"Photo-Manipulation-Boxes"; An Instrument for the Study of Plant Photobiology

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Abstract: An inexpensive device used to study the responses of seeds and small plants to light is described. The device allows the intensity, duration and wavelength of light to be controlled. Three different wavelengths of light may be simultaneously and independently manipulated. The key components of the instrument are light-emitting diodes (LEDs) and a light-tight box. This design uses commercially available LEDs with peak emissions in the red (660nm), far-red (735nm) and blue (470nm) bands, corresponding to the peak absorption bands of phytochrome and cryptochrome. The results of some preliminary experiments conducted with the instrument are described. The costs, advantages, limitations and potential of this system as an undergraduate teaching and research tool are discussed.

Key Words: Phytochrome, cryptochrome, germination, plant development, light, light-emitting-diodes, lettuce, *Arabidopsis*, methods.

Introduction

Light is not only an energy source for plants but also acts as a stimulus that controls plant development and gene expression throughout a plants life (Morgan and Smith 1981; Chory *et al*. 1996). Undergraduate botany, plant-biology and plant-physiology textbooks routinely include discussions of phototropism, photomorphogenesis, photoperiodism, and photoblastic seed germination (Salsibury and Ross 1996; Mauseth 1998; Moore *et al*. 1998; Hopkins 1999; Raven *et al*. 1999). Likewise, undergraduate plant-physiology laboratory manuals include exercises designed to demonstrate light induced response by plants (Ross 1974; Kaufman *et al*. 1975; Reiss 1994). The key to conducting a photobiology experiment is manipulating the quantity of light and the quality (wavelength) of light to which an organism is exposed. An ideal system would allow the simultaneous control of both quantity and quality of light. Here, I describe such a system.

Plant Pigments and Photobiology

One of the fundamental questions asked in photobiology is "Which wave-lengths of light induce a response?" For light to induce a response a pigment must absorb the light and thereby trigger a biochemical reaction. Different pigments absorb different wavelengths of light. Plants contain many pigments, serving diverse functions, but two pigments, phytochrome and cryptochrome, are crucial in mediating plant responses to light.

Phytochrome consists on of two tautomers; phytochrome-red (P_r) and phytochrome-far-red (P_{fr}). Phytochrome-red absorbs red wavelengths ($\cong$660nm) and when it does so P_r is converted to P_{fr}. Phytochrome-far-red absorbs far-red wavelengths ($\cong$730nm) and when it does so P_{fr} is converted to P_r. Phytochrome mediates many different plant responses to light including, photoperiodism, seed germination, leaf expansion, hypocotyl hook straightening and de-etiolation.

Cryptochrome absorbs light in the blue and ultra-violet regions. The responses mediated by cryptochrome include phototropism (the growth of a plant towards or away from light), hypocotyl elongation, and stomata opening. Other pigments are involved in plant development, but phytochrome and cryptochrome mediate so many different responses, that it is important to introduce students to the biology of these two pigments. Demonstrating that a given response only occurs following irradiation with red light, far-red light or blue light, is one method of implicating that these pigments mediate the response.

Controlling Quality of Light

There are three methods of obtaining a specific wavelength of light for use in an experiment: 1) Spectroscopy, the use of a prism or diffraction-grating to separate broad-band, white-light into a spectrum of colors; 2) Filtration, the passing of light through a semitransparent object which selectively absorbs or reflects certain wavelengths of light; and 3) Use of a narrow band light source, a source of light that emits only selected bands of wavelengths. Historically, all three of these methods have been important in the development of plant photobiology, but because spectroscopes capable of exposing entire seeds or plants are both large and expensive, they have rarely been used in undergraduate laboratories. The methods typically recommended for undergraduate laboratories combine the use of filters and narrow band light sources (Lercari 1991).

Fluorescent lamps do not emit far-red light and therefore can be used, either with or with out subsequent filters, as a source for "red" light. Incandescent lamps emit both red and far-red light, and when coupled with a filter that absorbs red and shorter wavelengths incandescent lamps can be used as a source of far-red and infrared light. Either fluorescent or incandescent lamps can be used in combination with filters that block long wavelengths as sources of blue light. There are difficulties in using these techniques. Because of the possible contamination by broad-band sunlight, the light sources should be placed in a darkroom or dark-box. This is a logistical problem in undergraduate labs where darkrooms are not available or convenient. Care must be taken to avoid cross contamination from the far-red to the red light source and visa-versa. Because incandescent lamps emit large amounts of heat it is important to take precautions to prevent temperature from being an uncontrolled covariate between red and far-red treated plants.

High quality glass filters can be expensive and consequently undergraduate labs often use inexpensive cellophane filters. However, cellophane filters are prone to fade with use, creating the potential for error. One manual recommends measuring the transmission spectrum of a representative strip of the cellophane before use (Ross 1974). Switching the exposure from one wavelength to another (as is required to demonstrate the photoreversibility) requires special precautions but can be accomplished without a darkroom (Wagner and Wagner 1995).

Light-emitting-diodes (LEDs) are solid state devices that emit light when a current is passed across their leads. LEDs emit a narrow range of wavelengths. Typical emission spectra are symmetrical around a single peak with half-band widths of 30nm or less. LEDs are available in a range of colors from 430nm (Blue) to 940nm (Infrared). LEDs are inexpensive, ranging in cost from fifty cents to ten dollars each. LEDs are reliable; most LEDs are rated to last a minimum of 100,000 hours. Each LED emits a relatively small amount of light, but a bank of LEDs can generate high light intensities. Over the past few years, light-emitting diodes have become increasingly popular as narrow-band light sources for plant studies (Bula *et al*. 1991; Lee and Palsson 1994; Robin *et al*. 1994; Tennessen *et al*. 1994; Brown *et al*. 1995; Miyashita *et al*. 1995; Schuerger *et al*. 1997; Tanaka *et al*. 1998). Percival Scientific Incorporated now markets a plant-growth-chamber that use LEDs as the sole light source (see; http://www.percival-scientific.com/prod03.htm). Li-Cor now offers the optional LED light source for use with their photosynthesis measuring systems (see; http://www.licor.com/).

I have created a series of boxes that use LEDs to emit light into a light-tight chamber. Each box contains three LEDs, one each with peak emissions at 470nm, 630nm and 735nm. The boxes are designed so that each individual LED can be dimmed with a potentiometer and controlled independently. This system allows the researcher to control the color of light, the intensity of light and the length of exposure for objects inside the box. The boxes are small so a large number of treatments can be applied in a small area. Plants can be left in the dark boxes between treatments, reducing the need for dark rooms. For lack of a better name I refer to these boxes as "Photo-Manipulation-Boxes" or PMBs.

