reveal increased peroxidase and invertase specific activities during gravistimulation in red-treated tissue by 120 min stimulation. These findings are consistent with a light-regulatory role of ethylene biosynthesis and ethylene response during the later phases of stem gravitropic curvature.

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**EFFECTS OF GRAVISTIMULATION AND RED LIGHT ON
ETHYLENE BIOSYNTHESIS AND PEROXIDASE
AND INVERTASE LEVELS IN PEA STEMS (*PISUM SATIVUM*)**

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Abstract

Ethylene is a gaseous plant hormone known to elicit many responses during growth and development. In etiolated seedlings (dark-grown) ethylene causes the hook to close and inhibits growth by shortening the stem. Exposure to red-light opens the hook due to an immediate reduction in ethylene production. During gravitropism, red light enhances gravity perception by changing the shape of the curving region. The interaction and effects of ethylene, red-light, and gravity vary with respect to growing conditions, plant material used and quantity of light. Peroxidase and invertase are two enzymes that function in cell wall loosening and may play an integral part during gravistimulation.

Etiolated and red-pretreated seven-day-old pea segments (*Pisum sativum* L. cv. Alaska) were used for all studies at time intervals of 0, 30, 60, 90, 120 min gravistimulation. Gas chromatograph measured the ethylene precursor ACC and its stable conjugate (CACC). Spectrophotometric analysis included peroxidase and invertase activity as well as protein determination. Isoelectric focusing studies on peroxidase activity were also investigated.

A significance difference is seen in the red-treated ACC level beginning at time zero to 90 min gravistimulation ($P \leq 0.02$). An appreciable difference is observed between the etiolated and red-treated CACC at time zero ($P \leq 0.032$). A significant difference is seen at 120 min in CACC content between the upper and lower tissue ($P \leq 0.04$). A significant difference in peroxidase specific activity and protein levels is found between the etiolated and red-treated lower tissue at 120 min ($P \leq 0.03$). Isoelectric focusing analysis revealed an isozyme beginning at 30 min ($pI=3.5$) in the red-treated not seen in the etiolated until 90 min gravistimulation. Peroxidase isozymes at pI 8.3 and 9.5 are more prominent in the red versus the etiolated during gravistimulation.

The present study indicates that exposure to red-light 18 hours prior to gravistimulation enhances ACC levels and peroxidase activity in pea segments grown in open chambers. Differences in ACC observed in the study may be related to the change in growth pattern and sharp angle of curvature followed by a counter-reactive phase (net-slowing of curvature) in which increases in ethylene have been observed upon exposure to red-light.

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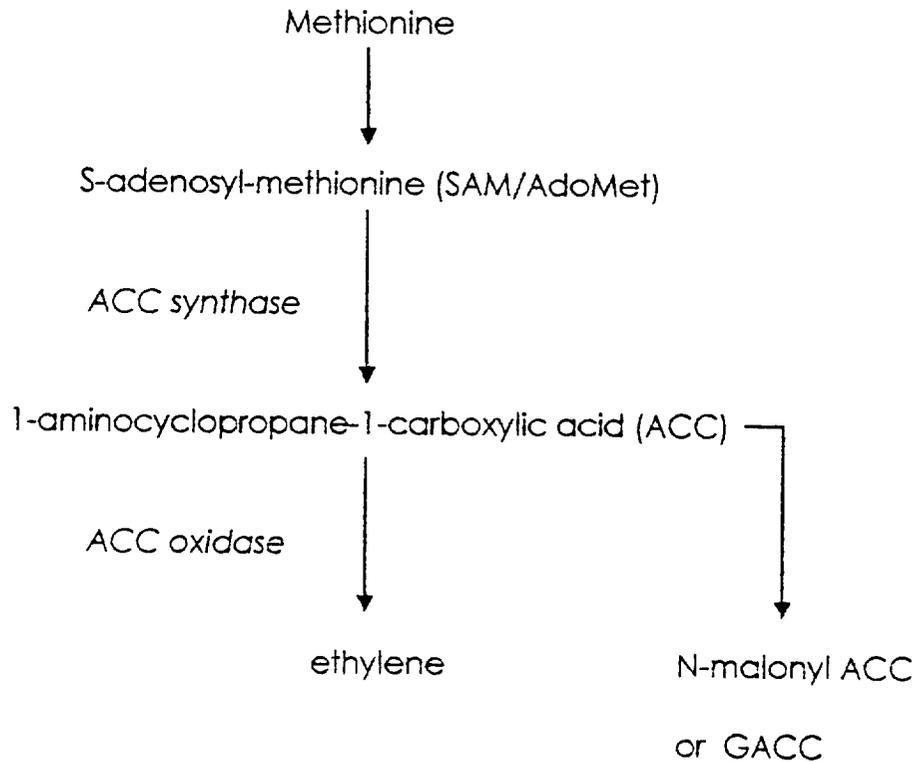
Introduction

Ethylene biosynthesis

Ethylene is a gaseous plant hormone that regulates many aspects of growth and development. The rate of endogenous ethylene produced is normally low, with increases occurring during certain stages of development, such as, seed germination, fruit ripening, leaf senescence and after pollination (Salisbury and Ross, 1992; Nadeau, *et al.* 1993). Additionally, ethylene exhibits a "triple response" in pea seedlings consisting of inhibition of stem elongation, increased stem thickening, and a horizontal growth pattern (Salisbury and Ross, 1992).

Ethylene, a volatile compound, is the simplest known olefin and under physiological conditions is lighter than air. It can be transported across dead, as well as, living tissue with the diffusion rate varying according to tissue type (Abeles, *et al.* 1992). The highest rates of ethylene production are associated with meristematic, stressed, or ripening tissues (Abeles, *et al.* 1992). Ethylene is biologically active in trace amounts (0.1 to 1.0 $\mu\text{l/l}$) and is most accurately measured using gas chromatography (Salisbury and Ross, 1992). The ease and sensitivity of this technique has made ethylene one of the most studied plant hormones.

The metabolic pathway for ethylene synthesis has been established and is as follows (Adams and Yang, 1979; Yang and Hoffman, 1984).



The enzymes involved in the pathway include: ACC synthase which aids in the conversion of SAM to ACC and ACC oxidase (formally ethylene-forming enzyme) which oxidizes ACC to ethylene (Kende, 1989). The formation of ACC synthase serves as the rate-controlling step in ethylene biosynthesis during the conversion of SAM to ACC (Yang and Hoffman, 1984). This was demonstrated using amino-ethoxyvinylglycine (AVG), an ACC synthase inhibitor, in which the conversion of SAM to ACC was blocked with no other observed alterations on the system (Yang and

Hoffman, 1984). Recently, ACC synthase has been purified from wounded tomato pericarp, zucchini, winter squash, and ripened apple fruits with the data supporting the theory there may be more than one ACC synthase genes that are specifically activated (Yang, *et al.* 1990). Auxin can stimulate ethylene production by boosting ACC synthase activity. Immunochemical and chromatographical studies performed by Imaseki, *et al.* (1990) have shown that wound-induced and auxin-induced ACC synthases are products of two different genes expressed by specific stimulus. Thus, ACC synthase is regulated by different genes depending on the stimulus, i.e. developmental, wounding, or auxin-induced.

ACC oxidase is responsible for the conversion from ACC to ethylene. This oxygen-dependent step follows a binding mechanism in which ACC oxidase first binds oxygen and then ACC (Yang, *et al.* 1990). Activity of the enzyme *in vitro* requires the presence of Fe^{2+} and ascorbate and is enhanced by CO_2 levels (Vioque and Castellano, 1994; Hyodo, *et al.* 1993). In a study by Hyodo, *et al.* (1993) their data suggests that wound-induced ACC oxidase is positively regulated by endogenous ethylene while ACC synthase is negatively regulated during wounding and auxin-induced ethylene production. This differs from the autocatalytic ethylene production during fruit ripening and senescence in

which Yang (1987) found that endogenous ethylene expedites the formation of both ACC synthase and ACC oxidase. Therefore, during wounding, the ethylene produced may act to regulate the rate of ethylene production by suppressing ACC synthase and enhancing ACC oxidase.

