2004

Effect of light duration on pulvinar motor organ movements in Phaseolus vulgaris

Thomas William Rinehart
University of Northern Iowa

EFFECT OF LIGHT DURATION ON PULVINAR MOTOR ORGAN MOVEMENTS IN *PHASEOLUS VULGARIS*

A Thesis

Submitted in Partial Fulfillment

Of the Requirements set forth

By the Board of the Presidential Scholars

Thomas William Rinehart

University of Northern Iowa

April 2004

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

While plants are rooted and do not have the ability to alter their surroundings by moving into a more suitable one, they are not completely vulnerable. Many plants exhibit the diurnal pattern of skotonastic downfolding and photonastic unfolding—a behavior exhibited even in the dark—implying that it is a "biological clock" mechanism. While these movements are important, the leaves of some plants can orient themselves quickly and in a reversible fashion in response to environmental conditions and stimuli (Darwin 1881). Of more interest on a crop science level are those plant movements that occur as a result of the transport of osmotically active solutes and water, which is triggered primarily by high photon flux density (PFD) and drought via a well known, but not completely understood, mechanism.

Observations of the effect of PFD differences have been made extensively in many leguminous plants—especially bean plants. These high light fluxes result in a variety of phototropic responses in bean plants. Further, while sunlight consists of a wide range of light wavelengths, several distinct regions of light have been associated with specific movements. Blue light is typically associated with upward leaf movements and upward pulvinar torque in restrained leaflets (Burkholder and Pratter, 1963; Williams and Raghavan, 1966; Fondeville et al., 1967; Satter et al., 1981; Watanabe and Sibaoka, 1983; Donahue and Berg, 1990; Koller and Ritter, 1994; Koller 2001). However, directional blue light studies have shown that while illumination of the upper cells of the pulvinus result in upward paraheliotropic movements, exposure of blue light to the lower pulvinar surface results in downward pulvinar movements (Nishizaki, 1987, 1988; Koller et al., 1996).

Red light also plays a key role in phototropic movements—it is believed to be a key component in photonastic folding, but has similar effects to those of blue light when exposed to the pulvinus at an intensity significantly greater than the intensity of red light in the light transmitted from the sun (Koller et al., 1996; Koller, 2001). Red light pulses at solar levels have also been show to cause a continuous decline in leaf angle (Church, 2003). Additionally, while much work has been done regarding the effects of blue light and red light, preliminary research with green light (around 720nm) indicates that it causes upward phototropic movements, much the same as blue light, when exposed to the plant at levels around those of full sunlight (Church, 2003).

After noting similarities in the directional movements of both stomatal guard cells and pulvinar motor cells in response to specific wavelength light stimuli (Iino, Ogawa, and Zeiger, 1985; Zeiger et al., 1987; Zeiger, 2000) Nishizaki,proposed a model for the mechanism by which blue light causes hydraulic movements in pulvinar plant cells. When guard cells are exposed to blue light, it is hypothesized that the carotenoid zeaxanthin absorbs the light and is excited. This excitation likely causes a certain photochemical reaction, which in turn triggers a cascade to transmit the signal. After crossing the chloroplast envelope, this signal activates a serine/threonine kinase, which phosphorylates proton pumps. Depending on zeaxanthin concentration and blue light flux density, a corresponding amount of protons are pumped, resulting in solute uptake, changes in turgor pressure, and the eventual opening on stomata (Zeiger, 2000).

When applied to a model for blue-light reaction in the pulvinus, the mechanism is somewhat related. Upon illumination with blue light, an unknown photoreceptor molecule activates the proton pumps in cell membranes of pulvinar protoplasts. This

photoreceptor is more than likely able to undergo a chemical interconversion between two forms; existing as molecule A (inactive), and molecule B (active). After illumination with blue light, molecule A undergoes a chemical conversion to molecule B, the form which inhibits proton pumps and causes depolarization of the motor cell membrane. As the blue light stimulus subsides, molecule B begins the conversion back to the inactive state, molecule A, allowing repolarization of the motor cell membrane (Nishizaki, 1996). This polarizing activity is important in the activation of certain selective ion channels. The ion channels allow the movement of ions into or out of the pulvinar cell in response, ultimately resulting in cell swelling during osmotic water uptake, and cell shrinking during osmotic water loss. The coordinated shrinking and swelling of the pulvinus manifests as paraheliotropic light movements.

With a well established model in place, the main objective of this study will be to determine if the pulvinar motor organs have a threshold radiation level to elicit paraheliotropic movements. Plants will be illuminated with full spectrum light at varying intensities and periods. Observation will take place minute-by-minute in an effort to denote certain lighting conditions which might trigger paraheliotropism.