The Design of Photo-Manipulation-Boxes

Three identical electronic circuits are included in each box; one circuit to control each LED (figure 1). Each circuit requires few solder joints and minimal knowledge and skill to construct. A 9-volt battery provides current for the circuit. The battery is connected via a switch to a 7806 integrated circuit. The 7806 is a voltage regulator that accepts inputs between 6 and 15 volts and outputs a constant 6 volts. The output current passes through a small resistor and into a potentiometer. The potentiometer controls the brightness of the LED by either forcing all the current through the LEDs or allowing some of the current to pass to the ground. Two LEDs are connected in series. The first emits light into the box, the second, an inexpensive green LED, indicates whether the circuit is open or closed. Each circuit is isolated and has its own power source. Therefore the intensity of light emitted by each LED can be regulated independently, allowing the researcher to simultaneous expose seeds to controlled amounts of one to three wavelengths of light. A 9-volt battery will power this circuit for up to 2-4 hours. For prolonged use, Radio Shack's 9-Volt Battery Eliminator (Cat No. 273-1552A) can replace the 9-volt battery and provide current via a 110-volt alternating-current outlet.

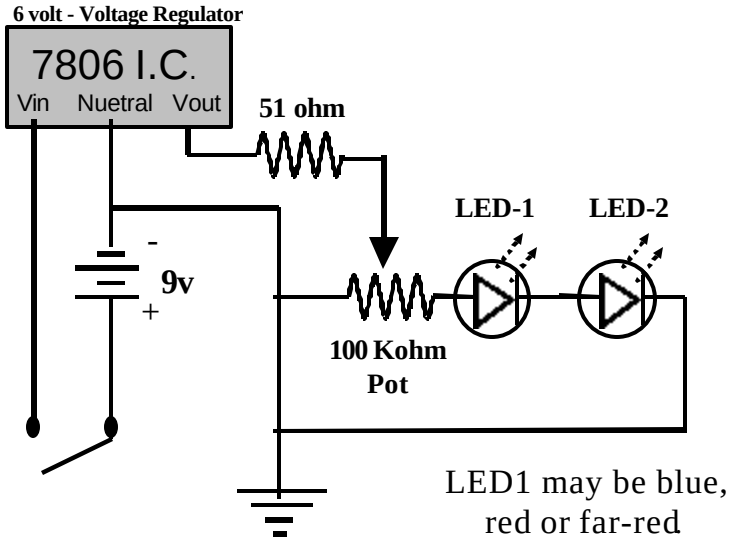

Figure 1 *Three copies of the circuit shown above are found in each PMB, The color of LED-1 varies between the circuits.*

The boxes are made of ½ plywood with 1/8" fiberboard used to form bases and lids. The box is divided into bottom and top halves. The bottom half is a 2.5" deep by 4.5" wide, by 4.5" long box (see figure 2.). The top half is divided into two compartments by a 1/8" fiberboard which is joined into the edges of the box. The upper compartment protects the LED circuits. The roof of the lower compartment has the LEDs mounted in it. The light-emitting portion of the LED extends into the lower compartment, the leads into the upper compartment. Black electrical-tape is applied around the LED leads to assure a light-tight seal between the upper and lower compartment.

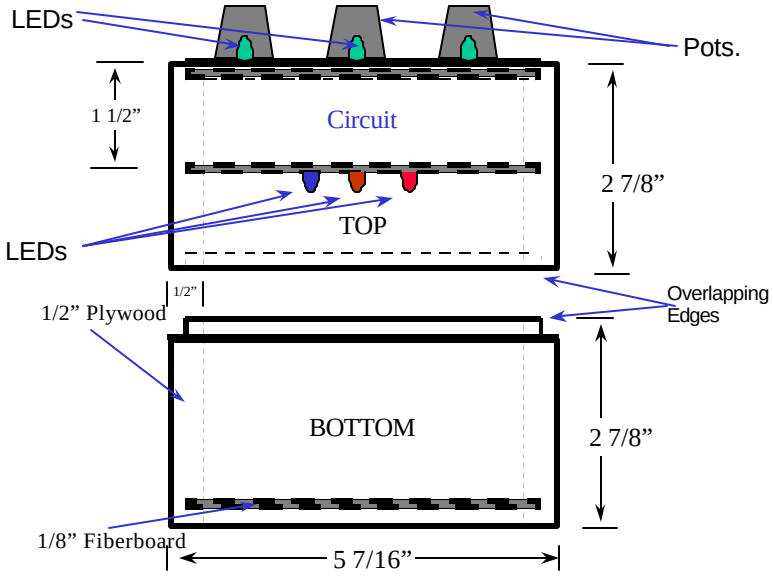

Figure 2. *The design of the PMB enclosures.*

The two halves of the box are machined to have overlapping joints. These joints are lined with gray weather-seal foam-tape, so that when the top half is pressed against the bottom half, a light-tight seal is created and an irradiation chamber is formed. A pair of chest latches attach the top and bottom halves of the boxes. The inner walls of the irradiation chamber are lined with a reflective aluminum foil tape. This insures that all joints are light tight and helps diffuse the light more evenly through the chamber. The box is sized so that the irradiation chamber can hold one 10cm diameter petri dish or two 6cm diameter petri dishes. Additionally the chamber has enough depth to allow the light to spread from the LEDs. There is also room for seeds to germinate and grow to a height of an inch or two.

Each PMB contains approximately $34 dollars worth of parts (Table 1). The single most expensive item in the design is the far-red LED, costing over ten dollars each. The second most expensive item is the blue LED at $6.58 each. If the researcher is not interested in experimenting with blue light, the parts for a box containing only red and far-red LEDs can be purchased for less than $25. Some cost savings are possible by using a combination of three-LED and two-LED PMBs. A PMB enclosure without any LEDs is acceptable for dark controls. PMBs are more expensive than colored cellophane as a method of generating near monochromatic light. However, it is hoped that the ease of use, increased reliability, and greater versatility will justify the increased expense.

