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Effect of Blue Light and Temperature on Leaf Expansion, Stem Elongation, and Growth

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EFFECT OF BLUE LIGHT AND TEMPERATURE ON

LEAF EXPANSION, STEM ELONGATION, AND GROWTH

by

Tracy A. O. Dougher

A dissertation submitted in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Science

Approved:

UT AH STATE UNIVERSITY Logan, Utah

1999

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ABSTRACT

Effect of Blue Light and Temperature on Leaf Expansion, Stem Elongation, and Growth

by

Tracy A.O. Dougher, Doctor of Philosophy Utah State University, 1999

Major Professor: Dr. Bruce G. Bugbee Department: Plants, Soils, and Biometeorology

Short height and high yield per unit energy in controlled environments are essential to the success of a food production system for spaceflight. Temperature and light quality can be manipulated in controlled environments to reduce plant height and increase yield. Although the effects of temperature on height and yield are well studied at ambient $CO₂$, temperature effects at elevated $CO₂$ with a hydroponic root zone are not well characterized. We studied soybean yield and height under two lamp types over a broad range of temperatures. Temperature had little effect on yield or height, but lamp type had a significant effect on canopy height. This first study highlighted the importance of understanding spectral quality in controlling plant growth, especially canopy height.

Numerous studies have compared lamp types and suggested that profound differences in leaf area, canopy height, yield, and total dry mass responses were due to blue light differences. Unfortunately, the most energy-efficient light sources have the least

blue light. We have a poor understanding of the specific morphological and histological effects of blue light on leaves and stems. Three species, soybeans, wheat, and lettuce, were grown at five blue light fractions (0, 2, 6, 12, and 26%) and two light levels (200 and 500 μ mol m⁻² s⁻¹). Phytochrome photoequilibria were constant among treatments. Blue light responses were species dependent. Wheat leaf area, dry mass, and stem length were insensitive to blue light fraction. Increasing blue light to 26% decreased soybean stem length, but leaf area was greatest at 6% blue. Lettuce leaf area, stem length, and dry mass were highly sensitive to blue light fraction between 0% and 6% under high pressure sodium lamps, but were insensitive between 6% and 26% under metal halide lamps. These results may be complicated by sensitivity to other wavelengths. The decrease in soybean stem length with increasing blue light was caused by an inhibition of cell division, while the decrease in leaf area was caused primarily by a decrease in cell expansion. Increased lettuce leaf area with increasing blue light fraction was caused by both cell division and expansion. This research indicates that lamps high in blue photons are not only energetically wasteful, but do not benefit, and in some cases reduce, plant growth. However, some blue light is necessary for controlling plant height in soybean and even required for proper growth and development in lettuce.

(197 pages)

IV

For my amazing families, the Ohlers and the Doughers.

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Tracy A.Ohler Dougher

CONTENTS

 vii

 $viii$

LIST OF TABLES

X

LIST OF FIGURES

XI

Xll

xiii

ABBREVIATIONS

- PPF = Photosynthetic Photon Flux
- PPE = Phytochrome Photoequilibrium
- LED = Light Emitting Diode
- **MH** = Metal Halide
- HPS = High Pressure Sodium
- DIF = Difference between day temperature and night temperature
- PAR = Photosynthetically Active Radiation
- YPF = Yield Photon Flux
- PE = Photosynthetic Efficiency
- RWC = Relative Water Content

CHAPTER 1

INTRODUCTION

This research, funded by the NASA Advanced Life Support (ALS) program, sought to improve production efficiency of crop plants in controlled environments. Astronauts in an ALS system depend on plants for food, air revitalization, and water purification. Plants are grown under controlled conditions so that light intensity, spectral quality, CO₂, plant nutrients, and temperature can be altered to optimize yield per unit of input energy. Growth volume is limited, so plant height needs to be minimized. There is considerable potential to manipulate temperature and light quality to reduce height without reducing yield.

Temperature

Temperature affects both yield and height in many species. Most of these results are from studies done in the field or growth chambers at ambient $CO₂$ and in soil. In controlled environments, the reduced plant water potential usually associated with high temperatures can be minimized by growing plants hydroponically at high humidity and elevated $CO₂$. Elevated $CO₂$ reduces photorespiration, which generally increases with temperature. Thus crops grown in controlled environments should have higher temperature optima than field-grown plants. Optimal field temperatures for many species have been characterized, but the results may be affected by reduced plant water potential and increased photorespiration.