MATERIALS AND METHODS

Plants and Growth Conditions

Commercially distributed seeds of *Phaseolus vulgaris* L. cv. Redkloud were grown in a 1:1:1 mixture of soil:perlite:peat in 7.5 cm plastic pots. The seeds were germinated in a controlled environment at 26 degrees C in the dark. Approximately two days after emergence, the seedlings were transferred to the greenhouse in Cedar Falls, Iowa, USA, and grown under sunlight in the months from December to March. Midday temperatures were about 26-28°C, and maximum PFD values were near 1300 μmol m^{-2} s^{-1}. Plants were watered every three days with tap water. Testing was conducted on the pulvinus approximately 5-10 days after full expansion of the first unifoliate leaf..

Chamber Configuration

All trials were conducted inside a previously constructed lightproof chamber built by Jeff Church, with a few modifications. The chamber contained an array of three ultra-bright white LEDs (LED Museum, Rochester, VT), and a digital camera (QuickCam VC, Logitech, Freemont, CA). The camera was positioned approximately 20 cm from the plant, and was oriented in each study so that the field of image capture contained both the pulvinus and the leaf of the plant.

The top of the chamber consisted of a track system for orienting the light (Oriel, Stratford, CT), and a 75-watt xenon arc lamp (Photon Technology International, Brunswick, NJ). A heat mirror (Optical Coatings Limited, Inc., CA) was positioned in the light path at an angle of 57 deg to horizontal to eliminate infrared and far red light. Light then entered a black box, where the horizontal light beam struck a mirror oriented 45 deg

to parallel, and was directed down into the chamber with the plant. Light intensity was controlled by adjusting the wattage dial on the arc lamp's power supply.

Experiments

The day before a plant was to be used, it was placed in the growth chamber (Conviron, Winnipeg, Manitoba, Canada) to induce drought conditions. Plants were then transferred to the lightproof container the night before the experiment. In order to eliminate any sort of experimental bias occurring because of the quick transition from dark to light, the three white LEDs (LED Museum, Rochester, VT) were set on a timer to turn on at 6:00 AM the day of experiment, at an intensity of about 5 μmol m^{-2} s^{-1}. The dark and light intervals tested are given in Fig. 6. During light periods, PFD values were about 850 mol m^{-2} s^{-1}. These values were measured at the end of the trial with a quantum sensor (400-700nm, LI-COR, Lincoln, NE) placed at the same location as the pulvinus .

Image Capture

All images were captured from the camera inside the chamber using the standard Quickcam software. Images were captured every minute for the 5, 10, and 20 minute trials, and every 30 seconds for 1 and 2 minute interval trials. All images were in TIFF format.

Measuring Leaf Angles

Leaf angles were measured using Scion Image (NIH, Scion Corporation, Rockville, MD). This software allowed the measurement of the angle between the petiole and the leaf (Figure 7). After copying the list of measurements, the values were imported into Sigma Plot (SPSS Science, Chicago, IL), where leaf angle was plotted

against time. If movies were desired, QuickTime Pro (Apple Computer, Cupertino, CA) was used to convert a series of images into video files of MOV or AVI format.