Table 1. *Parts and suppliers for construction of PMB enclosures.*

Part	Part #	Supplier	Cost Each	#per box (B-R-FR)	cost per box (B-R-FR)	#per box (R-FR)	cost per box (R-FR)
Blue LED 470nm Panasonic part LNG992CFBW	P465-ND	D-K	6.58	1	6.58	0	0.00
Red LED 665nm Panasonic part LN261CAL(UR)	P408-ND	D-K	0.93	1	0.93	1	0.93
Far-Red LED 735nm Quantum Devices part QDDH73502 (preferred) or QDDH73520	QDDH73502	QD	10.97	1	10.97	1	10.97
Green LED 565nm Panasonic Part LNG305CFT	P461-DN	D-K	0.31	3	0.93	2	0.62
Voltage Regulator 7806, 6 Volt / 500mA NJR Corp Part NJM78M06FA-ND	NJM78MO6 FA-ND	D-K	0.41	3	1.23	2	0.82
51 ohm ½W Resistor Yageo 5% Carbon film Resistor	51H-ND	D-K	0.02	3	0.06	2	0.04
Potentiometer 100 Kohm 0.1 W-linear taper	31CR501	M	1.12	3	3.36	2	2.24
SPDT Slide Switch	10SM007	M	0.34	3	1.02	2	0.68
9V Battery Connectors	270-324	RS	0.38	3	1.14	2	0.76
Snap-in LED Holders	276-079	RS	0.12	6	0.72	4	0.48
General Purpose IC Board	276-150A	RS	1.19	0.5	0.60	0.5	0.60
Screws (for attaching switches)	48SM006	M	0.04	6	0.24	4	0.16
Electrical Tape (estimate	?	?	0.25	1	0.25	1	0.25
Solder (estimated cost)	?	?	0.02	1	0.02	1	0.02
				Cost of Electronics	27.54		18.14
				Cost of Enclosure	5.97		5.97
				Total Costs per Box	33.51		24.11

Electronic Suppliers

D-K = Digi-Key Corporation -- 701 Brooks Ave. South, Thief River Falls, MN; M = Mouser Electronics -- 958 N. Main, Mansfield, TX 76063-4827; RS = Radio Shack

Replication of the Grand-Rapids Lettuce Seed Experiment using PMBs

When Grand-Rapids variety lettuce seeds are maintained in the dark, they have low rates of germination; however when exposed to white light, they have high rates of germination. More precisely, red-light (≅660nm), and not other wavelengths, will induce the lettuce seeds to germinate. Far-red light (≅730nm) inhibits lettuce seed germination. This reaction is photoreversible, if seeds are exposed first to red light and then to far-red light, they will not germinate. However, if another exposure of red light follows the seeds will again exhibit high germination rates. (Flint and McAlister 1935, 1937; Borthwick *et al*. 1954). The observation of red/far-red photoreversibility in the induction of both lettuce seed germination and cocklebur flowering led to the proposal of the existence of a photoconvertible pigment. Eventually this pigment was identified to be phytochrome.

I have also used the PMBs to demonstrate photoreversible lettuce seed germination. For each treatment, two pieces of filter paper (Fischer Brand -- qualitative, grade p5, medium porosity, 9.0cm diameter) and 30 Ward's Light-Sensitive lettuce seeds were placed in a 10cm diameter petri dish (Pyrex glass was used in this case, but plastic petri dishes will work). 5ml of water were added to each dish and the dish was immediately sealed in a PMB. The potentiometers were adjusted so that the maximum amount of light was emitted from the LEDs. During the next 24 hours the seeds were exposed to one of six sequences of light treatments: A) 1 hour of far-red light, 23 hours of darkness; B) 1 hour of far-red light, 0.25 hours of red light, 22.75 hours of darkness; C) 1 hour of far-red light, 0.25 hours of red light, 0.5 hours of far-red light, 22.25 hours of darkness; D) 1 hour of far-red light, 0.25 hours of red light, 0.5 hours of far-red light, 02.5 hours of red light, 22 hours of darkness; E) 24 hours of darkness; or F) 24 hours of ambient light (fluorescent lamps and indirect light from a nearby window). Twenty four hours after the beginning of the light treatment the boxes were unsealed and the number of seeds with radicles extending from the seed coat was recorded. The mean results of three replicates of each treatment are illustrated in figure 3.

Note, that the methods above do not require a dark room or "safe-light." Phytochrome is insensitive to light as long as the seeds are dry. Only after the seeds absorb water and the phytochrome goes into solution is the phytochrome active. If seeds are placed into dark boxes before they have imbibed an appreciable amount of water it is, probably safe to ignore the effects of ambient light during experimental set-up. The one hour of far-red treatment included sufficient time for the seeds to both imbibe water and respond to the inhibitory effect of far-red light.

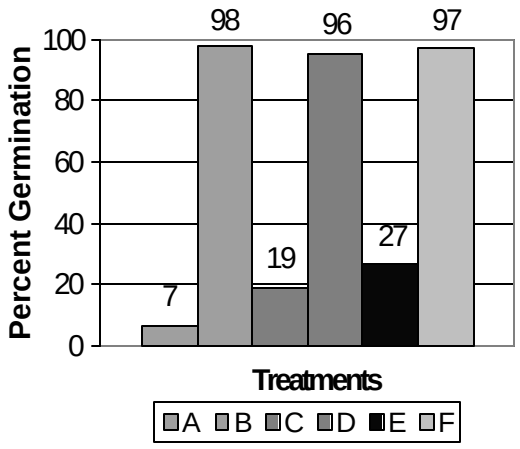

Figure 3*. Effect of red and far-red light on lettuce seed germination. The light treatment were:* ***A*** *--1 hour far-red, 23 hours darkness;* ***B*** *-- 1 far-red, 0.25 hours red, 22.75 hours darkness;* ***C*** *-- 1 hour far-red, 0.25 hours red, 0.5 hours far-red, 22.25 hours darkness;* ***D*** *-- 1 hour far-red, 0.25 hours red, 0.5 hours far-red, 02.5 hours red, 22 hours darkness;* ***E*** *-- 24 hours darkness;* ***F*** *– 24 hours ambient light (fluorescent lamps and indirect light from a nearby window).*

A student might reasonably conclude from the above experiment that far-red light inhibits and any other light promotes germination. The PMBs can be used to demonstrate that blue light neither promotes nor inhibits lettuce seed germination. With the exception of the light treatments, the methods of this experiment are identical to the one described above. In this case the seeds were exposed to one of the six following sequences of light treatments: A) 1 hour of far-red light, 0.5 hours blue light, 22.5 hours of darkness; B) 1 hour of red light, 0.5 hours of blue light, 22.5 hours of darkness; C) 1 hour of far-red light, 23 hours of darkness; D) 1 hour of red light, 23 hours of darkness; E) 24 hours of darkness; or F) 24 hours of ambient light (fluorescent lamps and indirect light from a nearby window). The results show that blue light does not reverse the inhibition of germination by far-red light or the promotion of germination by red light (figure 4).