Light

ALS systems will rely on electric lamps or filtered sunlight for plant growth lighting, which provides an opportunity to alter light quality relative to sunlight. Light quality is known to affect plant morphology. High pressure sodium (HPS) lamps and more recently, red light emitting diodes (LEDs) are widely used to improve energy efficiency , but they have little to no blue light. Lamps with a higher blue content such as metal halide (MH) and cool white fluorescent are less energy efficient. Blue photons are energetically expensive to generate. In spite of several decades of research, the mechanisms underlying the effects of blue light on leaf and stem morphology are still poorly understood. Small amounts of blue light appear to be important for normal development of some species; however, too much blue light may be detrimental.

2

CHAPTER 2

LITERATURE REVIEW

Temperature

Stem morphogenesis

High temperatures increase the elongation of soybean stems. Thomas and Raper (1978) found that at day temperatures of 14 to 18° C stems were relatively short. Increasing temperature from 18 to 30 \degree C at ambient CO₂ increased stem length by at least 20 cm for each 4°C . Increasing night temperature had a much smaller effect on stem elongation than increasing day temperature . A maximum stem length occurred at 30/26°C and a minimum at $14/10^{\circ}$ C.

Elevating $CO₂$ can alter temperature optima. Sionit et al. (1987a) compared $CO₂$ levels from 350 to 1000 μ mol mol⁻¹ and found that stem height increased with increasing CO₂ but height was more responsive to temperature. Previous studies on soybeans at the Utah State University Crop Physiology Lab showed that increasing CO₂ level from 350 to 1000 μ mol mol⁻¹ increased canopy height from 28.5 to 39.5 cm when grown at 26/22 °C day/night temperature.

Erwin and Heins (1995) showed that plant height could be affected by the difference between day and night temperatures (DIF). The typical condition of warm days and cool nights results in a positive DIF. Controlled environments and greenhouses can be controlled to maintain cool days and warmer nights, which results in what is called negative DIF. The more positive the DIF, the taller the plant. However, some largeseeded species and legumes show little sensitivity to DIF (Erwin, 1991; Erwin and Heins, 1995) . Thomas and Raper (1978) examined several combinations of day/night temperatures in ambient $CO₂$. A reexamination of their data indicated that holding day temperature constant and increasing night temperature from 10 to 26°C (decreasing DIF) increased stem length in soybean. This result is opposite that of Erwin (1991) and Erwin and Heins (1995). However, increasing night temperature only increased stem length by 20 cm. In the same study, increasing day temperature and holding night temperature constant (increasing DIF) more significantly increased stem elongation . This result agrees with Erwin (1991) and Erwin and Heins (1995).

Leaf morphogenesis

Thomas and Raper (1978) found that warm night temperatures and cool day temperatures reduced soybean leaf area. Maximum and minimum leaf areas were achieved at $26/10^{\circ}$ C and $14/26^{\circ}$ C, respectively. Increasing CO₂ from 330 to 740 μ mol mol⁻¹ can also increase leaf area but the response appears to be temperature dependent (Baker et al., 1989; Sionit et al., 1987a; Ziska and Bunce, 1995).

Effect of temperature on yield

Temperature affects soybean yield, yield components, and harvest index (Thomas and Raper, 1978; Sionit et al., 1987b). A day/night temperature of 26/20 °C appears to be optimal for soybean growth and yield in the field (Raper and Kramer, 1987) and controlled environment at ambient $CO₂$ (Gibson and Mullen, 1996). High $CO₂$ typically increases temperature optima, and yield is more responsive to $CO₂$ at higher temperatures

(Campbell et al., 1990; Ziska and Bunce, 1995; Sionit et al., 1987b; Baker et al., 1989).

Interaction of temperature and light quality

Numerous studies of temperature have been conducted in controlled environments, each under different lamp types (Thomas and Raper, 1978; Downs and Thomas, 1990; Bunce, 1991; Gibson and Mullen, 1996). However, the interaction of light quality and temperature on morphogenesis and growth is not well characterized .

Light Quality

Stem morphogenesis

Internode elongation may be controlled by both phytochrome balance and blue light (Ritter et al., 1981). Changes in the red to far red ratio (R:FR), specifically wavelengths affecting phytochrome 660:730nm, caused by neighbor detection, shading, and end-of-day light quality are well documented (Pausch et al., 1991; Smith, 1982; Ballare et al., 1995). The typical phytochrome response associated with low R:FR is an increase in apical dominance and increased internode elongation.