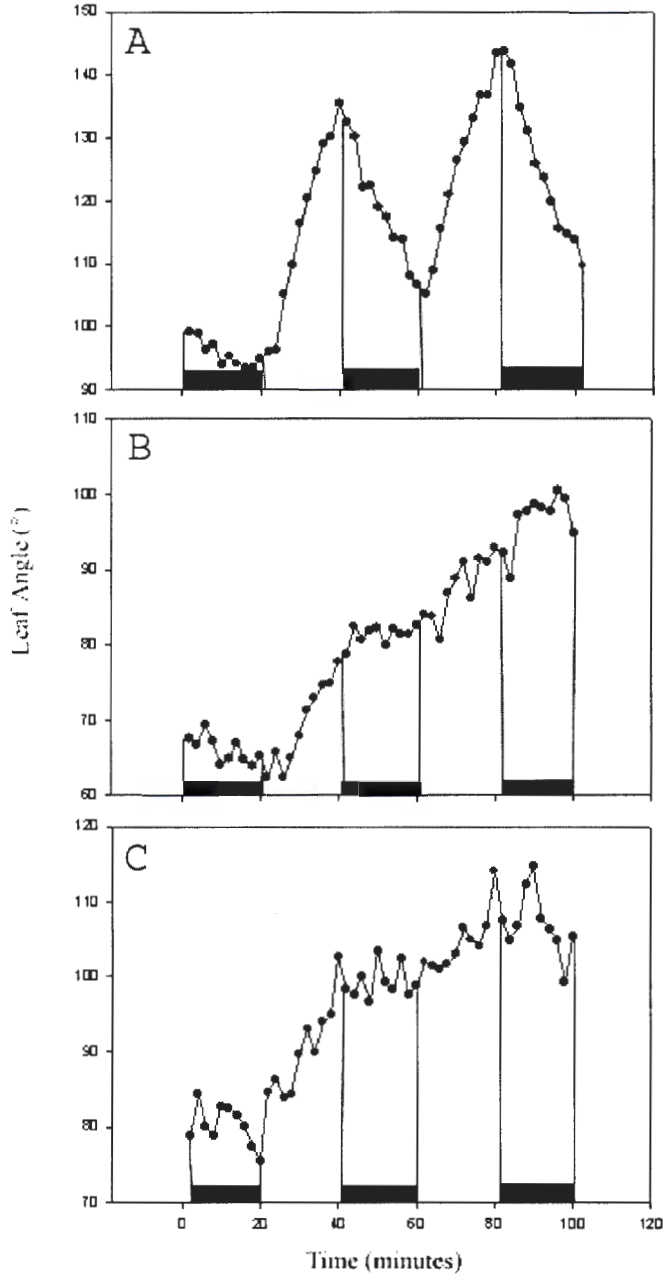

Figure 1. Effect of full spectrum light at twenty-minute intervals. A, B, and C are each representative of the movement exhibited by one leaf on three independent plants. Starting angles were -22.99°, -46.47°, and -36.84° in respect to horizontal for A, B, and C, respectively.

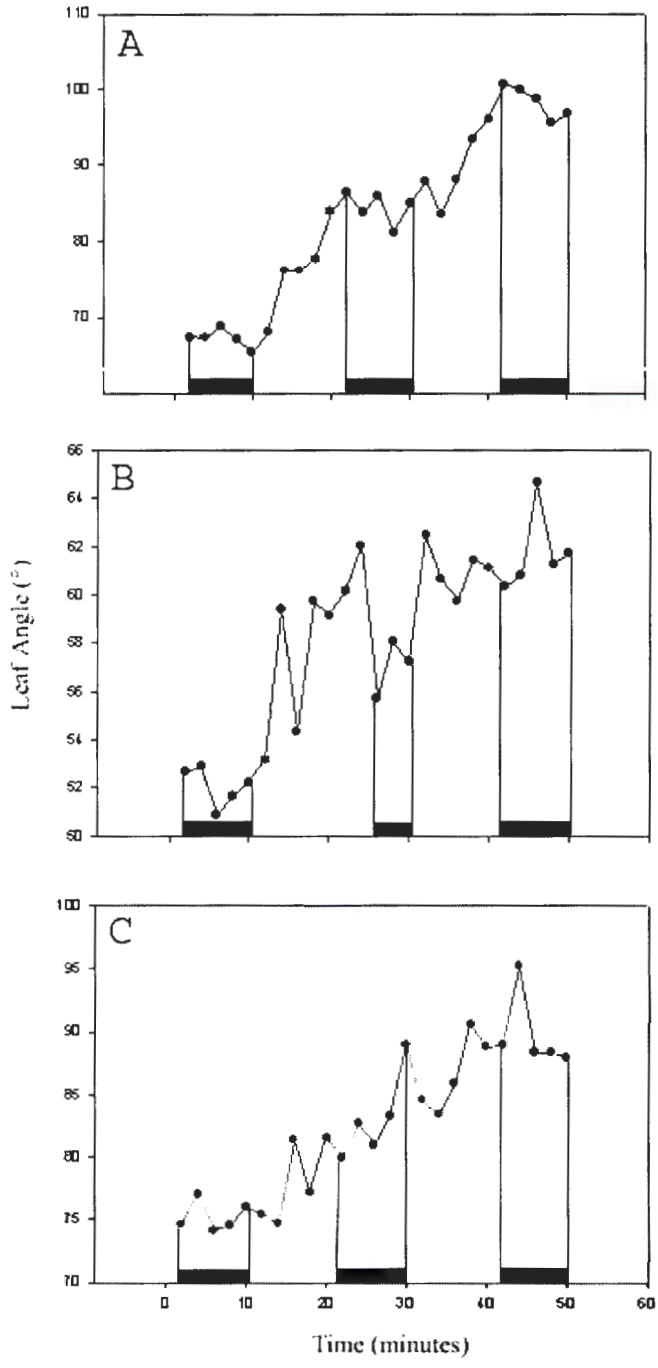

Figure 2. Effect of full spectrum light at ten-minute intervals. A, B, and C are each representative of the movement exhibited by one leaf on three independent plants. Starting angles were -68.2°, -67.48°, and -46.33° in respect to horizontal for A, B, and C, respectively.