In addition to being the precursor to ethylene, ACC can also be metabolized into its stable conjugate 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC). Because MACC is a poor ethylene precursor, it is believed that it is an irreversible end product of ACC and may aid in regulating ethylene synthesis by removing excess ACC (Yang and Hoffman, 1984). The enzyme that catalyzes the reaction is ACC malonyltransferase and its normal function is to inactivate potentially toxic substances such as D-amino acids (Abeles, *et al.* 1992). It has been observed that MACC does accumulate when high rates of ACC synthesis are induced by stress factors, thereby this system may be an adaptive response which may allow the plant to cope with the stressful situation (Liu, *et al.* 1985).

Recently it has been found that ACC can also be conjugated into γ -glutamyl ACC (GACC) which is synthesized by tomato fruit and seed extracts (Martin, *et al.* 1995). The current protocol for determining

endogenous levels of conjugated ACC releases all bound ACC and does not separate MACC from GACC. It is due to this nature the author will refer to conjugated ACC as CACC (Conjugated = MACC + GACC) to avoid any confusion.

Ethylene synthesis can be induced by a diverse number of external factors and an increase in production can bring about many physiological changes. Increases brought about in living tissue by stress include: insect infestation, extreme temperatures, flooding and drought, chemicals, irradiation, and mechanical wounding such as cutting, bruising, and pressure (Abeles, 1973; Yang and Hoffman, 1984). During their work with ethylene, Yang and Hoffman (1984) have found that after stress, a typical lag phase of 10-30 min occurs before ethylene production is stimulated and usually reaches a peak after several hours. They have also demonstrated the methionine to ACC pathway is the same for stress induced ethylene production with the conversion of SAM to ACC being the rate-limiting step.

Phytochrome

Plants are able to perceive light by using three different photoreceptors independently or in tandem. These include: a UV-B absorbing pigment, cryptochrome a blue absorbing pigment, and

phytochrome which exists in two forms, absorbing red (P_r) and far-red (P_{fr}) light with a maximum absorbance of 666 nm and 730 nm, respectively (Salisbury and Ross, 1992). Phytochrome, a covalently linked linear tetrapyrrole chromophore, is a well characterized cytosolic plant photoreceptor. The two forms of phytochrome are photoreversible with red light converting P_r to P_{fr} and far-red light changing P_{fr} back to P_r (Salisbury and Ross, 1992). Phytochrome is synthesized as P_r , a biologically inactive form, and becomes active upon conversion to P_{fr} where it initiates many responses. An alteration in gene expression, either activation or repression of specific genes, is one of the more rapid effects of P_{fr} (Viestra, 1993). However, the conversion back to P_r will cancel many phytochrome-mediated responses, allowing phytochrome to act as a light regulated switch during plant development (Viestra, 1993). This phenomenon may be one reason it is the most studied photoreceptor.

A plant's response to light can vary based on length of exposure and the fluence rate. There are two categories in which responses to red light can be grouped: those affected by low fluence (LFR) which have a threshold of $10^{-1} \mu E m^{-2}$ and those affected by very low fluence (VLFR) with a threshold of $10^{-3} \mu E m^{-2}$. The LFR is the established far-red reversible phytochrome response, whereas, VLFR is not nullified by far red light and

requires less P_{fr} for saturation (Salisbury and Ross, 1992).

Dark-grown seedlings irradiated with low fluences of red light undergo a variety of responses, including transcriptional regulation of nuclear genes for several chloroplast proteins, mesocotyl elongation inhibition, promotion of coleoptile growth and seed germination (Chory, *et al.* 1989; Salisbury and Ross, 1992). Red-light pretreatment has been shown to change the growth and development pattern in etiolated bean seedlings causing a shift in the hook region from hypocotyl to epicotyl and increasing fresh weight (Vangronsveld, *et al.* 1988). Briggs and McArthur (1970) demonstrated a higher concentration of phytochrome was located in the pea epicotyl hook when compared to other parts of the seedling. Other various responses to red-irradiation include stimulation of anthocyanin synthesis, non-gradient stem elongation, plumular expansion, and hook opening (Kang and Burg, 1972; Shinkle, *et al.* 1992; Goeschl, *et al.* 1967).

The most extensive phytochrome studies have been carried out using etiolated (dark-grown) seedlings. Advantages of this system include the absence of complications that arise from chlorophyll and the phytochrome responses tend to be more prominent than in light grown plants. In etiolated seedlings, the accumulation of more P_r and its

subsequent conversion to P_{fr} enhance the phytochrome responses (Thompson and White, 1991). Recently, it has been suggested different genes of phytochrome predominant in etiolated plants than do in light-grown plants. Based on expression patterns and stability after photoconversion to P_{fr} , the genes have been classified as Type I (*phyA*) and Type II (*phyB-E*) which are expressed in etiolated tissue and light-grown plants, respectively (Vierstra, 1993). The most distinguishing feature between the two types is Type I undergoes proteolytic degradation after illumination and conversion to P_{fr} , while Type II is relatively stable in light (Thompson and White, 1991).

Gravistimulation

The earth's gravitational pull influences the direction of growth in plants. The roots are usually positively gravitropic (downward curvature) while stems and flowers are negatively gravitropic (upward curvature) (Salisbury and Ross, 1992). The response mechanism as it relates to gravity can be divided into three steps: gravity perception, signal transduction, and asymmetric growth by differential cell growth. The manner in which gravity is perceived is still under investigation. In roots, removal of the root cap inhibits a gravitropic response until the cap is either regenerated or replaced (Salisbury and Ross, 1992). The perception mechanism may be

enhanced by the starch grains found in the amyloplasts, where numerous studies have demonstrated a correlation between the rate of amyloplast settling and presentation time (Salisbury and Ross, 1992).

The sites of gravity perception and response is the same in stems, including the coleoptiles, hypocotyls, and mature stem (Salisbury and Ross, 1992). It is also strongly suggested, although not conclusive, that amyloplasts are also the statoliths in stems. During gravistimulation, the upper side of the stem ceases to grow or even shrink, whereas the lower side continues to grow normally. The rate of growth between the upper and lower surfaces can differ by a factor of 10 or more (Salisbury and Ross, 1992). One hypothesis on signal transduction, as it relates to gravitropism, is the Cholodny-Went theory. Their model is based on the transport of auxin from the upper side (lighted) to the lower side (shaded) where it acts on the epidermis to loosen the cell wall for fast growth (Salisbury and Ross, 1992). There has been considerable data for and against this theory, with the main question being is an auxin gradient a result of the bending or a cause? Studies by Salisbury, *et al.* (1988) measured hypocotyl bending as a function of time after gravistimulation and bending of the upper and lower sides as a function of auxin concentration. Their data suggests the sensitivities of the upper and lower

sides to applied auxin are strongly influenced by gravistimulation, with the lower side increasing the number of available auxin binding sites and affinity (Salisbury *et al.* 1988).

Ethylene, Phytochrome, and Gravity Interactions

There are several reports in the literature that examine the interaction of ethylene/light; ethylene/gravity; and light/gravity responses in higher plants. As stated earlier, ethylene has a profound effect on etiolated seedlings causing hook closure and stem shortening. However, exposure to red light in dark-grown bean and pea seedlings causes the hook to open by reducing the amount of endogenous ethylene produced (Goeschl, *et al.* 1967; Kang and Burg, 1972). In etiolated lettuce seedlings, Janes and Loercher (1976) found that red light acted independently of ethylene to cause a closure of the normally open hook. A restricted sensitivity in etiolated bean seedlings 7 to 10 days old and a decrease in ethylene production after exposure to red light was observed by Vangronsveld, *et al.* (1988). He also noted a near 50% decrease of free-ACC content within 2 h after irradiation which decreases ethylene biosynthesis by lowering the available ACC for conversion. Most of these effects are reversed by far-red light and tend to diminish off over a period of time.

The effect of light on the rate of ethylene production has been known to vary depending on the quantity of light (brief or extended), the plant material involved (intact or excised), and the growing conditions of the plant (closed or open chambers). The technique used to determine ethylene biosynthesis may also affect the rate, such as, direct ethylene measurements versus measuring the precursors. Hence, red-light not only decreases ethylene production in some systems but, it has been shown to increase or have no effect at all on biosynthesis. The extensive current literature fails to reveal a unifying theme in regards to light and its subsequent effect on ethylene production.