Blue light appears to profoundly influence stem elongation in many species, such as soybeans (Wheeler et al., 1991; Hunt et al., 1989; Britz and Sager, 1990); chrysanthemum (Rajapakse et al., 1992); white clover (Gautier et al., 1997); sorghum (Britz and Sager, 1990; Warrington and Mitchell, 1976); mustard, spinach, and lettuce (Tibbitts et al., 1983); potato (Yorio et al., 1995); and pepper (Brown et al., 1995). A threshold intensity, above which blue light has no further effect, has been reported for

soybean at a photosynthetic photon flux (PPF) of 30 μ mol m⁻² s⁻¹ (Wheeler et al., 1991) and for lettuce at 15 μ mol m⁻² s⁻¹ (Hoenecke et al., 1992). Beyond these thresholds, stem and hypocotyl elongation, respectively, did not decrease significantly. Both studies were at moderate (500 μ mol m⁻² s⁻¹) to low (100 μ mol m⁻² s⁻¹) light levels but reports claim that these thresholds may be PPF independent (Wheeler et al., 1991; Hoenecke et al., 1992; Tibbitts et al., 1983).

Other studies have attempted to explain unusual results by suggesting that a balance between red and blue light regulates stem length (Drumm-Herrel and Mohr, 1984; Mohr, 1987; Britz and Sager, 1990). Casal and Smith (1989) suggest a high phytochrome photoequilibrium (PPE) is necessary to trigger blue light effects, but PPE does not interact with blue light once triggered. No conclusive studies have been completed.

Leaf morphogenesis

Leaf morphology under differing lamp types is less well studied. Leaf morphology is here defined as the ratio of leaf area to leaf mass (specific leaf area, m^2 kg⁻¹). Specific leaf area (SLA) can be affected by cell size and number. The effects that are apparently related to blue light may also be attributed to other wavelengths associated with the type of lamps compared. Hoenecke et al. (1992) found that addition of blue light to red LEDs increased leaf area of lettuce, but the lamps in this study completely lacked FR. Brown et al. (1995) later found these same results in pepper plants, but also found a combination of red and far red LEDs still had a leaf area less than metal halide, which has spectral qualities similar to sunlight. Under filtered and unfiltered broad-spectrum metallic iodure

lamps, Gautier et al. (1997) saw no change in total leaf area of white clover with decreased blue light. However, area of leaves on the main stolon was increased.

Morphology can also be altered by leaf number, which affects total leaf area. Yanagi et al. (1996) found that leaf number of lettuce was greater under red than under blue LEDs. Leaf length was similar under these two light sources, but leaf width was less under red LEDs. A combination of red and blue LEDs produced the highest leaf widths and lengths.

Total PPF can interact with the effects of light quality. Tibbitts et al. (1983) found that mustard, lettuce, and spinach tended to have larger leaves under HPS than MH at low PPF (320 μ mol m⁻² s⁻¹) but results were mixed at high PPF (700 μ mol m⁻² s⁻¹). Wheat showed no response to lamp type in these studies. However, Barnes and Bugbee (1992) found reduced leaf length in wheat with increasing blue light at a PPF of 200 μ mol m⁻² s⁻¹. At low R:FR and low blue light, as in shade settings, reduced leaf expansion occurs in shade-avoiding species (Dale, 1988). In this setting, however, R:FR and blue light effects are not separable. In soybean, specific leaf mass (mass per unit area) was significantly less under low pressure sodium lamps than under daylight fluorescent lamps, suggesting broader, thinner leaves (Britz and Sager, 1990).

Effects of morphology on light interception and yield

Wells et al. (1993) found that leaf and stem morphology altered light interception, growth, and, ultimately, yield in field-grown soybeans. PPF interception increased with increasing plant height. Cultivars with narrow leaves also had reduced light interception

7

and seed yield (Wells et al., 1993). In field grown wheat, light interception and yield of dwarf isolines were reduced by 13% compared to tall isolines (Gent, 1995). Blue light effects on stem cells