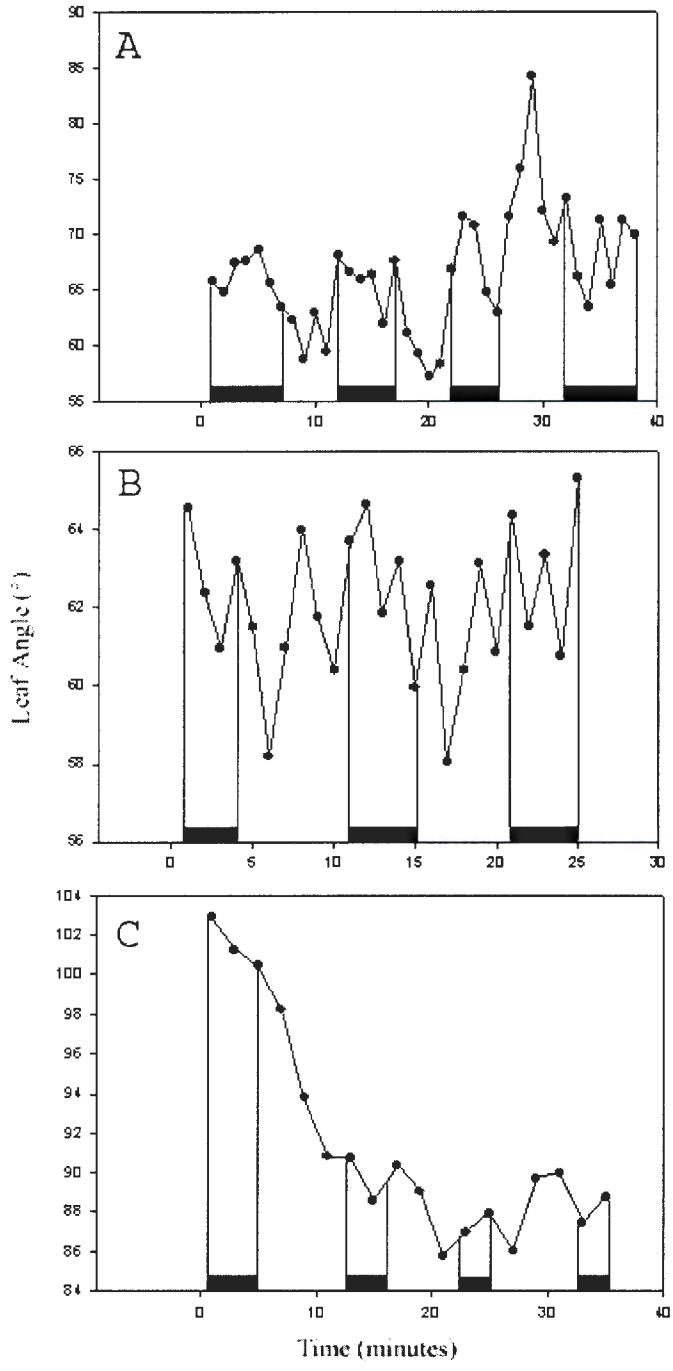

Figure 3. Effect of full spectrum light at five-minute intervals. A, B, and C are each representative of the movement exhibited by one leaf on three independent plants. Starting angles were -23.75°, -37.69°, and -34.38° in respect to horizontal for A, B, and C, respectively.