The interaction between light and gravitropism also has conflicting results. Britz and Galston (1982) concluded that although gravity perception was enhanced by red light, the kinetics and extent of the response was the same as dark-grown controls. Yet others have found that after red-light pretreatment an inhibition of gravitropic curvature was observed (McArthur and Briggs, 1979; Hart and McDonald, 1980). Harrison and Pickard (1996) compared the patterns of curvature in dark-grown and green pretreated etiolated pea epicotyls with those receiving red irradiation. They found green-pretreated and dark-grown epicotyls exhibit a distinct biphasic pattern of curvature consisting of 15 min lag phase

after gravistimulation followed by upward curvature lasting 45-60 min and a slowing during the counter-reactive phase or straightening of the tip. The red-pretreated stems, given irradiation 18 h prior to gravistimulation, had a reduced biphasic pattern of curvature that resulted in a net increase in curvature (Harrison and Pickard, 1996). This can attribute to a change in shape of the curving region and the growth pattern along the stem. They also noted after one hour of gravistimulation a net slowing of curvature is apparent during the strong counter-reaction phase in etiolated plants. The red-irradiated experience a net slowing approximately 45 minutes after the etiolated and the light grown exhibit little or no counter-reactive phase (Harrison and Pickard, 1996). While it is apparent phytochrome may influence the gravitropic response in plant shoots, the exact nature of their interaction is unclear.

The role and function of ethylene in gravitropism remains controversial. Inhibitors of ethylene biosynthesis or action retard shoot gravitropism, thus suggesting ethylene may mediate gravitropic curvature in cocklebur shoots (Wheeler and Salisbury, 1981). However, others have found that gravitropism can occur without changes in ethylene production and in the presence of ethylene inhibitors (Harrison and Pickard, 1986; Lee *et al.* 1990). Red light can inhibit endogenous ethylene

production and lowers the plant's sensitivity to exogenous ethylene (Kang and Burg, 1972; Harrison and Pickard, 1996). Kang and Burg (1972) noted a reduction in ethylene caused an increase in lateral auxin transport and red light enhanced gravity perception in pea seedlings. The decrease in ethylene production by red light enhanced lateral auxin transport, which has a known role in the asymmetric growth response, attributing to the increased curvature noted by Harrison and Pickard (1996). The available data linking the interaction of light, ethylene, and gravity are limited and vary depending on what system is employed for the study.

Peroxidase and Invertase

Peroxidases (E.C.1.11.1.7) are iron containing enzymes that exist in several isoforms. Plant peroxidases are usually glycoproteins of 30 to 60 kD and they catalyze the oxidation of a substrate and the reduction of H_2O_2 (van Huystee, 1987). These enzymes have been implicated in lignin synthesis, auxin oxidation, ethylene biosynthesis, wound healing, and disease resistance (Salisbury and Ross, 1992; Gijzen, *et al.* 1993; van Huystee, 1987). Plants contain several different peroxidase isozymes which have similar catalytic functions but, vary according to structure, catalytic parameters, and are encoded by different genes (Taiz and Zeiger, 1991). These isozymes are classified according to their pI values.

The two major groups of peroxidase are guaiacol peroxidases (GPX's) and ascorbate peroxidases (APX's). Guaiacol peroxidase utilizes both guaiacol and pyrogallol as electron donors in assays of their activity (Amako, *et al.* 1994). Upon oxidation, guaiacol forms a soluble brown product, tetraguaiacol, which is readily visible (Fielding and Hall, 1978). The GPX's take part in a wide array of physiological processes and their expression is usually correlated with responses to development (Amako, *et al.* 1994). The APX have a high degree of specificity for ascorbic acid and play a role in scavenging hydrogen peroxide which can accumulate to toxic levels unless removed (Mittler and Zilinskas, 1991). Research in the area of peroxidase as it relates to gravitropism is limited, but it may have a role in loosening the cell wall to allow for asymmetric growth.

Like peroxidase, invertase (EC 3.2.1.26) has several isoforms and exists in the cytosol, vacuoles and cell walls. Invertase is unique in that it is one of two enzymes known to hydrolyze sucrose into glucose and fructose in higher plants (Walker and Pollock, 1993). The two types of invertase are classified according to pH. The alkaline invertases have a pH optima of 7.5 and can be found in the cytosol; the acidic invertases have a pH optima of 5 or less and are located in the cell wall and vacuoles (Salisbury and Ross, 1992). It is postulated invertase acts to

provide growing tissues with hexose for metabolism and increases the concentration gradient between source and sink by facilitating phloem unloading (Walker and Pollock, 1993).

Invertase is thought to be upregulated during gravistimulation. In oat leaf-sheath pulvini, it has been demonstrated that invertase is differentially upregulated in the top compared to the bottom halves of the gravistimulated tissue (Wu, *et al.* 1993). It is theorized that the hexoses, glucose and fructose, provide the substrate for starch synthesis and cell-wall biosynthesis in the elongating cells of the gravistimulated tissue (Wu, *et al.* 1993). These sugars also aid in maintaining turgor pressure during gravitropism.

Research Objectives

The main objectives of this research was to:

- Examine ethylene biosynthesis in etiolated and red-pretreated apical pea segments via the precursor ACC and its conjugated form CACC (MACC + GACC).
- Compare the ACC/CACC levels in the upper and lower tissue at 60 and 120 min gravistimulation in the red-pretreated plants.
- Analyze the peroxidase and invertase levels in the two light variables to determine if an increase occurs during gravistimulation and if light has an overall effect.
- Determine if the upper and lower tissues differ in respect to light treatments and quantity of the two enzymes at 60 and 120 min.

Materials and Methods

Plant material and light conditions

Experiments were carried out on 7-day-old etiolated pea seedlings (*Pisum sativum* L. cv. Alaska) sown in vermiculite. The seeds were sterilized for 10 min in 10% NaOCl and rinsed under running tap water for 10 min then grown in darkness in a Rheem environmental chamber (Rheem Scientific Ashville, USA) at a constant temperature of 23 °C with varying levels of humidity. After germination, they were watered daily under a dim green safelight made from a 22-W white fluorescent tube wrapped with four layers each of yellow and green cellophane to produce a low irradiance at wavelengths not absorbed by phytochrome (520-555 nm). Light readings were measured in $W\ m^{-2}$ and converted to fluence rate based on the energy of a photon. The safelight provided a fluence rate of $0.40\ \mu\text{mol}\ m^{-2}\ s^{-1}$. Red light illumination was administered to etiolated seedlings 18 h before experimentation for 6 min 20 sec and consisted of three 22-W white fluorescent tubes wrapped in three layers of red cellophane with a wavelength of 675 nm. The fluence rate was calculated to be $43\ \mu\text{mol}\ m^{-2}\ s^{-1}$. Plants were gravistimulated for a total of 120 min for all procedures with samples taken at time zero and 30 min intervals.

ACC and CACC measurements

ACC and CACC levels in etiolated and red-pretreated stems were determined before and at 30 min intervals during gravistimulation. For extraction, approximately 0.5 g sub-apical segments were homogenized with a mortar and pestle in 2 ml boiling 80% ethanol and centrifuged at 10,000 g in a Sorvall RC-5B Refrigerated Superseed centrifuge (Dupont Co., Wilmington, USA) for 10 min. Sub-apical segments were cut lengthwise, using approximately 0.25 g tissue, for the upper and lower samples. The supernatant was equally divided between two micro-centrifuge tubes and concentrated in a Labconco Centrivap console (Riov and Yang, 1982 a,b). Free ACC was analyzed by reacting rehydrated material with 600 μ l of H₂O and 100 μ l of 10 mM HgCl₂ that reduced the effects of interfering proteins (Lizada and Yang, 1979). The second sample of each pair was hydrolyzed in 400 μ l of 6 N HCl for one hour to release bound ACC (Vangronsveld, *et al.* 1988). The sample was neutralized with 150 μ l 13.25 N NaOH followed by addition of 100 μ l 10 mM HgCl₂, and 50 μ l H₂O to total a volume of 700 μ l. To every sample, 0.1 ml of a 2:1 mixture of saturated NaOH and NaOCl was added and after 3 min for total hydrolysis of ACC to ethylene, 0.5 ml headspace was injected into a gas chromatography (Varian 3700 GC equipped with an

Alumina F column). The value obtained from the free ACC was subtracted from the bound ACC data to give the CACC results.