The phototropism and elongation of stems is well studied under blue light. Blue light rapidly suppresses elongation in dark grown seedlings of many species (Cosgrove, 1981; Kigel and Cosgrove, 1991; Liscum et al., 1992). Dark-grown (etiolated) soybean seedlings had downregulated levels of β -tubulin compared to light-grown seedlings (Bustos et al., 1989). This downregulation was correlated with cell elongation because Ptubulin is a building block of microtubules. The effect of light quality, if any, on the regulation of β -tubulin production is not known. Blue light affects the orientation of the microtubules. In stem tissue, microtubules align longitudinally rather than transversely under blue light, but the importance of this response is not clear (Short and Briggs, 1994). Longitudinal alignment may inhibit cell elongation. Blue light may cause changes similar to a stress response , protecting cells from invasion by changing the properties of the cell wall (Horwitz and Gressel, 1987; Voigt and Munzner, 1994). Possible blue light photoreceptor precursor genes affecting stem elongation have been elucidated (Ahmad and Cashmore, 1993; Short and Briggs, 1994).

Blue light effects on leaf cells

Blue light effects on leaf expansion may be caused by changes in cell number and/or cell size. Leaf size is mostly determined by cell number (Dale and Milthorpe, 1983; Wenzel et al., 1997). However, there is no clear evidence that blue light affects cell

division more than cell expansion. Blue light delays cell division in *Ch/amydomonas reinhardtii* (Munzner and Voigt, 1992). Blue light increased leaf epidermal cell area in birch plantlets *in vitro* over red light (Saebo et al., 1995). In *Phaseo/us vulgaris,* blue light did not decrease cell number for primary leaves but accounted for most of the increase in leaf area of trifoliates (Dale, 1988). This was true for various fluence rates. In the primary leaves of *P. vulgaris,* cell division is complete before most cell expansion begins (Van Volkenburgh et al., 1985), so cell expansion can be studied without cell division affecting results. Using this technique, Van Volkenburgh et al. (1985) found cell elongation was due to a proton efflux associated with the acid growth hypothesis. Staal et al. (1994) found that red and blue light stimulated a proton efflux in pea epidermal cells, also agreeing with the acid growth hypothesis. However, these tests were done on a mutant strain and *in vitro* cells. In wheat, Guerra et al. (1985) found that the lignin precursors phenylalanine ammonia lyase and tyrosine ammonia lyase were lowest under LPS lamps, which contain no blue light. A decrease in these lignin precursors suggests a decrease in lignin synthesis, which would decrease cell wall rigidity and allow for more cell expansion. Blue light is known to affect turgor pressure in soybean pulvini (Donahue et al., 1990) and leaf stomata (Short and Briggs, 1994), probably via a potassium shift. It is unlikely, though, that continuous blue light could sustain a lower turgor pressure .

Literature Cited

Ahmad, M. and A. R. Cashmore. 1993. *Hy4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366: 162-166.