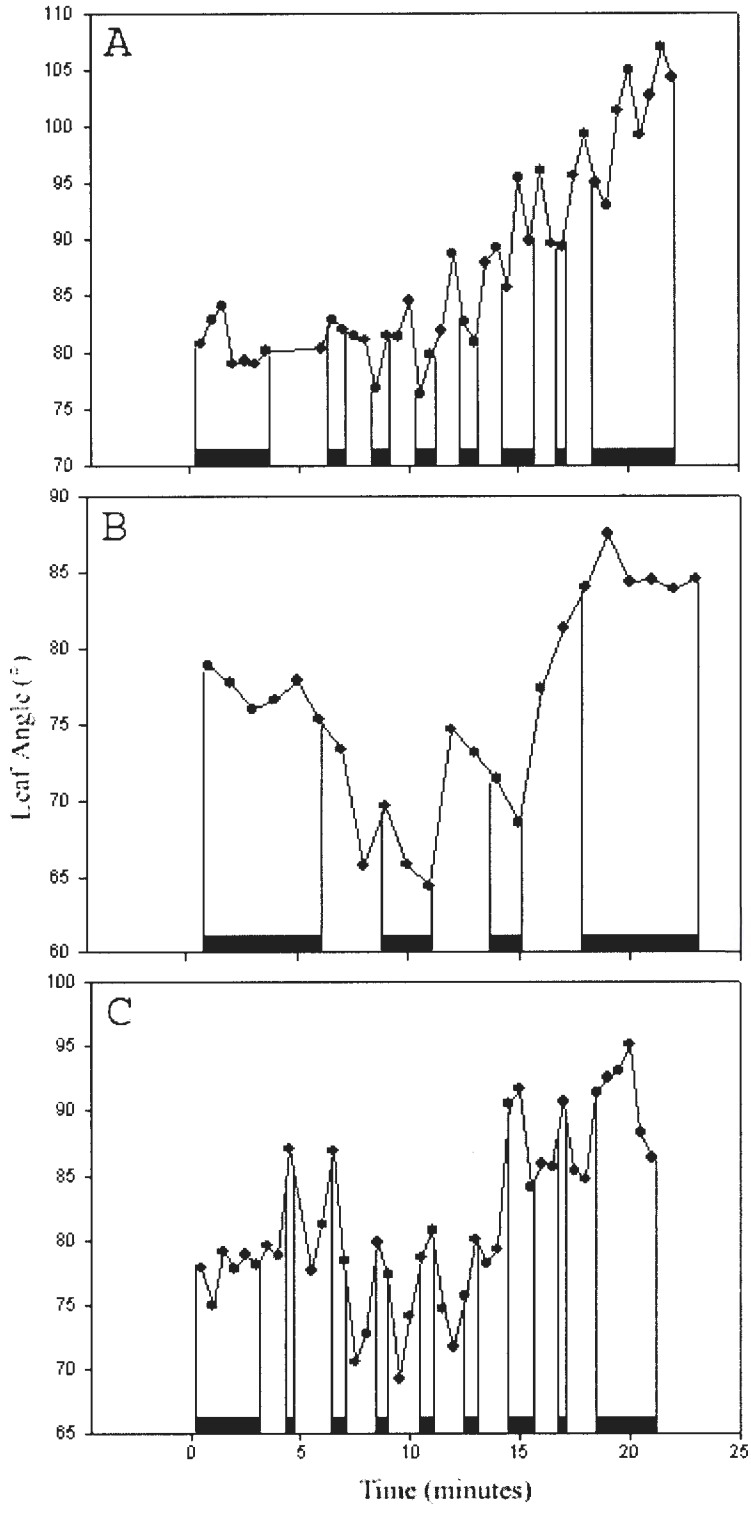

Figure 4. Effect of full spectrum light at two-minute intervals. A, B, and C are each representative of the movement exhibited by one leaf on three independent plants. Starting angles were -34.82°, -42.27°, and -47.20° in respect to horizontal for A, B, and C, respectively.