Many variations of the above experiments are possible. Temperature could be varied by placing the entire PMB in a temperature controlled chamber. Buffers could be used to vary the pH of the petri dishes. Osmotic potential could be varied among the dishes. Plant hormones could be added to the dishes. The timing of light exposures could be varied to investigate how long after a red exposure seed germination becomes inevitable.

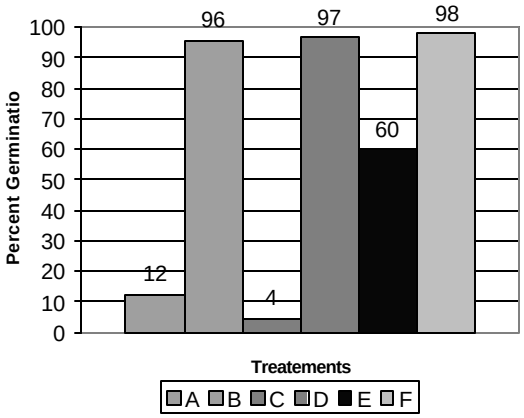

Figure 4*. Seeds were treated as in figure 4., with the exception of the light treatments. The light treatments were:* ***A*** *-- 1 hour far-red, 0.5 hours blue, 22.5 hours darkness;* ***B*** *-- 1 hour red, 0.5 hours blue, 22.5 hours of darkness;* ***C*** *-- 1 hour far-red, 23 hours of darkness;* ***D*** *-- 1 hour far-red, 23 hours of darkness;* ***E*** *– 24 hours darkness;* ***F*** *– 24 hours ambient light (fluorescent lamps and indirect light from a nearby window).*

Arabidopsis **Seed Germination**

Because of its historic importance, undergraduate texts routinely describe the phytochrome-mediated germination of lettuce seeds, but lettuce is only one of many species exhibiting this behavior. For example, the germination of *Arabidopsis thaliana* seeds is also controlled by red and far-red light (Shropshire *et al.* 1961). Moreover, *Arabidopsis* contains at least five different types of phytochrome: PhyA, PhyB, PhyC, PhyD and PhyE. Each type of phytochrome differs in its protein structure and function. (Quail 1991, 1997; Quail *et al.* 1995). Mutants of *Arabidopsis* are available with altered genes for each type of phytochrome (McCullough and Shropshire 1970; Cone *et al.* 1985; Shinomura *et al.* 1994; Botto *et al.* 1995; Briggs and Liscum 1997; Poppe and Schäfer 1997; Shinomura 1997). Even the different ecotypes of *Arabidopsis* may have significant differences in their phytochrome genes (Yanovsky *et al.* 1997).

With slight modifications to the methods used with lettuce seeds, PMBs can be used to demonstrate that wildtype *Arabidopsis* seeds are sensitive to red and far-red light. I have used the PMBs to compare the responses of the Columbia and Landsberg erecta ecotypes of *Arabidopsis* to red and far-red light. Two sheets of filter paper (Whatman -- qualitative, grade b, medium porosity, 5.5cm diameter) and 30 seeds of an ecotype were placed in a 6cm diameter petri dish. Another petri dish was prepared with seeds of the other ecotype, 2ml of water were added to each dish, and the dishes were sealed side by side in a single PMB. Seven PMBs were so prepared, and one of the seven following sequence of light treatments were applied to seeds in each: A) 1 hour of darkness, 1 hour of far-red light, 46 hours of darkness; B) 1 hour of darkness, 1 hour of far-red light, 0.5 hours of red light, 45.5 hours of darkness; C) 1 hour of darkness, 1 hour of far-red light, 0.5 hours of red light, 0.5 hours of far-red light, 45 hours of darkness; D) 1 hour of darkness, 1 hour of far-red light, 0.5 hours of red light, 0.5 hours of far-red light, 0.5 hours of red light, 44.5 hours of darkness; E)1 hour of darkness, 1 hour of far-red light, 0.5 hours of red light, 0.5 hours of far-red light, 0.5 hours of red light, 0.5 hours of far-red light, 44 hours of darkness; F) 48 hours of darkness; and G) 48 hours of ambient light (fluorescent lamps and indirect light from a nearby window). For all treatments, germination was assessed 48 hours after the water was added to the dish. Three replicates of this experiment were performed. *Arabidoposis* seeds are slower to imbibe water and germinate than are lettuce seeds. The initial hour of darkness at the start of each of the light treatments allowed the seeds to become fully imbibed before light treatments began. Given equal amounts of red light the Columbia ecotype has higher rates of germination than Landsberg erecta ecotype (figure 5 & 6). One possible future use of the PMBs is to compare the responses of phytochrome mutants of *Arabidopsis* to varying regimes of light.

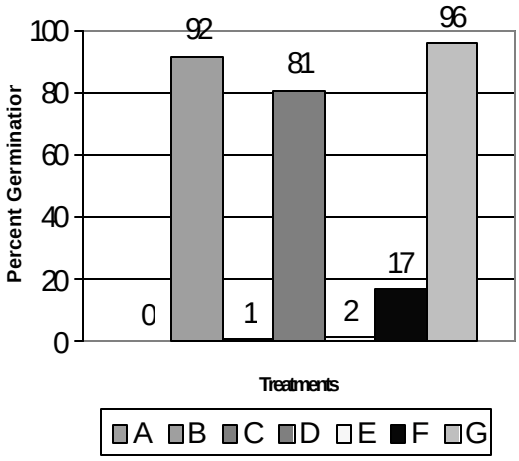

Figure 5*. Effect of red and far-red light on the germination of Arabidopsis thaliana variety Columbia seeds. The light treatments were:* ***A*** *-- 1 hour darkness, 1 hour far-red, 46 hours darkness;* ***B*** *-- 1 hour darkness, 1 hour of far-red, 0.5 hours red, 45.5 hours darkness;* ***C*** *-- 1 hour darkness, 1 hour far-red, 0.5 hours red, 0.5 hours far-red, 45 hours darkness;* ***D*** *-- 1 hour darkness, 1 hour far-red, 0.5 hours red, 0.5 hours far-red, 0.5 hours red, 44.5 hours darkness;* ***E*** *--1 hour darkness, 1 hour far-red, 0.5 hours red, 0.5 hours far-red, 0.5 hours red, 0.5 hours far-red, 44 hours darkness;* ***F*** *-- 48 hours darkness;* ***G*** *– 48 hours ambient light (fluorescent lamps and indirect light from a nearby window).*