9

- Baker, J. T., L.H. Allen, Jr., K.J. Boote, P. Jones, and J.W. Jones. 1989. Response of soybean to air temperature and carbon dioxide concentration. Crop Sci. 29:98-105.
- Ballare, C. L., A. L. Scopel, and R. A. Sanchez . 1995. Plant photomorphogenesis in canopies, crop growth, and yield. HortScience 30:1172-1181.
- Barnes, C. and B. Bugbee. 1992. Morphological responses of wheat to blue light. J. Plant Physiol. 139:339-342.
- Britz, S. J., and J. C. Sager. 1990. Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad spectrum or blue-deficient light sources . Plant Physiol. 94:448-454.
- Brown, C. S., A. C. Schuerger, and J. C. Sager. 1995. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. J. of the Amer. Soc. of Hort. Sci. 120:808-813.
- Bunce, J. A. 1991. Control of the acclimation of photosynthesis to light and temperature in relation to partitioning of photosynthate in developing soybean leaves. J. of Exp. Bot. 42:853-859 .
- Bustos, M. M., M.J. Guiltinan, R.J. Cyr, D. Ahdoot, and D.E . Fosket. 1989. Light regulation of b-tubulin gene expression during internode development in soybean *(Glycine max* (L.) Merr.) . Plant Physiol. 91: 1157-1161.
- Campbell, W. J., J. L.H . Allen, and G. Bowes. 1990. Response of soybean canopy photosynthesis to CO₂ concentration, light, and temperature. J. of Exp. Bot. 41:427-433 .
- Casal, J.J. and H. Smith. 1989. Effects of blue light pretreatments on internode extension growth in mustard seedlings after the transition to darkness: analysis of the interaction with phytochrome. J. of Exp. Bot. 40:893-899.
- Cosgrove, D. J. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67 :584- 590.
- Dale, J. E. 1988. The control of leaf expansion. Ann. Rev. of Plant Physiol. 39:267-295.
- Dale, J.E. and F.L. Milthorpe. 1983. General features of the production and growth of leaves, p.151-178. In: J.E . Dale and F.L. Milthorpe (eds.). The growth and functioning of leaves. Cambridge University Press, Cambridge, U.K.
- Donahue, R. A., V. S. Berg, and T. C. Vogelmann. 1990. Assessment of the potential of the blue light gradient in soybean pulvini as a leaf orientation signal. Physiologia Plantarum 79:593-598 .
- Downs, R. J. and J. F. Thomas. 1990. Morphology and reproductive development of soybeans under artificial conditions. Biotronics 19:19-32.
- Drumm-Herrel, H. and H. Mohr. 1984. Mode of coaction of phytochrome and blue light photoreceptor in control of hypocotyl elongation. Photochem. and Photobiol. 40:261-266 .
- Erwin, J. 1991. Temperature effects on plant growth . Proc. 12th Ann . Conf on Hydroponics 1: 1-6.
- Erwin, J. E. and R. D. Heins, 1995. Thermomorphogenic responses in stem and leaf development. HortScience 30:940-949.
- Gautier, H., C. Varlet-Grancher, and N. Baudry. 1997. Effect of blue light on the vertical colonization of space by white clover and their consequences for dry matter distribution . Annals of Bot. 80:665-671.
- Gent, M. P. N. 1995. Canopy light interception, gas exchange, and biomass in reduced height isolines of winter wheat. Crop Sci. 35:1636-1642.
- Gibson, L. R. and R. E. Mullen. 1996. Influence of day and night temperature on soybean seed yield. Crop Sci. 36:98-104.
- Guerra, D., A. J. Anderson, and F. B. Salisbury. 1985. Reduced phenylalanine ammonialyase and tyrosine ammonia-lyase activities and lignin synthesis in wheat grown under low pressure sodium lamps. Plant Physiol. 78 : 126-130.
- Hoenecke, M. E., R. J. Bula, and T. W. Tibbitts. 1992 . Importance of'blue' photon levels for lettuce seedlings grown under red-light-emitting diodes . HortScience 27:427- 430.
- Horwitz, B. A. and J. Gressel. 1987 . First measurable effects following photoinduction of morphogenesis, p.53-70. In: H. Senger (ed.). Blue light responses: Phenomena and occurrence in plants and microorganisms, Vol. 2. CRC Press Inc., Boca Raton, Fla.
- Hunt, P. G., M. J. Kasperbauer, and T. A. Matheny. 1989. Soybean seedling growth responses to light reflected from different colored soil surfaces. Crop Sci. 29:130-133.
- Kigel, J. and D. J. Cosgrove. 1991. Photoinhibition of stem elongation by blue and red light. Plant Physiol. 95:1049-1056.
- Liscum, E., J.C. Young, K.L. Poff, and R.P. Hangarter, 1992 Genetic separation of phototropism and blue light inhibition of stem elongation. Plant Physiol. 100:267-271.