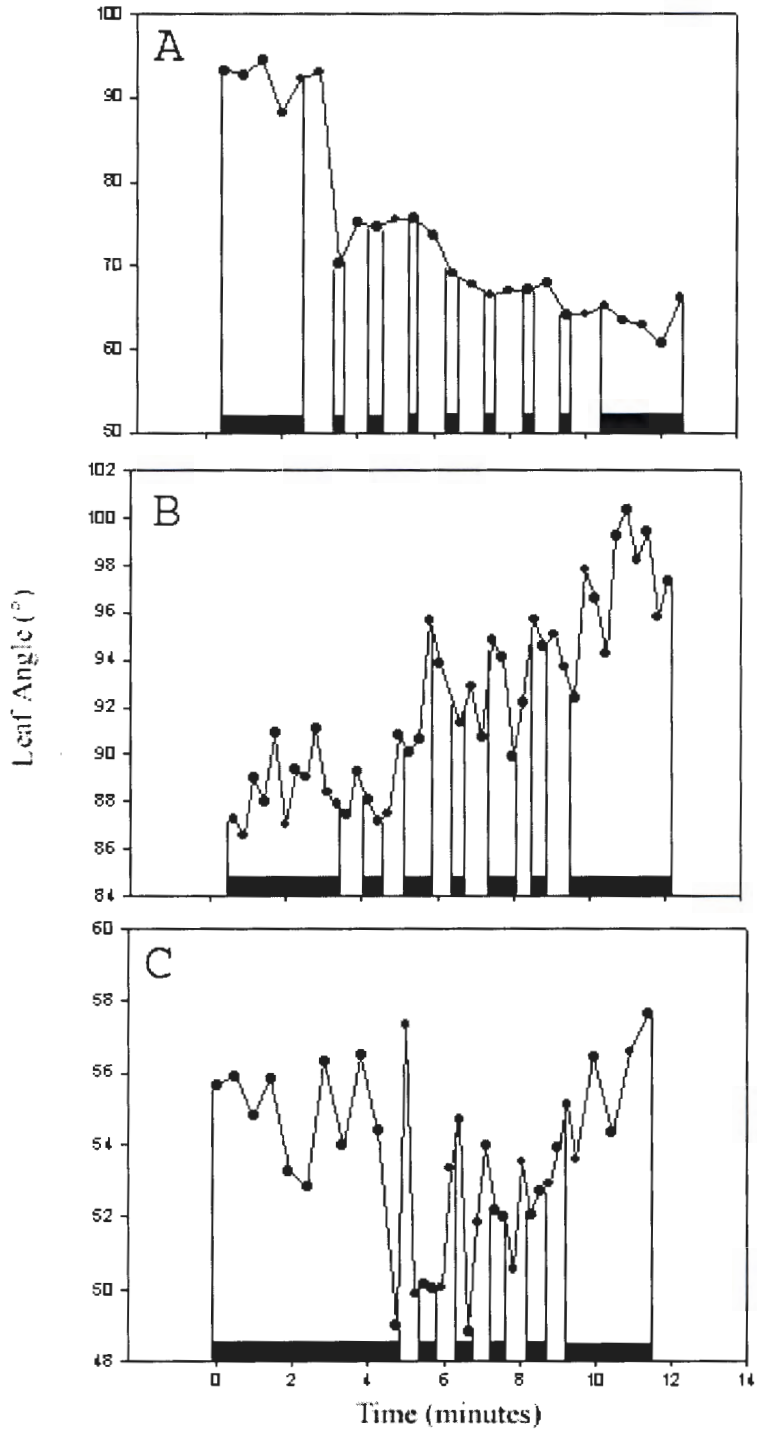

Figure 5. Effect of full spectrum light at one-minute intervals. A, B, and C are each representative of the movement exhibited by one leaf on three independent plants. Starting angles were -27.6°, -34.99°, and -68.5° in respect to horizontal for A, B, and C, respectively.

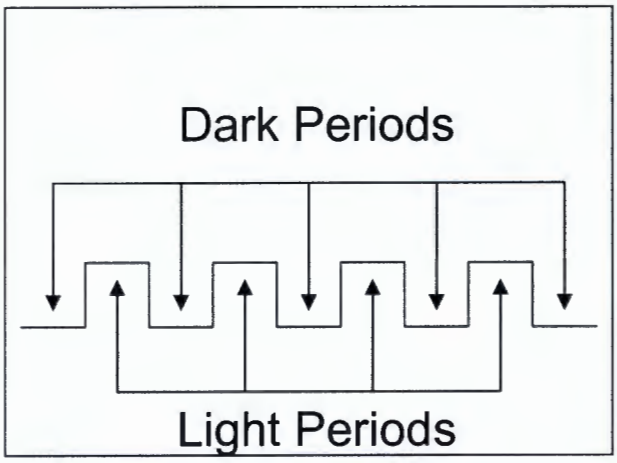

Figure 6. Example of a typical interval pattern during experimentation.

Figure 7. A characteristic example of image measurement of the angle between petiole and leaf using Scion Image.

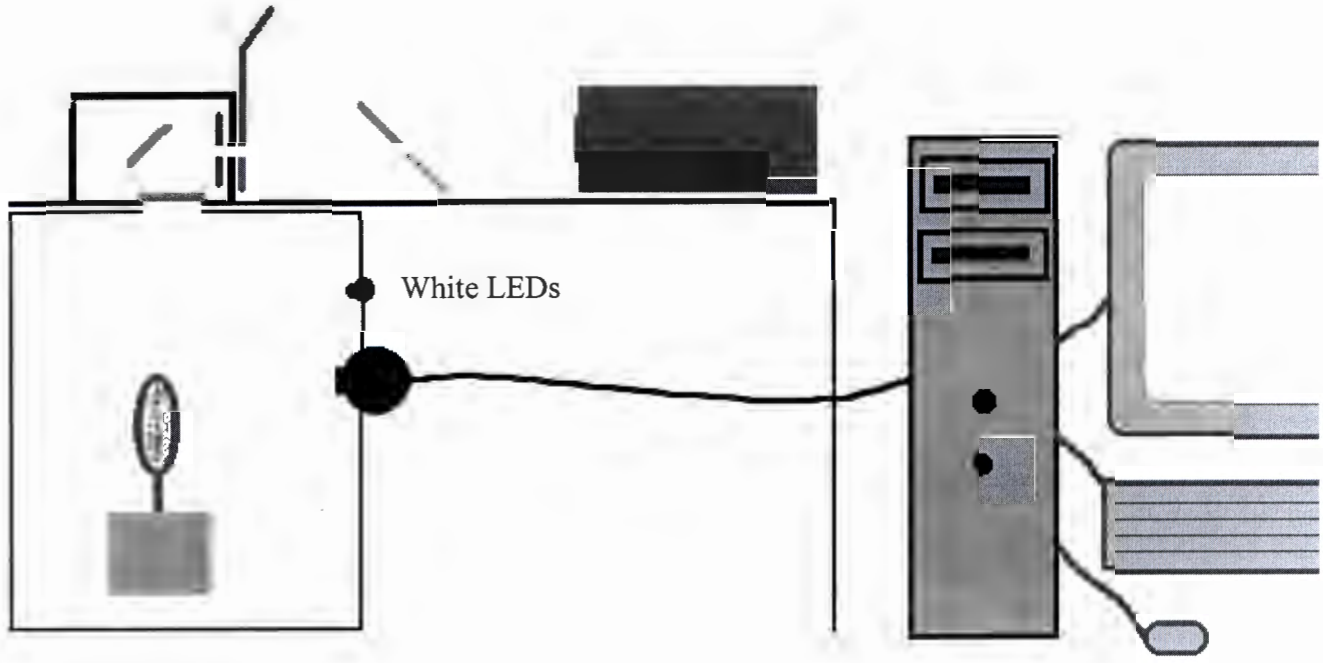

Figure 8. Chamber setup (Image modified, courtesy of Jeff Church).