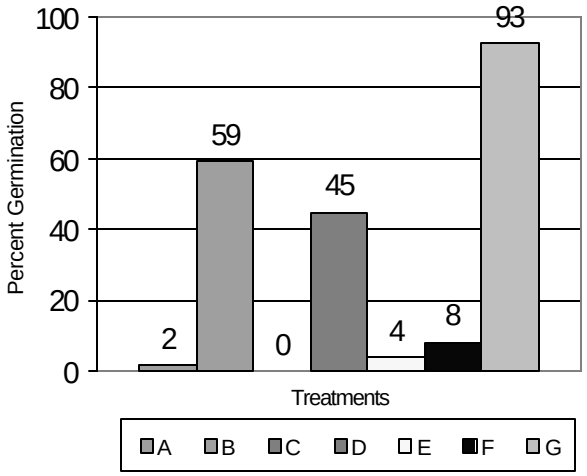

Figure 6. *Effect of red and far-red light on Arabidposis thaliana variety Landsberg erecta. Treatments were the same as in figure 5, but using the Landsberg erecta variety instead of the Columbia variety of Arabidopsis thaliana.*

De-etoliation and Hypocotyl Elongation of Lettuce Seedlings

Seed germination is only one of many stages of plant development that are controlled by light. For example, seedlings grown in total darkness will lack chlorophyll and have long thin hypocotyls. PMBs were used to test which color of light promotes chlorophyll synthesis and inhibits hypocotyl elongation. Two sheets of filter paper were placed in each of twelve 10cm diameter petri dishes. Ten lettuce seeds and 5ml of water were added to each dish. The dishes were covered and immediately sealed in separate PMBs. The red LED was turned on for one hour (to stimulate germination) and then all LEDs were turned off for an additional 71 hours. For the next 24 hours the seeds were exposed to one of four treatments: 24 hours of blue light, 24 hours of red light, 24 hours of far-red light or 24 hours darkness. The boxes were then opened, the length of the hypocotyl was measured and the color of the cotyledons was noted. The cotyledons of the far-red and dark treated seeds were yellow, the cotyledons of the blue and red treated seeds were pale green. Far-red and blue light both inhibited hypocotyl elongation relative to the dark controls, but red light had little effect on hypocotyl elongation (fig 7). A number of variations of this experiment are possible.

Controlling Quantity of Light

Photobiologists are concerned not only, with which color of light induces a response, but also how much light is needed to induce a response. Quantities of light can be defined either in terms of the amount of energy (joules or watts) contained in the light or in the number of quanta (photons) of light. Regardless of the units used, it is important to differentiate between fluence rate and fluence. Fluence rate is the energy (or photons) striking a surface in given amount of time. Fluence is the total energy (or photons) striking a surface. So fluence rate is measured in joules$*m^{-2}*s^{-1}$ or µmol of photons$*m^{-2}*s^{-1}$, whereas fluence is measured in units of joules$*m^{-2}$ or µmol of photons$*m^{2}$.

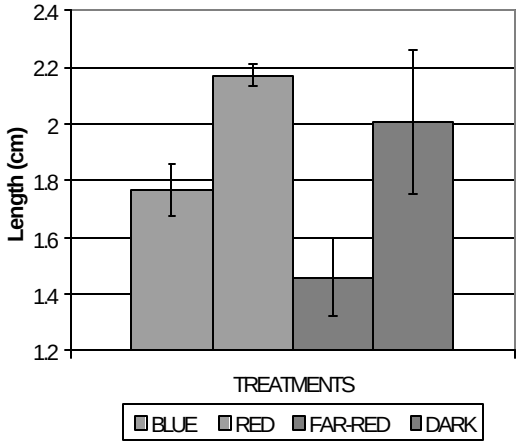

Figure 7 *The hypoctyl length of dark grown lettuce seedlings after exposure to 24 hours of blue, red or far-red light.*

Historically the Bunson-Roscoe law of reciprocity was thought to apply to most, if not all, photobiological reactions. The law of reciprocity states that the degree of response is proportional to fluence regardless of fluence rate. The causal explanation of the law of reciprocity, assumes that photochemical reactions are triggered by the absorption of photons, and therefore the more photons absorbed the greater the response. Of course, if fluence rates are so high that all possible photon-absorption-sites are saturated with photons at all times, increasing the fluence rate even further will not induce a greater response.

Today we recognize that there are major exceptions to the "law" of reciprocity. Most phototropic responses obey the law as long as fluences are low, but depart from the law when both fluences and fluence rates are high (Zimmermann and Briggs 1963; Salsibury and Ross 1996). Phytochrome mediated responses can be divided into very low fluence responses (VLFR), low fluence responses (LFR) and high irradiance responses (HIR) (Mandoli and Briggs 1981; Heim and Schäfer 1984; Botto *et al.* 1995; Salsibury and Ross 1996). Phytochrome A appears to mediate VLFRs and , phytochrome B & C may meditiate LFRs and HIRs (Qin *et al.* 1997). However, if only a single pigment is involved in

mediating a response it is likely that there is a range of fluences within which the law of reciprocity will hold.

The Length of Exposure and Germination of Light Sensitive Lettuce Seeds

One method of changing the fluence is to vary exposure time but keep fluence rate constant. When provided with a constant current and kept at near constant temperatures LEDs emit light at near constant fluence rates. Therefore, doubling the time an LED is on will result in nearly doubling the fluence. This allows the researcher to make inferences about the effect of change in fluence without having to accurately measure fluence rates. However it should be noted, that even given the same current, different LEDs may emit light at different fluence rates. Therefore, caution should be taken in comparing the effects of length of exposure to different LEDs. My experience with the PMBs is that for the purpose of teaching laboratories, the differences between LEDs can be ignored; however the more sophisticated researcher could control for such errors by replicating the experiment using a randomized block design to control for differences between PMBs.