- Mohr, H. 1987. Mode of coaction between blue/UV light and light absorbed by phytochrome in higher plants, p. 133-144. In: H. Senger (ed.). Blue light responses: Phenomena and occurrence in plants and microorganisms, Vol. 1. CRC Press Inc., Boca Raton, Fla.
- Munzner, P. and J. Voigt. 1992. Blue light regulation of cell division in *Ch/amydomonas reinhardtii .* Plant Physiol. 99 : 1370-1375 .
- Pausch, R. C., S. J. Britz, and C. Mulchi. 1991. Growth and photosynthesis of soybean *(Glycine max (L.)* Merr.*)* in simulated vegetation shade: Influence of the ratio of red to far-red radiation. Plant, Cell, and Env. 14:647-656.
- Rajapakse, N.C., R.K. Pollock, M.J. McMahon, J.W. Kelly, and R.E. Young. 1992. Interpretation of light quality measurements and plant response in spectral filter research. HortScience 27:1208-1211.
- Raper, D., and P.J. Kramer. 1987. Stress physiology, p. 590-591. In: J.R. Wilcox (ed.). Soybeans: Improvement, production, and uses, 2nd ed. Agronomy Publishers, Madison, Wis.
- Ritter, A., E. Wagner, and M.G. Holmes. 1981. Light quantity and quality interactions in the control of elongation growth in light-grown *Chenopodium rubrum* L. seedlings. Planta 153:556-560.
- Saebo, A., T. Krekling, and M. Appelgren. 1995. Light quality affects photosynthesis and leaf anatomy of birch plantlets in vitro. Plant Cell, Tissue and Organ Culture 41 :177-185 .
- Short, T. W. and W. R. Briggs. 1994. The transduction of blue light signals in higher plants. Ann. Rev. of Pl. Phys. and Mol. Biol. 45:143-171.
- Sionit, N., B. R. Strain, and E. P. Flint. 1987a. Interaction of temperature and $CO₂$ enrichment on soybean: Growth and dry matter partitioning. Can. J. of Pl. Sci. 67:59-67.
- Sionit, N., B. R. Strain, and E. P. Flint. 1987b. Interaction of temperature and $CO₂$ enrichment on soybean: Photosynthesis and seed yield. Can. J. Plant Sci. 67:629-636.
- Smith, H. 1982. Light quality, photoperception, and plant strategy. Ann. Rev. of Plant Physiol. 33:481-518 .
- Staal, M., J.T.M. Elzenga, A.G. van Elk, H.B.A. Prins, and E. Van Volkenburgh. 1994. Red and blue light-stimulated proton efflux by epidennal leaf cells of the Argenteum mutant of *Pisum sativum*. J. of Exp. Bot. 45:1213-1218.
- Thomas, J. F. and J. C.D. Raper. 1978. Effect of day and night temperatures during floral induction on morphology of soybeans. Agronomy Journal 70:893-898.
- Tibbitts, T. W., D. C. Morgan, and I. J. Warrington. 1983. Growth of lettuce, spinach, mustard, and wheat plants under four combinations of high-pressure sodium, metal halide, and tungsten halogen lamps at equal PPFD. J. of the Amer. Soc. for Hort. Sci. 108:622-630.
- Van Volkenburgh, E., R. E. Cleland, and M. G. Schmidt. 1985. The mechanism of lightstimulated leaf cell expansion, p. 223-237 . In: N.R. Baker, W.J. Davies, and C.K. Ong (eds.). Control of leaf growth. Cambridge University Press, Cambridge, U.K.
- Voigt, J. and P. Munzner . 1994. Blue light-induced lethality of a cell wall-deficient mutant of the unicellular green alga *Chlamydomonas reinhardtii.* Plant Cell Physiol. 35:99-106.
- Warrinton, I.J. and K.J. Mitchell. 1976. The influence of blue- and red-biased light spectra on the growth and development of plants. Agricultural Meteorology 16:247-262.
- Wells, R., J. W. Burton, and T. C. Kilen. 1993. Soybean growth and light interception: response to differing leaf and stem morphology . Crop Sci. 33 :520-524.
- Wenzel, C.L., P.M. Chandler, R.B. Cunningham, and J. B. Passioura. 1997. Comparative leaf epidennal anatomy of mutants of barley *(Hordeum vulgare* L. 'Himalaya') which differ in leaf length. Annals of Bot. 79 :47-52.
- Wheeler, R. M., C. L. Mackowiak, and J. C. Sager. 1991. Soybean stem growth under high-pressure sodium with supplemental blue lighting. Agronomy Journal 83:903-906.
- Yanagi, T., K. Okamoto, and S. Takita. 1996. Effects of blue, red, and blue/red lights of two different PPF levels on growth and morphogenesis of lettuce plants. Acta Hort. 440:117-122.
- Yorio, N. C., C.L. Mackowiak, R.M. Wheeler, and J.C. Sager. 1995. Vegetative growth of potato under high-pressure sodium, high-pressure sodium SON-Agro, and metal halide lamps. HortScience 30:374-376.
- Ziska, L. H. and J. A. Bunce. 1995. Growth and photosynthetic response of three soybean cultivars to simultaneous increases in growth temperature and CO₂. Physiologia Plantarum 94:575-584.