DISCUSSION

When compared to control plants that were exposed to dark-only or light-only conditions, the plants exposed to light and dark intervals exhibited large and predictable responses. Previous research has shown that certain wavelengths of light have very conventional pulvinar responses; however, not much has been recorded about the effects of light period and its corresponding effects on the plant.

Twenty-Minute Intervals

When looking at the results of the twenty-minute interval trials, the results display precisely what was expected to happen according to the hypothesized blue light mechanism (Nishizaki, 1996). After the initial period of dark, during the light-exposure part of the interval, the graph is very smooth, with upward leaf movement during periods of light exposure, and downward leaf movement during periods of low light condition, as simulated by the LEDs positioned in the plant chamber. Of particular interest in these trials is the fact that on a long, strong light exposure, the plants react exactly as we would expect them to, except for one factor. The erratic movement at the onset of light exposure is a precursor of some mechanism that is seen more profoundly on shorter light intervals.

Ten-Minute Intervals

Similar to the twenty minute interval trials, on a ten-minute interval the plants still exhibited the upward response to light, and the downward leaf movement during the dark periods. However, the erratic movements occurring during the initial exposure to light were back, this time to a much greater degree. Instead of spiking three to four degrees,

the range of unpredictable movement during this period occurred at a maximum swing of five to ten degrees.

Five-Minute Intervals

During five-minute light intervals, some interesting trends started to become obvious. For one, when light exposure first occurred, the leaf angle typically started down and continued on a downward pattern until the next dark cycle, at which point the leaf would accelerate at an upward angle. The unique part of this pattern is the fact that these movements are opposite of what is expected, and what had been observed to this point. Further, some interesting cyclic patterns within each individual segment of the light trials were noted.

Two-Minute Intervals

With the two-minute intervals, the shorter periods for observation (30 seconds instead of 1 minute) allowed the before and after each portion of the intervals to be more closely observed. Less consistent results were common for the two-minute intervals, and when acceleration occurred in either direction, it seemed to be much sharper and faster.

One Minute Intervals

Characteristic trends from trial to trial were virtually nonexistent during the one-minute intervals; however, the short periods of observation were again very important to tying the trends in other parts of the experiment together. Generally, the overall leaf movement was very minimal, although acceleration from observation to observation would often spike largely. Also, while overall direction was different from trial to trial, it seemed that once a plant started moving a certain direction, it would continue for the rest of the trial.

Multiple Responses

The patterns exhibited in these trials, as well as previous trials (Church, 2003), seem to confirm the fact that at least two different "systems" are used. The first clue to this came during Church's trials examining the effects of light wavelength on pulvinar motor function. It was noted that before upward acceleration occurred during blue light bombardment, there was a short downward spike. Conversely, during red light exposure, there was a shallow, slow upward acceleration before the characterstic downward movement upon exposure to red light (Church, 2003). While these movements were attributed to the possible lack of ATP due to drought conditions or "shock" from the change of no-light to bright-light conditions, there is evidence from the trials that this is not the case.

If we look only at the first few moments of any of the trials, we can see clearly that almost all of the plants reach a point of slow stabilization, often leveling off after an initial decrease in leaf angle before being bombarded with bright light. However, when the plants were exposed to high light conditions, some would accelerate up (1A and 2A), some would have a downward spike (2C, 3B), and some would show a quick increase followed by a prolonged decrease in leaf angle (1B, 1C, and 2B). This inconsistency of plants under full spectrum lighting seems to refute the fact that plants could be lack ATP due to drought conditions. Further, shock from transfer was deliberately avoided by transferring the plants to the dark chamber the night before the trials, and then exposing the pulvini to low light for several hours before the trial.

While not entirely conclusive from this study, one idea for these movements stems from this episodic movement at the onset of light. Based on trends noted in this

trial, it seems feasible that before exposure to light, the upper and lower sections of pulvinus "pull" on the vascular tissue, resulting in a tension which helps to maintain the leaf angle. When light hits the upper pulvinus, the cells shrink, releasing ions and water on a somewhat fast time scale. Since the pulvinus is an enclosed organ, the ions and water must be taken up by the bottom cells, which occurs at a slower rate; partially due to time it take for the ions and water to reach these cells, and possibly in part due to rate of uptake by pulvinar motor cells. The leaf continues to move up and down erratically while the top and bottom sections of the leaf balance out ion and water uptake, at which point a new tension is established on the vascular tissue and a direction is determined. At shorter light intervals this balance is not effectively established, and so indeterminate leaf direction continues.