Enzyme extraction and peroxidase assay

Etiolated and red-pretreated pea stem segments at time zero and during gravistimulation were ground with a mortar and pestle at room temperature (24°C), using a phosphate buffer (pH 6.3) containing 1 mg/ml PVP that stabilized the enzyme activity. The final tissue to volume ratio was 1:1 except for the upper and lower tissue samples which were approximately 1:3 (w/v). The samples were spun at 9500 g for 3 min in a Beckman 12 microfuge and the supernatant was collected for peroxidase and invertase studies. Peroxidase activity was quantified, based on the rate of oxidation of guaiacol, using 50 µl of the supernatant fraction in 1 ml of substrate (0.1 M phosphate buffer pH 6.3, 0.5% guaiacol, and 0.05% peroxide). The absorbance was read at 470 nm using a Perkins and Elmer Lambda 4A UV/vis spectrophotometer 3 min after the addition of the extract. One unit of activity was defined as a ΔA_{470} (min) at 25 °C.

Invertase assay

Using a modified assay from Sigma Chemical Co., invertase activity was based on the production of glucose resultant from the breakdown of

sucrose by glucose oxidase. A 50 μ l aliquot of the enzyme extraction was placed in 1 ml of substrate (0.1 M phosphate buffer pH 6.3, 0.5% guaiacol, 0.1 M sucrose solution, 93.5 units/ml glucose oxidase (Sigma Chem. Co.), and 60 units/ml peroxidase) at time zero and the absorbance was recorded at 470 nm after 3 min. One unit of invertase was defined as the amount that will hydrolyze 1 μ mol of sucrose in 1 min at 25 °C (Sigma Chem. Co.).

Determination of total protein content

Protein concentration was estimated by the Bradford method (1976) using BSA concentrations of 0.2, 0.4, and 1.0 mg/ml to establish the standard curve. Each assay tube contained 1 ml of Bradford reagent and 20 μ l of sample. After a 5 min incubation period, the absorbance was read at 595 nm and total protein was calculated. The absorbance was recorded for etiolated and red-pretreated samples at time zero and at 30 min intervals during gravistimulation.

Electrophoretic Analysis

Electrophoretic studies were performed on etiolated and red-pretreated samples at time zero and at 30 min intervals using an isoelectric focusing system (Fisher Biotech) and Servalyt Precote gels, pH 3-10. Protein

samples of 10 μ l were run at a constant power supply (Fotodyne DNA Sequencing System, Model 4200) with an initial voltage of 200 and ending with settings of 2000 V and 4 W. The temperature was kept constant at 4 °C using an Isothermal Controlled Electrophoresis FB 1001 (Fisher Biotech). The gels were stained for peroxidase with the same solution used in the spectrophotometer assay.

Results and Discussion

ACC and CACC

Measurements of ACC and CACC levels in etiolated and red-pretreated stem segments were used to determine changes in ethylene biosynthesis. Comparison of the ACC levels in etiolated and red-pretreated pea segments during gravistimulation revealed no significant differences between the two variables (Figure 1). At time zero, both treatments had approximately the same amount of ACC ($G=0.0166$ and $R=0.0178$). By 120 min, the red-treated level increased to 0.0371 nmol/g ACC and the etiolated to 0.0249 nmol/g ACC. This is statistically insignificant ($P \leq 0.08$), but may indicate a weak tendency for red-light to stimulate ethylene production during gravistimulation. An appreciable difference in the levels of ACC in the red-treated is seen beginning at 90 min ($P \leq 0.02$) and continuing through 120 min gravistimulation ($P \leq 0.004$).

There are conflicting reports in terms of red light and its subsequent effect on ethylene production. Many have reported decreases in ethylene production after red irradiation (Goeschl, *et al.* 1967; Imaseki, *et al.* 1971; Harrison and Pickard, 1996), but increases have also been reported. Rohwer and Schierle (1982) found that red-light promoted ethylene production in the shoot of pea seedlings. These differences may

be attributed to the manner in which the plants were grown (open versus closed chambers), experimental conditions (excised or intact) and/or the plant material used. It is postulated that light may exert a dual role in ethylene production by: (a) stimulating ethylene biosynthesis via promotion of ACC oxidase activity if the ACC level is not a limiting or (b) inhibiting production by decreasing the ACC level via ACC malonylation (Jiao, *et al.* 1987) . Increased ACC levels can be associated with stress, wounding, and other developmental processes. Steed and Harrison (1993) found a slight increase in heat treated pea segments beginning 30 min after excision.

Numerous reports have linked phytochrome's ability to enhance the gravitropic response in plants (Britz and Galston, 1982; McArthur and Briggs, 1979; Kang and Burg, 1972). The reasoning to support the data involves a reduction in ethylene production, which in turn, allows for the lateral movement of auxin to the lower side. This effect is measurable up to 8 hr after exposure (Kang and Burg, 1972). Increases in auxin (IAA) can induce the formation of ACC synthase leading to augmentation of ACC which ultimately results in increased ethylene production (Salisbury and Ross, 1982). The present data indicates etiolated pea segments exhibit a uniform rate of ACC production in open chambers during gravistimulation

using excised tissue and the red-treated reveal a trend of increased ACC content 18 h after red-light treatment under the same conditions.

The level of CACC (bound ACC) for the two treatments was also investigated. A significant difference ($P \leq 0.032$) was found at time zero between the etiolated and the red-pretreated (Figure 2). However, by 120 min gravistimulation no significant difference is apparent between the two treatments ($P \leq 0.14$). The data imply no significant differences within the light treatments but, the etiolated exhibit an overall decrease in CACC content during gravistimulation (G0-G120 $P \leq 0.20$). Overall, CACC levels were higher than the ACC tissue levels indicating a high capacity for conjugation.

A net increase in CACC levels usually indicates the removal of excess ACC which corresponds to a decrease in ACC and ethylene production. In the present study, the etiolated tissue exhibits a net decrease in CACC, with no appreciable increases in ACC content. One explanation for this may be that once the CACC was formed it was transported to the vacuoles for storage (Abeles, *et al.* 1992). A reported decrease in MACC content was attributed to a basipetal transport to the roots which served as a sink in the second node of pea plants (Fuhrer and Fries, 1985). The difference between the light treatments at time zero may

be related to the fact red-light stimulated ethylene biosynthesis to the point that ACC levels rose steadily but not to the point of saturation which results in ACC malonylation. This statement supports the results observed in the red-treated tissue in which an overall increase in ACC synthesis is paralleled with the steady rate in CACC levels.

During gravistimulation, the lower side grows at a rate faster than the upper side. It is believed that ethylene production is confined to the lower half of the stem and acts to inhibit growth on the upper side (Wheeler, *et al.* 1986). Due to the ambiguous role light may have in ethylene production, the differences in ACC/CACC levels were investigated in the upper and lower halves of the red-treated pea segments (Figure 3). At 60 min gravistimulation, slight accumulation of ACC occurred in the lower tissue. There was a greater level of CACC accumulation in the lower than in the upper, but the difference was not significant ($P \leq 0.15$). By 120 min gravistimulation the ACC/CACC levels are the opposite of what was observed at 60 min with more in the upper tissue. A significant difference is seen in the upper and lower CACC content at 120 min ($P \leq 0.04$).

According to Wheeler, *et al.* (1986) ethylene was confined to the lower half in cocklebur stems for 12 h during gravistimulation. They have

proposed that the stimulation of ethylene production via auxin accumulation in the lower tissue, aids in inhibiting growth on the upper side by movement of the precursors (ACC) to the top. It has also been speculated that the asymmetric gradient be the result of strong ethylene binding sites, i.e. gravistimulation may result in an increase in available binding sites in the top half leaving no free ethylene left to measure. In another study by Philosoph-Hadas, *et al.* (1996) they observed after 4 h gravistimulation a ratio of ethylene production existed between the upper and lower halves of approximately 4:1 in favor of the lower half in snapdragon segments. They also reported asymmetric distribution of ACC/MACC in the upper and lower following 4 h gravistimulation that closely correlated with sharp angle of curvature. The present data is in agreement at 60 min gravistimulation with their findings. The reversion of ACC/CACC levels back to the upper tissue by 120 min could be the result of the strong counter-reaction (straightening of the tip) followed by a net slowing that occurs approximately 90 minutes after gravistimulation in red-treated seedlings (Harrison and Pickard, 1996). During this time period, increases in ethylene are noted by Harrison and Pickard (1996) which parallel increased ACC levels in the upper side (Figure 1). This occurrence can then inhibit growth on the lower side just as it did in the upper tissue

during the 60 minute time period.