CHAPTER 3

EFFECT OF LAMP TYPE AND TEMPERATURE ON DEVELOPMENT, CARBON PARTITIONING, AND YIELD OF SOYBEAN¹

ABSTRACT

Soybeans grown in controlled environments are commonly taller than field-grown plants. We studied canopy height, carbon partitioning, and yield of soybeans under two lamp types and a range of temperatures. In controlled environments, including liquid hydroponics, height of the dwarf cultivar 'Hoyt' was reduced from 46 to 33 cm when plants were grown under metal halide lamps compared to high pressure sodium lamps at the same photosynthetic photon flux. Metal halide lamps reduced total biomass 14% but did not significantly reduce seed yield. Neither increasing temperature nor altering the difference between day/night temperature affected plant height. Increasing temperature from 21 to 27°C increased yield 32%. High temperature significantly increased carbon partitioning to stems and increased harvest index.

INTRODUCTION

Short-stature, high yielding cultivars are desirable in controlled environments because space is often limited. However, soybeans grown in controlled environments are taller than field-grown plants (Downs and Thomas, 1990). Red :far red ratios, specifically phytochrome 660:730 nm, have been implicated as the cause of internode elongation

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(Pausch *et al.*, 1991), although soybeans may also respond to a balance of red and blue light (Britz and Sager, 1990). Wheeler *et al.* (1991) reported that there was a threshold intensity of blue light (30 μ mol m⁻² s⁻¹) necessary to reduce stem elongation. However, elongation is also dependent upon the total photosynthetic photon flux (PPF) from lamps (Tibbitts *et al.,* 1983). Differences in the spectral quality of high pressure sodium (HPS) and metal halide (MH) lamps could alter the stem length and thus alter carbon partitioning of soybeans.

Another factor affecting stem length is temperature, which is easily manipulated in a controlled environment. The reduced plant water potential usually associated with high temperatures can be minimized by growing plants hydroponically at high humidity and elevated CO₂. Elevated CO₂ reduces photorespiration, which generally increases with temperature. Thus crops grown in controlled environments should have higher temperature optima than field-grown plants. Optimum field temperatures for soybean have been characterized (Raper and Kramer, 1987), but the results may be affected by reduced plant water potential and increased photorespiration.

Our objective was to study soybean canopy height, carbon partitioning, and yield under HPS versus MH lamps at varied and constant day/night temperatures in a CO₂enriched, hydroponic, controlled environment.

MATERIALS AND METHODS

Dwarf soybean cv. 'Hoyt' (maturity group 2.5) canopies were grown in Plexiglas chambers (0.47 x 0.36 x 0.61 m) at a density of 36 plants m⁻² (6 plants per chamber). This

choice of density was based on preliminary trials, which indicated that higher densities increased stem elongation and lower densities increased time to canopy closure . An extensive controlled environment screening showed 'Hoyt', a determinate cultivar, to be the shortest and highest yielding. All indeterminate cultivars were unacceptably tall. Chambers were positively pressurized for an open gas exchange system as described by Bugbee (1992). Seeds were germinated in moist diatomaceous earth (Isolite) and transplanted when the hypocotyls had elongated to at least 4 cm (about 6 days). Plants were transferred to an aerated nutrient solution, 21 cm deep, in a 30 L tub. Closed cell foam plugs in a blue Styrofoam lid supported the plants. Nutrient solution was replenished to maintain solution level. Nutrient solution electrical conductivity (14 ± 4.4) mS m⁻¹) and pH (5.6 \pm 0.6) were monitored and controlled as necessary. Ammonium sulfate was added as needed to counteract the rise in pH caused by nitrate uptake.

Five day/night temperature regimes were used to test a range of temperatures typically utilized in controlled environments: $29/25$, $26/22$, $24/24$, $23/19$, and $21/21$ °C. Root temperatures were kept constant at the average daily temperature of the shoot: 27, 24, 24, 21, and 21°C, respectively. Shoot air and root-zone temperatures were measured with thermocouples and maintained by computer-controlled heaters. Each set of temperature treatments (5 chambers) was placed under either MH or HPS lamps. All chambers were in a single growth room with light treatments separated by a heavy Mylar sheet. A photosynthetic photon flux of 450 μ mol m⁻² s⁻¹ was maintained at the top of the canopy. This supplied approximately 40 and 140 μ mol m⁻² s⁻¹ of blue light in HPS and MH lamps, respectively. Intensity was maintained within 4% by shading each chamber

with neutral density filters. Aluminized Mylar around the chamber was maintained at canopy height to minimize the edge effect caused by side lighting. The photoperiod was 12 hours. Carbon dioxide concentration was enriched to 1100 μ mol mol⁻¹ based on known optimum enrichment levels for controlled environments.