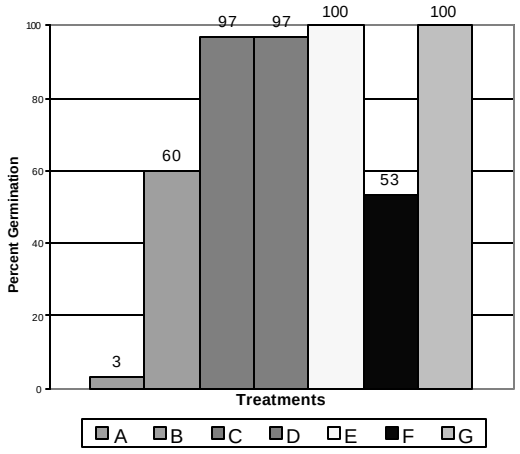

Figure 8 *The effect of short exposures of red light on lettuce seeds previously exposed to one hour of far-red light. The light treatments were:* ***A*** *– 1 hour far-red, 23 hours darkness;* ***B*** *– 1 hour far-red, 2 minutes red, 22 hour, 58 minutes darkness;* ***C*** *-- 1hour far-red, 4 minutes red, 22 hours 56 minutes darkness;* ***D*** *-- 1 hour far-red, 8 minutes red, 22 hours 52 minutes darkness;* ***E*** *– 1 hour far-red, 16 minutes red, 22 hours 44 minutes darkness;* ***F*** *– 24 hours darkness;* ***G*** *– 24 hours ambient light (fluorescent lamps and indirect light from a nearby window) for 24 hours. All potentiometers were adjusted to maximize the light emission from the LEDs.*

Using the PMBs, I have investigated the minimum exposure time necessary to promote lettuce seed germination following exposure to far-red light (fig. 8.), or to inhibit lettuce seed germination after exposure to red light (fig. 9). Only a few minutes were needed to saturate the pigments and reverse the effects of the previous hour of exposure. It should be noted that this data represents a single measure from each of five different PMBs and therefore differences between the LEDs could contribute to the pattern observed here.

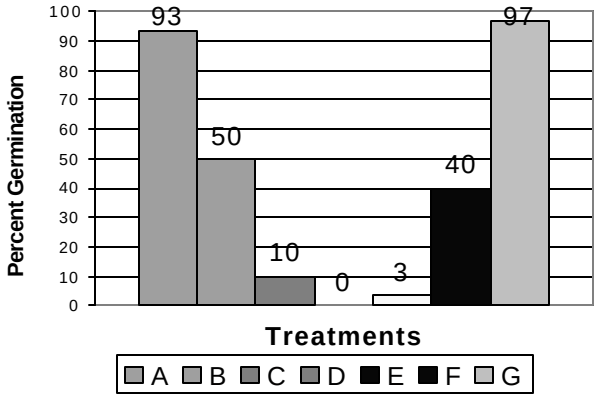

Figure 9 *The effect of short exposures of far-red light on lettuce seeds previously exposed to one hour of red light. The light treatments were:* ***A*** *-- 1 hour red, 2 minutes far-red, 22 hours 58 minutes darkness;* ***B*** *-- 1 hour red, 4 minutes far-red, 22 hour, 56 minutes darkness;* ***C*** *-- 1 hour red, 8 minutes far-red, 22 hours 52 minutes darkness;* ***D*** *-- 1 hour red, 16 minutes far-red, 22 hours 44 minutes darkness;* ***E*** *-- 1 hour red, 32 minutes far-red, 22 hours 28 minutes darkness;* ***F*** *– 24 hours darkness;* ***G*** *– 24 hours ambient light (fluorescent lamps and indirect light from a nearby window). All potentiometers were adjusted to maximize the light emission from the LEDs.*

The Law of Reciprocity and Lettuce Seed Germination

The other method of controlling fluence is to vary fluence rate and keep exposure times constant. This requires the use of a "light meter" to measure fluence rates accurately. For the moment, I will assume that the reader has access to an "appropriate" light meter and discuss its use with PMBs. I have modified the bottom half of one of the boxes of a PMB by drilling a hole in the center of the bottom plate. The hole has diameter slightly larger than the aperture of my light sensor, but smaller than the case of the sensor. Grey weather-seal-tape is glued around the outside edge of the hole. A spring-loaded clamp is used to press the light sensor against the weather seal creating a light–tight seal. Thus I have created a light sensor box that is identical to the bottom half of the other boxes with the exception that it has light sensor mounted where the petri-dish would normally set.

To calibrate the fluence rate for a PMB box I attach the top half of a PMB to the light sensor box, turn on an LED, and use the potentiometer and light meter to adjust the light to the desired fluence rate. The LED can then be turned off, and the PMB box can be reattached to its own bottom half. As long as the potentiometer is not readjusted when current is restored to the LED will emit light at the previously measured fluence rate. This can be verified by measuring the fluence rate before and after an experiment. Because the amount of current provided by batteries declines with use it is recommended to use Radio Shack's 9-Volt Battery Eliminator instead of a battery for this application.

PMBs can be used to demonstrate that fluence rather than fluence rate is the critical variable in determining seed germination. Three experiments were performed. In all three experiments, 30 Ward's light-sensitive lettuce seeds were placed on two sheets of filter paper in a 10cm diameter petri-dish, 5ml of deionized water were added to each dish and the dish was immediately sealed in a PMB box. The red LED was then turned on for one hour. Immediately after the red treatment the far-red LED was turned on, but how long it was turned on varied between treatments. In all three experiments, seed germination was assayed 24 hours after the seeds were sealed in the PMB.

In the first experiment the fluence rate was varied and the length of irradiance was held constant at 10 minutes. To accomplish this prior to use of each PMB, the fluence rate of the far-red LED was adjusted to a given value in a range between 0.5 and 16ìmol*m^{-2}*s^{-1}. The light meter used was an International Light, IL1400A with a far-red filter and calibrated to measure light with a peak wavelength of 735nm. The far-red fluence was then calculated by multiplying the fluence rate by the seconds of irradiance. The results are graphed on figure 10 as triangles. In the second experiment, the fluence rate of the far-red LED was adjusted to approximately 5ìmol*m^{-2}*s^{-1} and the length of irradiance was varied from 1 minute to 32 minutes. The results are graphed on figure 10 as diamonds. In the third experiment, the fluence rate of the far-red LED was adjusted to approximately 10ìmol*m^{-2}*s^{-1} and the length of irradiance was varied from 15 seconds to 16 minutes. The results are graphed on figure 10 as squares.