The overall results of ACC/CACC levels in excised pea segments grown in open chambers are:

- ACC levels in etiolated have a uniform rate of production and the red-treated show an increase in ACC content during gravistimulation.
- A significant difference exists between the light treatments at time zero in CACC levels. Overall, CACC values were higher than ACC tissue levels indicating a high incidence of conjugation.
- The lower tissue of the red-treated stems at 60 min has slightly more ACC/CACC with a reversion seen by 120 min which may be attributed to the action of the counter-reaction phase.

Figure 1. ACC levels in gravistimulated red-pretreated and etiolated pea segments at time zero and at 30 min intervals. Means \pm SE.

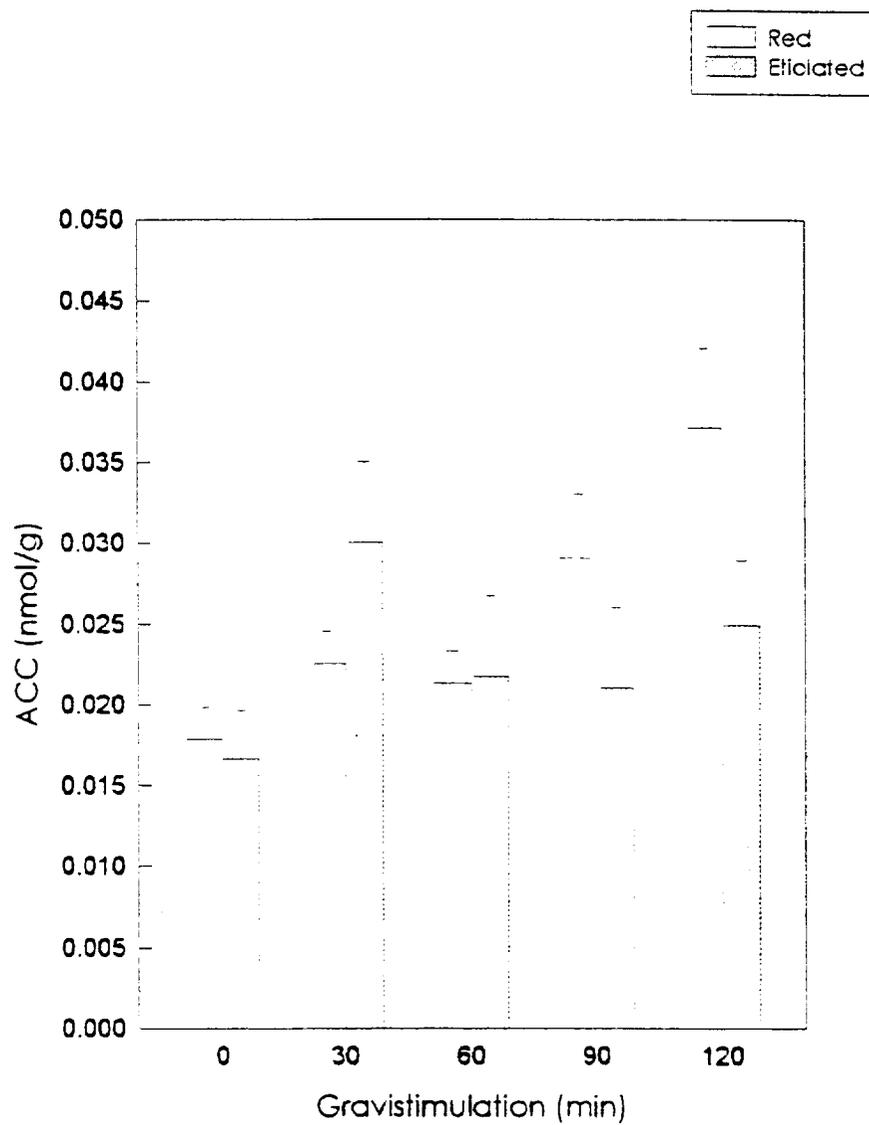


Figure 2. Comparison of CACC levels in gravistimulated red-pretreated and etiolated pea segments at time zero and at 30 min intervals. Means \pm SE.

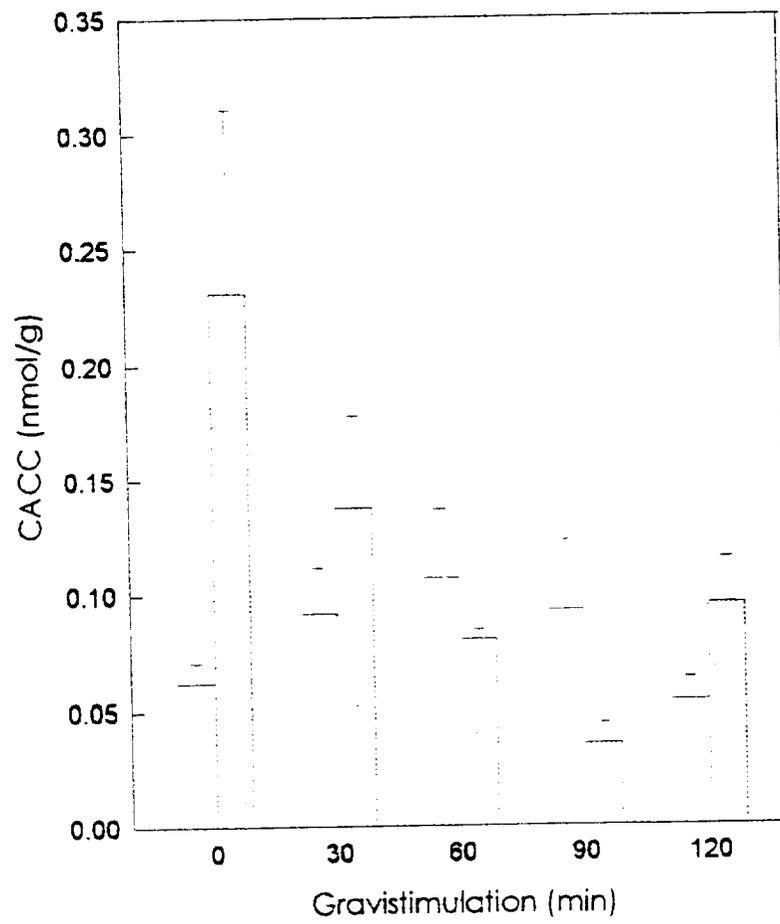
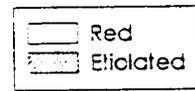
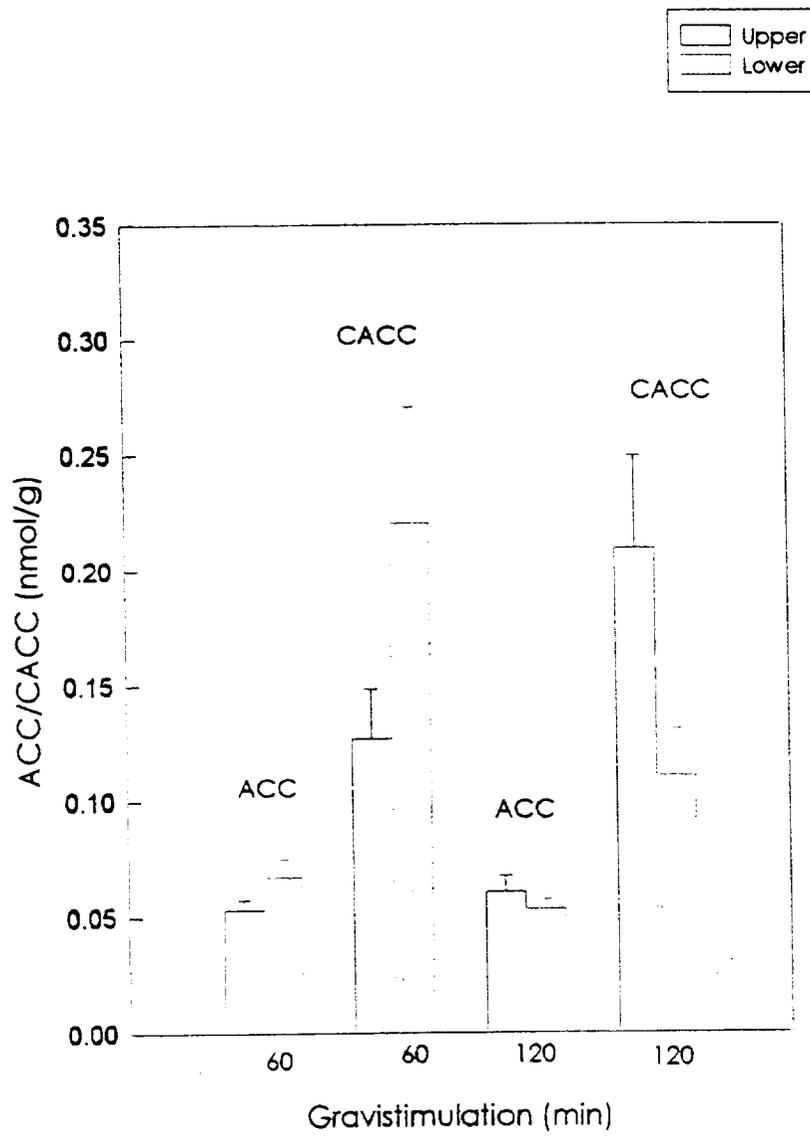