Conclusion and Future Experiments

The findings of this study provide a new explanation for the indeterminate and erratic leaf movements found not only in this trial, but the past trials of Jeff Church as well. The strange downward acceleration can be contributed to the model of vascular tissue tension, and rate of ion uptake. While this new hypothesis describes these movements, further experimentation is necessary to find out the rates of movement, and other workings of this model.

Future experiments would be well suited to discovering critical action lengths by significantly reducing periods of image capture. Periods of capture from five to 10 seconds would be very interesting, especially during light intervals producing the most erratic responses. Additionally, measurement error would be reduced during image

analysis if some sort of beads could be attached to the end of the leaf, the end of the petiole, and the pulvinus.

In regards to light, future experiments could include an automatic timer and shutter system to achieve shorter intervals of light. Also, it would be interesting to develop high intensity LED arrays in the blue, red, and green spectrum and repeat the white light trials. The data from these studies could be applied to the current model to determine precise rates of ion and water transport delay, and confirm this action in the pulvinus.

LITERATURE CITED

Burkholder, P.K., and Pratter, R. 1936. Leaf movements of *Mimosa pudica* in relation to the intensity and wavelength of incident radiation. American Journal of Botany. 23; 212-220.

Church, Jeff. 2003. Effect of light wavelength, intensity, and duration on leaf movements of *Phaseolus* species. University of Northern Iowa, unpublished.

Darwin, C. 1881. The power of movement in plants. Appleton, New York.

Donahue, R., and Berg, V.S. 1990. Leaf orientation of soybean seedlings. II. Receptor sites and light stimuli. Crop Science. 30; 638-643.

Fondeville, J.C., and Schneider, M.J., Borthwick, H.A., Hendricks, S.B. 1967. Photocontrol of Mimosa pudica L. leaf movement. Planta. 75; 228-238.

Iino, M., Ogawa, T., and Zeiger, E. 1985. Kinetic properties of the blue light response of stomata. Proc. Natl. Acad. Sci. USA. 82; 8019-8023.

Koller, D., and Ritter, S. 1994. Phototropic responses of the pulvinule and associated laminar reorientation in the trifoliate leaf of bean *Phaseolus vulgaris* (Fabaceae). Journal of Plant Physiology. 143; 52-63.

Koller, D., Ritter, S., and Fork, D.C. 1996. Light-driven movements of the trifoliate leaves of beans (*Phaseolus vulgaris* L.). Spectral and functional analysis. Journal of Plant Physiology. 143; 52-63.

Koller, D. 2001. Dynamic aspects of the response of the pulvinus in the leaf of bean plants (*Phaseolus vulgaris* L.): A kinetic analysis. Israel Journal of Plant Science. 48; 1-7.

Nishizaki, Y. 1987. Light-induced changes of electrical potential in pulvinar motor cells of *Phaseolus vulgaris* L. Plant Cell Physiology. 28; 1163-1166.

Nishizaki, Y. 1988. Blue light pulse-induced transient changes of electric potential and turgor pressure in the motor cells of *Phaseolus vulgaris* L. Plant Cell Physiology. 29; 1041-1046.

Nishizaki, Y. 1996. Effects of Blue Light on Electrical Potential and Turgor in Pulvinar Motor Cells of *Phaseolus*. Journal of Plant Res. 109; 93-97.

Satter, R.L., Guggini, S.E., Lonergan, T.A. and Galston, A.W. 1981. The effects of blue and far red light on rhythmic leaf movements in *Samanea* and *Albizzia*. Plant Physiology. 67; 965-968.

Wantanabe, S., and Sibaoka, T. 1983. Light- and auxin-induced leaflet opening in detached pinnae of *Mimosa pudica*. Plant Cell Physiology. 24; 641-647.

Williams, C.W. and Raghavan, V. 1966. Effects of light and growth substances on the diurnal movements of the leaflets of *Mimosa pudica*. Journal of Experimental Botany. 17; 742-749.

Zeiger, E., Iino, M., Shimazaki, K., and Ogawa, T. 1987. The blue-light response of stomata: Mechanism and function. In E. Zeiger, G.D. Farquhar and I.R. Cowan, eds., Stomatal Function. St.anford: Stanford: UP, pp. 209-227.

Zeiger, E. 2000. Sensory transduction of blue light in guard cells. Trends in Plant Science: Research News. Vol. 5 No 5; 183-185.