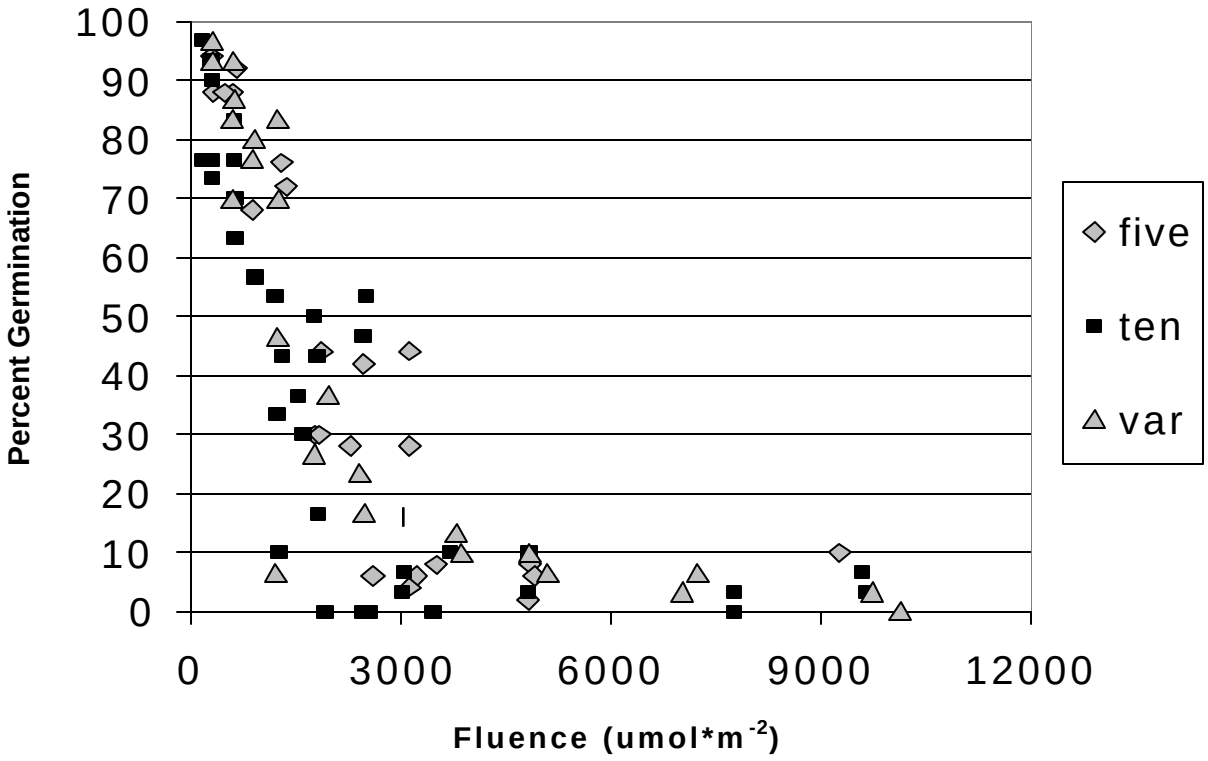

Figure 10 *Lettuce seeds were exposed to one hour of red light and then to varying fluences of far-red light. Percent germination was recorded 24 hours after the start of the light treatments. Diamonds indicate samples that were exposed to a fluence rate of approximately 5ìmol*m^{-2}*s^{-1} and the length of irradiance varied from 1 to 32 minutes. Squares indicate samples that exposed to a fluence rate of approximately 10ìmol*m^{-2}*s^{-1} and the length of irradiance varied from 15 seconds to 16 minutes. Triangles indicate samples for which the length of irradiance was approximately 10 minutes and the fluence rate was varied from 0.5 to 16ìmol*m^{-2}*s^{-1}.*

The law of reciprocity predicts equal fluences will result in equal responses despite difference in fluence rates. While there is some scatter in the data, they are generally consistent with the hypothesis (fig 10). At equal fluences, the seeds exposed to 10µmol*m^{-2}*s^{-1} do not consistently have higher or lower germination rates than those exposed 5µmol*m^{-2}*s^{-1}.

A statistically knowledgeable student might wish to formally test the hypothesis that the slopes of the regression lines are equal. Percentages are not continuous normally distributed variables. An arcsin transformation of the proportion of seeds germinated creates a variable that more closely meets the assumptions of parametric statistics. The log of fluence and the arcsin transformed variable are linearly related (fig. 11). Sokal and Rohlf describe a method of testing whether a population of slopes of regression lines are statistically different (Sokal and Rohlf 1995). Applying that method finds that the slopes are not significantly different ($F_{(2,77)}$=0.831 p=0.439), which is consistent with the prediction of the law of reciprocity.

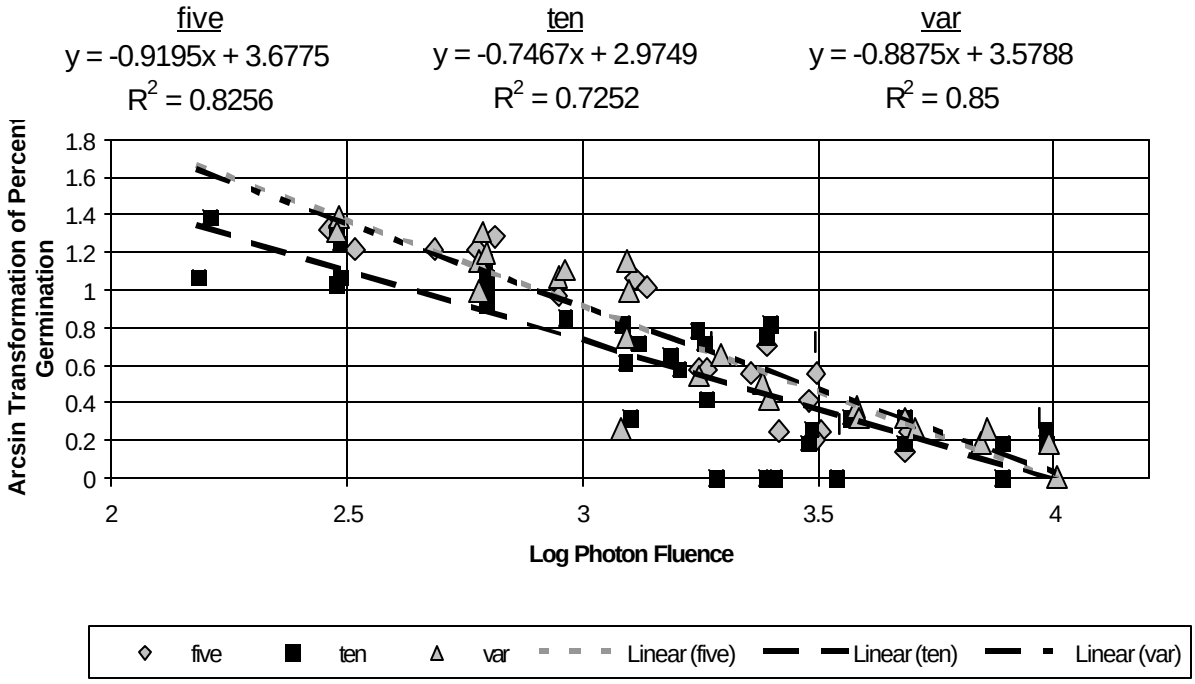

Figure 11 *Symbols the same as figure 10. Percent germination was arcsin transformed and plotted against the log of the fluence. Dashed lines indicate best-fit regression lines. The slopes of these lines are not statistically different.*

Light Meters and Measuring Fluence Rates

The light meter used in the experiment described above was a research quality instrument and therefore relatively expensive (≅$2000). The author recognizes that such instruments are not available in most undergraduate teaching laboratories. If used with an understanding of their limitations, the instruments more typically available in undergraduate laboratories may be suitable for use in student exercises.