Figure 3. Tissue levels of ACC/CACC in the upper and lower red-pretreated pea segments at 60 and 120 min gravistimulation. Means \pm SE.



Peroxidase and Invertase Analysis

To determine if changes occur in peroxidase and invertase activity during gravistimulation, spectrophotometric analysis was performed on etiolated and red-treated samples. IEF gel electrophoresis of peroxidase activity was also determined. The upper and lower tissue segments for the etiolated and red-treated samples were examined at 60 and 120 min gravistimulation. The total protein content determined from the Bradford assay was used in the calculation of specific activity for both invertase and peroxidase.

The results indicate no difference in peroxidase or protein activity between and within the light treatments during gravistimulation (Table 1). The average peroxidase activity remains fairly consistent through-out the time course. Specific activity at time zero through 90 min gravistimulation is greater in the etiolated when compared to the red-treated, but lacks significance ($R0-G0 P \leq 0.10$; $R90-G90 P \leq 0.13$). The total amount of protein increased slightly in the etiolated from $0.694 \mu\text{g}/\mu\text{l}$ at time zero to $0.747 \mu\text{g}/\mu\text{l}$ at 120 min. The red-treated began at $0.787 \mu\text{g}/\mu\text{l}$, reached a peak of $0.826 \mu\text{g}/\mu\text{l}$ at 60 min, and fell to $0.613 \mu\text{g}/\mu\text{l}$ by 120 min. In general, the etiolated samples reduced specific activity over time and the red-treated increased during gravistimulation.

Peroxidase activity in the etiolated upper tissue is slightly less than the lower tissue during 60 and 120 min gravistimulation (Table 2). The peroxidase content on lower side of the red-treated at 120 min is higher than the upper, but not significant ($P \leq 0.31$). Protein content in the upper half is reduced compared to the lower etiolated segments at both time intervals, but is not significant ($P \sim 0.20$). The red-treated data is the opposite, with the lower sides having decreased protein content at 60 and 120 min. A significant difference occurs in the protein level ($P \leq 0.02$) and the specific activity ($P \leq 0.03$) between the etiolated 120 min lower sample and the corresponding red-treated value. There is a difference of 0.051 units/ μg in the time interval between the lower side of the red-treated samples at 60 min gravistimulation and 120 min which is significant ($P \leq 0.007$). Overall, the etiolated upper tissues exhibit increased specific activity in relation to the lower tissue and the red-treated reveal an asymmetric distribution at 120 min favoring the lower side.

Regulation of isozyme expression or changes during gravistimulation was also monitored using isoelectric focusing (IEF). Extracts from the etiolated and red-treated samples at times 0, 30, 60, 90, and 120 min were separated and stained for peroxidase activity (Figure 5). Seven isozymes were found at pI's of approximately 3.5, 4.5, 8.3, 9.0, 9.5, 10.0, and 10.5. A

faint isozyme begins forming at pI 3.5 in the R30 (lane 4) and somewhat intensifies during gravistimulation. At this same point, the faint banding pattern is delayed in the etiolated until 90 min (lane 7). The isozymes are prominent in all treatments at $\sim pI$ 4.5 with the exception of the red-treated at 30, 60, and 120 min. Beginning at 30 min and up to 90 min, the red-treated isozyme intensity is much greater than the etiolated at the pI between 8.3 and 9.5. After 90 min the green appears to be equal with the red-treated samples.

The overall peroxidase specific activity in etiolated tissue fell below that of the red-treated segments and staining for peroxidase isozymes also favored the red-light (Table 1, Figure 4). Increases in ethylene have been known to enhance peroxidase activity in many plants (Abeles, *et al.* 1992). In this study, a trend for increased ACC content was observed in red-treated samples beginning at approximately 60 min (Figure 1). In a study by Abeles, *et al.* (1988), they provided evidence that ethylene induces synthesis of two peroxidases with isoelectric points and molecular weights of anionic, $pI=4.0$ (33kD) and cationic, $pI=8.9$ (60kD). Their data also indicates the 33kD peroxidase is inducible and the 60 kD is a constitutive enzyme. Hence, it can be postulated the rise in ACC content concomitant with red light acted to amplify peroxidase activity

which was evidenced in spectrophotometric and IEF analysis (Table 1 and Figure 1).

Changes in the upper and lower sides of the etiolated and red-treated segments were analyzed at 60 and 120 min during gravistimulation based on the changes that occurred in the ethylene precursors. Overall, there were six isozymes visible at isoelectric points of approximately 4.5, 8.3, 9.0, 9.5, 10.0, and 10.5. It is clearly evident that at the pI of ~ 4.5 the etiolated upper (lanes 2 & 6) and lower (lanes 4 & 8) have a isozyme that is not seen in the red-treated samples until 120 min in the upper half (lane 7) and then it is barely visible (Figure 5). In Figure 4, this isozyme pI (4.5) was present at time zero in red-treated, absent by 30 min, and became visible again by 60 min. This could be the result of an accumulative effect in the whole tissue versus the halves. However, the peroxidase isozymes at $pI \sim 8.3$ and 9.5 are more prominent in the red (lanes 1, 3, 5, 7) than the etiolated. Again, this was not clearly evident in Figure 4. The upper half in the etiolated tissue at 60 min ($pI \sim 8.3$) shows a reduced band when compared to lower at the same time interval (lane 2 vs. lane 4). The opposite is seen by 120 min gravistimulation in that tissue. The author speculates the reversions in activity noted through-out the study may be the result of the counter-reactive curvature phase.

The data reported for invertase and specific activity are found in Table 3. Invertase activity at time zero was low for etiolated and red-treated samples, 0.007 and 0.009 $\mu\text{mol}/\text{min}$, respectively. A slight increase of 0.002 was recorded in the red-irradiated samples over time, but the etiolated had a maximum of 0.012 $\mu\text{mol}/\text{min}$ at 30 min and then diminished to 0.008 $\mu\text{mol}/\text{min}$ by 120 min gravistimulation which are near the levels at time zero. No significant differences were revealed. In regards to specific activity, the etiolated had a burst of production at 60 min and then leveled off back to starting point. The increase from G0 to G60 was not significant ($P \leq 0.07$). In general, the red-irradiated samples expressed an insignificant increase during the time course. At 60 min gravistimulation a significant finding resulted between the red-treated and the etiolated at 60 min ($P \leq 0.42$). At this time the etiolated was at it's peak (0.018 units/ μg) and the red-irradiated had it's lowest value (0.010 units/ μg).

Invertase activity in the etiolated upper and lower tissue was slightly higher compared to the red-treated samples (Table 4). A decrease in both invertase and specific activity is observed in the etiolated tissue during gravistimulation, while the red-treated remain fairly steady throughout the time course. No major differences were found in this investigation.

The overall results of the peroxidase and invertase spectrophotometric analysis and IEF gel electrophoresis for peroxidase changes reveal:

- A consistent level of peroxidase activity is observed during 0-120 min gravistimulation in both treatments. The upper tissue of the etiolated segments demonstrate less activity compared to the lower tissue.
- There is no significant difference in protein activity between the 0-120 min gravistimulation parameters. But, a difference does exist among the 120 min lower etiolated and red-treated samples.
- Differences in peroxidase specific activity were observed in the lower halves of the red and etiolated tissue at 120 min and between the lower half of the red-treatment at 60 and 120 min gravistimulation.
- There were no discernible differences in invertase activity any of the investigations. A difference in the specific activity was noted at 60 min in the red-treated and etiolated tissue.
- IEF analysis revealed a peroxidase isozyme present at $pI \sim 3.5$ in the red-treated tissue which are not readily visible until 90 min in the etiolated tissue. This same pattern of isozyme expression is again seen

at $pI \sim 8.3$ and 9.5 . The etiolated upper and lower halves exhibit a band at $pI \sim 4.5$ that is not expressed in the red tissue.

Table 1. Peroxidase, protein content, and specific activity in etiolated and red-treated peas after 0, 30, 60, 90, 120 min gravistimulation. One unit of activity is equal to $\Delta A_{470} \text{ min}^{-1}$. Means \pm SE.

	Units (Δ_{470}/min)	Protein ($\mu\text{g}/\mu\text{l}$)	Specific Activity (units/ μg)
Etiolated			
0	0.0160 ± 0.002	0.694 ± 0.06	0.0243 ± 0.004
30	0.0162 ± 0.002	0.708 ± 0.05	0.0237 ± 0.004
60	0.0156 ± 0.002	0.740 ± 0.03	0.0212 ± 0.003
90	0.0151 ± 0.003	0.621 ± 0.08	0.0268 ± 0.005
120	0.0160 ± 0.002	0.747 ± 0.09	0.0204 ± 0.002
Red-treated			
0	0.0151 ± 0.002	0.787 ± 0.05	0.0186 ± 0.002
30	0.0161 ± 0.002	0.802 ± 0.09	0.0207 ± 0.001
60	0.0161 ± 0.002	0.826 ± 0.07	0.0197 ± 0.008
90	0.0160 ± 0.002	0.725 ± 0.02	0.0209 ± 0.002
120	0.0144 ± 0.003	0.613 ± 0.01	0.0239 ± 0.005

Table 2. Changes in peroxidase, protein content, and specific activity in etiolated and red-treated upper (\uparrow) and lower (\downarrow) tissue at 60 and 120 min gravistimulation. One unit of activity is equal to $\Delta A_{470} \text{ min}^{-1}$. Means \pm SE.

Blue=significance

	Units (Δ_{470}/min)	Protein ($\mu\text{g}/\mu\text{l}$)	Specific Activity (units/ μg)
Eticlated 60 \uparrow	0.0141 ± 0.003	0.435 ± 0.08	0.0289 ± 0.004
60 \downarrow	0.0154 ± 0.002	0.589 ± 0.03	0.0257 ± 0.003
120 \uparrow	0.0153 ± 0.002	0.506 ± 0.12	0.0289 ± 0.003
120 \downarrow	0.0158 ± 0.002	0.683 ± 0.03	0.0246 ± 0.002
Rec-treated 60 \uparrow	0.0161 ± 0.002	0.661 ± 0.16	0.0234 ± 0.001
60 \downarrow	0.0150 ± 0.003	0.574 ± 0.12	0.0234 ± 0.001
120 \uparrow	0.0143 ± 0.003	0.561 ± 0.08	0.0242 ± 0.003
120 \downarrow	0.0162 ± 0.002	0.547 ± 0.04	0.0285 ± 0.002

Figure 4. Representative IEF gel of red-treated and etiolated pea segments stained for peroxidase activity during gravistimulation. R= Red-treated; G= etiolated.

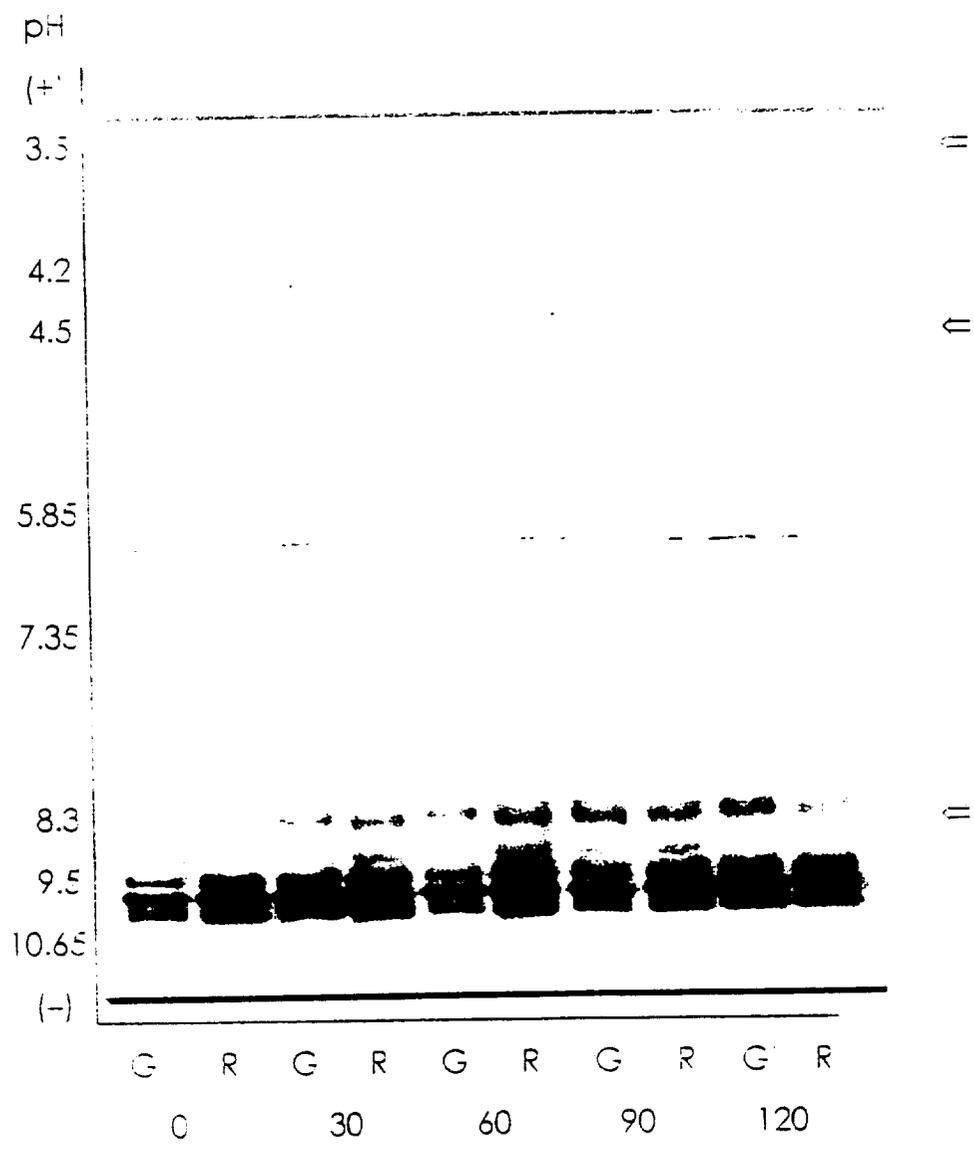


Figure 5. Representative IEF gel of upper (\uparrow) and lower (\downarrow) tissue in red-treated and etiolated pea segments stained for peroxidase activity at 60 and 120 min gravistimulation. R= Red-treated; G= etiolated.