For example, light meters that report light intensity in units of footcandles or lux are typically designed to mimic the sensitivity of the human eye. They contain filters to remove wavelengths that are outside the visible range, and the sensor and circuit are designed to respond based on how bright the light appears to the human eye, not the amount of energy in the light or the number of photons in the light. These meters may be used to make relative measurements of light intensity, and that may be sufficient for teaching purposes.

Plant physiology labs are often equipped with Photosynthetically Available Radiation (PAR) meters. PAR meters are equipped with filters that only allow 400-700nm wavelengths to reach the sensor. Obviously PAR meters should not be used to attempt to quantify fluence rates of far-red light (730nm). They can be used to measure the relative intensity red and blue lights. PAR meters are designed to measure sunlight or wide-band white light. Using a PAR meter to measure narrow band light will introduce

inaccuracies. In general, for a given wavelength the fluence rate of the light and the output of a PAR meter will be linearly related, but the slope of the linear relationship will vary among wavelengths. Therefore PAR meters can be used to return the relative fluence rates of red and blue LEDS but not to compare the absolute fluence rates of red to blue LEDs.

Many undergraduate labs are now equipped with computer-data-acquisition systems (such as those by Iworx, Pasco and Vernier) and many of those systems will include a light sensor. If the sensor is not encased behind a colored filter, it can probably be used to measure relative fluence rates of blue, red or far-red LEDs. Most such sensors will respond linearly to increased fluence rates regardless of wavelength but the slope of the linear relationships may be different depending upon wavelength.

All the above methods allow relative comparisons within wavelengths but not absolute comparisons among wavelengths. In many cases relative comparisons will be sufficient for teaching purposes. The author does not know of an inexpensive light meter that is calibrated to allow absolute measurements of narrow-band light in each of the blue, red, and far-red bands. However, such a system need not be too costly. Many inexpensive electronic sensors are available that would be sufficient for this use. No special optics or filters are needed in this application since the sensor can be enclosed in a box where only narrow band radiation will be present. The major difficulty is calibration of the sensor at each of the wavelengths, against a source of known fluence rate or against a previously calibrated meter. For example, the author has built and began to test a light-meter based upon Texas Instruments TSL230B light-to-frequency-converter integrated-circuit-chip. This meter contains less than $50 of parts and connects to the parallel port of a computer. Preliminary tests suggest that the home-made meter performs more than adequately for its intended use. It is hoped that in the near future this or some other inexpensive solution will be made commercially available.

Future Plans

I plan to continue to improve and develop the PMBs for use as teaching and research aids. The development of reliable and inexpensive light meters for use with the system is a top priority. A second priority is to develop more protocols and experiments for use with the PMBs. These should include exercises in phototropism, an exercise involving plant hormones, and an exercise involving phytochrome mutants.

A third goal is to improve the design of the PMBs themselves. While the current design works well, it could be improved. The switches currently used are among the least expensive available, but they have two weaknesses. The switches are open so dust can collect between the contacts causing the switch to fail. The switches are also square, requiring a square opening to be machined in the top cover. Toggle switches are more expensive, but they easier to install and the contacts are sealed.

The design could be improved to make adjustment of fluence rates easier. Under the current design, if the potentiometer is nearly all the way open, a small adjustment of the potentiometer results in large changes in fluence rate. If the potentiometer is partially closed, a small adjustment of the potentiometer results in small changes in fluence rate. The use of an adjustable voltage regulator and a different arrangement of potentiometers would both improve battery life and make adjustment of fluence rates easier.

In the current design the LEDs are hardwired and the only way to remove, replace or substitute an LED is to open the box and snip wires. It might be worthwhile to mount the LEDs in a socket that would allow easy removal, and substitution of the LEDs. This would allow the substitution of green, yellow and orange or even white LEDs for the ones currently installed, making the PMBs even more versatile. The suggested design changes may increase the cost of the PMBs slightly.

Conclusions

In this paper I have described a system for controlling light quality and quantity and gave some examples of experiments that can be performed using that system. The system is easy to use, and does not require dark rooms or special equipment to perform most experiments. The system is compact, and requires relatively little bench or storage space.

The system is versatile. In addition to the experiments I have described here there is the potential for many more experiment to be performed. For example, preliminary observations suggest that the blue LED can induce phototropic responses, and with minor modification (placing black paper over the reflective backs and sides) PMBs should be useful in conducting many different phototropic experiments. Seed germination, hypocotyl elongation and de-etiolation are only three of many different attributes of plant development controlled by light (for reviews see Mohr 1982; Schopfer 1984; Chory *et al.* 1996), PMBs might be useful in investigating many of those other attributes. Recently, the elongated internode mutant of *Brassica rapa* was recognized to be a phytochrome A mutant (Devlin *et al.* 1997). There are many *Arabidopsis* mutants that are impaired in phytochrome or cryptochrome function. PMBs could be used to compare the development of wild-type and mutant genotypes in varying light environments. The germination of light-sensitive lettuce seeds is normally inhibited by exposure to far-red light, but if lettuce seeds are first exposed to smoke, far-red light does not to inhibit germination (Drewes *et al.* 1995; van Staden

et al. 1995; Jäger *et al*. 1996a, b, c). I have been using the PMBs to investigate the chemical and genetic basis of the effects of smoke on the responses of light sensitive seeds.

The system is relatively inexpensive. All the experiments described in this paper were performed with a total of twelve PMBs. The parts for those twelve PMBs cost less than $400, even with the addition of a dozen 9-volt battery eliminators (≅$7 each) the cost remains under $500. The number of PMBs needed for use in a teaching laboratory would, of course, depend on the number students in the lab and how the teacher intends to use the PMBs. If the entire class participates in a single un-replicated experiment, five or six PMBs would be sufficient to demonstrate photoreversible germination of lettuce seeds. If the teacher wants more student involvement, a single PMB for each student would allow for large, complex and replicated experiments in which each student was responsible for a single data-point. I do not yet know the useful life span of a PMB (i.e. none have failed), but PMBs should be re-useable for many years.

It is hoped that other instructors will find the PMBs to be a useful and economical tool for undergraduate research and teaching.

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