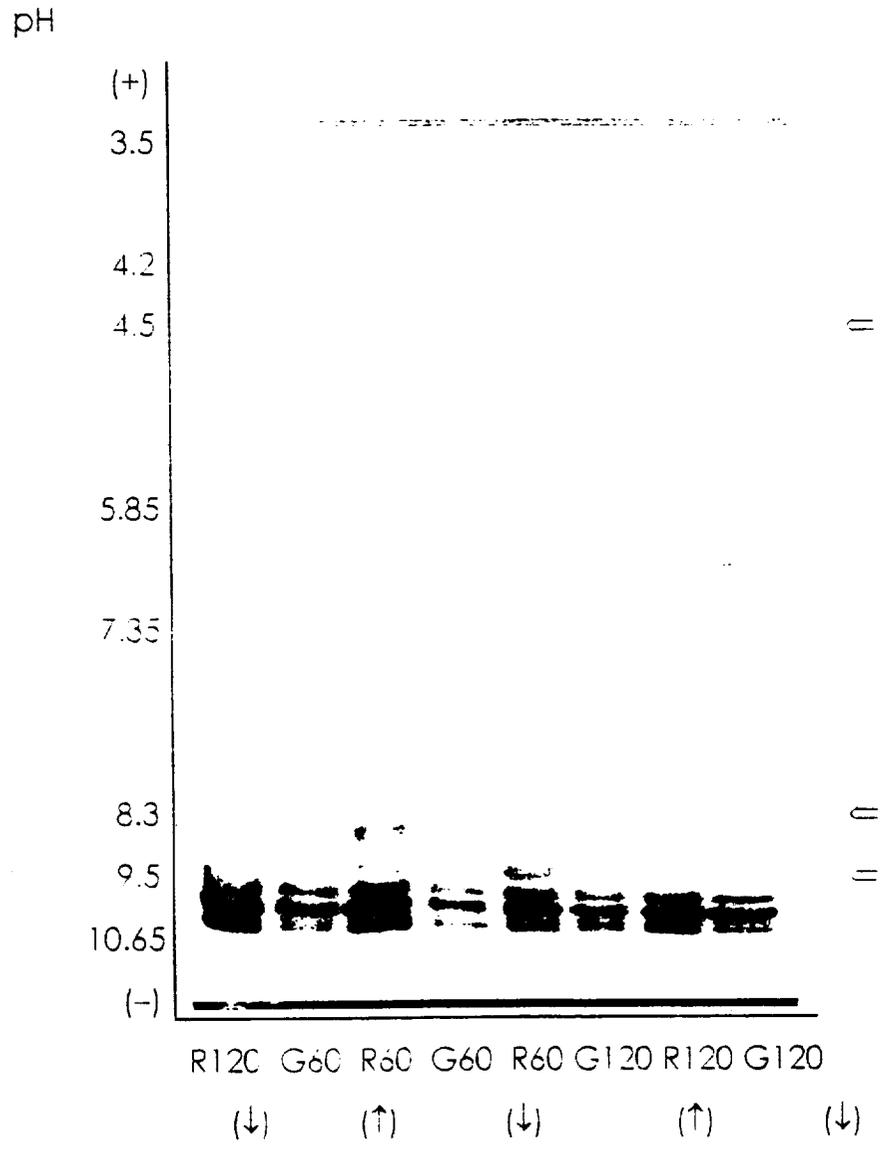


Table 3. Invertase, protein content, and specific activity in etiolated and red-treated pea segments after 0, 30, 60, 90, and 120 min gravistimulation.

One unit of invertase is defined as the amount of enzyme that will

hydrolyze 1 μmol of sucrose per minute at 25 °C at pH 6.3. Means \pm SE.

Blue=significance

	Units ($\mu\text{mol}/\text{min}$)	Protein ($\mu\text{g}/\mu\text{l}$)	Specific Activity (units/ μg)
Eticlated 0	0.007 ± 0.001	0.694 ± 0.06	0.010 ± 0.001
30	0.012 ± 0.003	0.708 ± 0.05	0.017 ± 0.004
60	0.011 ± 0.002	0.740 ± 0.03	0.018 ± 0.003
90	0.011 ± 0.002	0.621 ± 0.08	0.017 ± 0.005
120	0.008 ± 0.001	0.747 ± 0.09	$.0010 \pm 0.002$
Red-treated 0	0.009 ± 0.002	0.787 ± 0.05	0.011 ± 0.004
30	0.009 ± 0.002	0.802 ± 0.09	0.012 ± 0.003
60	0.008 ± 0.001	0.826 ± 0.07	0.010 ± 0.001
90	0.011 ± 0.002	0.725 ± 0.02	0.015 ± 0.002
120	0.011 ± 0.003	0.613 ± 0.01	0.018 ± 0.005

Table 4. Invertase, protein content, and specific activity in etiolated and red-treated upper (\uparrow) and lower (\downarrow) tissue at 60 and 120 min gravistimulation. One unit of invertase is defined as the amount of enzyme that will hydrolyze 1 μmol of sucrose per minute at 25 $^{\circ}\text{C}$ at pH 6.3. Means \pm SE.

	Units ($\mu\text{mol}/\text{min}$)	Protein ($\mu\text{g}/\mu\text{l}$)	Specific Activity (units/ μg)
Etiolated 60 \uparrow	0.007 ± 0.003	0.435 ± 0.08	0.018 ± 0.012
60 \downarrow	0.009 ± 0.003	0.589 ± 0.03	0.016 ± 0.007
120 \uparrow	0.005 ± 0.001	0.506 ± 0.12	0.011 ± 0.005
120 \downarrow	0.004 ± 0.001	0.683 ± 0.03	0.006 ± 0.001
Red-treated 60 \uparrow	0.004 ± 0.001	0.661 ± 0.16	0.007 ± 0.002
60 \downarrow	0.004 ± 0.001	0.574 ± 0.12	0.008 ± 0.003
120 \uparrow	0.003 ± 0.001	0.561 ± 0.08	0.005 ± 0.001
120 \downarrow	0.004 ± 0.001	0.547 ± 0.04	0.007 ± 0.001

Conclusions

Recent advances in space technology has provided insight into how various systems respond, grow, and adapt to their environment. Much attention has been focused on ethylene biosynthesis in recent years because of its vast array of effects on many growth processes. Therefore, it is only natural to investigate how light interacts with this gaseous hormone. It was my intention to try to elucidate the effect light and gravity have on ethylene biosynthesis via measuring the precursors ACC and its total conjugated forms, MACC and GACC. Peroxidase and invertase activity was also investigated because of their involvement in cell wall elongation.

ACC levels in etiolated tissue exhibited a uniform rate of production during gravistimulation. This indicates that during the time interval, etiolated stem segments are not significantly affected by gravitropism. It can also be assumed that wound ethylene did not influence biosynthesis due to lack of increased ACC content. The red-treated tissue show a weak, but significant tendency to stimulate ACC production during gravistimulation. The upper and lower tissue at 60 and 120 minutes reveal a reversion in favor of ACC/CACC levels from the lower side to the upper side during the time course. It is known that red light alters the growth

pattern during gravistimulation causing a sharper angle of curvature followed by a net slowing. Therefore, it is speculated the change in growth pattern followed by the counter-reactive phase, or auto-straightening of the tip, is responsible for this difference. Accompanying this modification, a change in hormone response is expected and was observed in this study. Another reasonable explanation for the reversion of ACC/CACC levels may involve the binding of ethylene to its receptor. However, the knowledge is limited regarding ethylene receptors making this theory much harder to validate.

Changes in peroxidase specific activity support the notion that red-light may increase production. This was supported by the IEF gel which revealed an isozyme in the red-treated but, not seen in the etiolated until 90 minutes gravistimulation. The invertase specific activity also increased in the red-treated during the time interval compared to the etiolated which peaked at 60 min and then returned to the level at time zero.

It is surmised that peroxidase and invertase have a role in cell wall loosening during gravistimulation. In this study, both enzymes steadily increased in activity in the red-treated samples over the time course. This could be correlated with the sharp angle of curvature induced by red light which in turn enhanced production.

In the present investigation, pea segments grown in open chambers exposed to a long duration of red-light 18 prior to gravistimulation, reveal a tendency to increase the production of ACC levels and peroxidase specific activity when compared to etiolated pea segments. A noted insignificant increase in invertase specific activity was also shown in the red-treated.

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