Days to first flower was recorded as appearance of visible flower color. Plants were harvested at physiological maturity as indicated by loss of green color from the pods (Gbikpi and Crookston, 1981). At harvest, canopy height, from stem base to the top of the leaves, was measured *in situ.* Then plants were extended to their full height and measured to the growing tip of the main stem and longest branch. These different length measures were used as a more specific indication of intemode elongation. Plants were separated into leaves, stems, pods, and roots, dried at 80°C for 48 hours, and weighed. Seed and pod number were recorded. Yield parameters and carbon partitioning (organ DW / total DW) were calculated from the harvest data. Main effects were tested using the light by temperature interaction error term with SAS ANOVA (SAS Institute, NC) (Appendix D). Net canopy photosynthesis (P_{net}) was calculated from the measured change in $CO₂$ (infrared gas analyzer in differential mode) times air flow rate through the chamber divided by chamber ground area (Bugbee, 1992).

RESULTS

Effect of Lamp Type

MH lamps significantly reduced canopy height but slightly increased relative branch length compared with HPS lamps (Table 3.1). The main stem of HPS plants was

87% the length of the longest branch while MH main stem was 75% of the longest branch . MH canopy height was greater than the longest branch length because canopy height included petiole lengths. Although not measured, petioles appeared to contribute more to height in MH canopies. Plants grown under MH lamps had 14% less biomass compared to plants under HPS lamps (Table 3.1). Reduced stem mass in MH plants was associated with an increase in harvest index (HI) (Table 3.2). All other component partitioning was similar. MH lamps also had 7% less seed yield than HPS lamps (Table 3.1). Lower pod number and seeds per pod accounted for the lower in seed yield under MH than HPS. P_{net} measurements were consistent with the yield differences between lamp types (Figure $3.1a$.

Effect of Temperature

Higher temperatures increased seed yield via increased number of pods per square meter and seeds per pod (Table 3.3). We were surprised to find that cooler temperatures reduced the duration of the seed fill period. Higher temperatures increased P_{net} early in the life cycle (Figure 3.1b) but no trend was apparent after 35d. Total biomass (Table 3.3) and HI (Table 3.4) tended to decrease with lower temperatures.

Percent leaf mass decreased with increasing temperatures probably because of hastened leaf senescence. Warm temperatures also tended to decrease percent root mass.

The day/night temperature scheme did not affect canopy height. Erwin and Heins (1995) showed that altering the difference (DIF) between day/night temperature changed plant height for some species but larger-seeded species showed little response to DIF

19

(Erwin, 1991). In our experiment, the canopies at $+4$ DIF (42 cm at 26/22 $^{\circ}$ C, 41 cm at 23/19°C) were not significantly taller than at zero DIF (38 cm at 24/24°C, 36 cm at $21/21$ °C).

DISCUSSION

While short-stature canopies are desired in controlled environments, high yield is also a priority. The mechanism underlying biomass differences with spectral quality, specifically orange bias (HPS) versus a balanced spectrum (MH), is unknown. However, a 14% biomass difference generated only a 7% difference in yield. Because plant height and seed yield commonly are positively correlated (Wells *et al.,* 1993), a slight difference in yield was to be expected. Taller plants under HPS lamps may have had better light interception.

Higher P_{net}, longer internodes, larger leaves (data not shown), and more rapid canopy closure (data not shown) suggest that there is better light distribution and capture in the HPS canopy. Increasing plant density under MH lamps might overcome canopy closure differences but this would probably increase stem elongation after canopy closure, which would reduce the height advantage for conserving space in a controlled environment.

A lack of significant effect on plant height indicates temperature can be manipulated to some extent to maximize yield without increasing canopy height. The high temperatures increased yield by increasing pod and seed number. Rapid canopy closure and higher photosynthesis contributed to the yield differences. High temperatures also

hastened development as evidenced by shorter time to final vegetative-stage (data not shown) and decreasing time to first flower. We are currently testing temperatures above 29°C (Appendix A).

Measurement of P_{net} is important for calculating oxygen production for a bioregenerative life support system. Regardless of treatment, there was a broad peak in P_{net} between days 25 and 45. Early life cycle rate of increase in P_{net} was caused by rate of canopy closure and radiation capture. Differences between lamp types and between temperatures were apparent during this part of the life cycle. The decrease in P_{net} was due to senescence and treatment had no effect on the rate of decrease . Therefore we are focusing on environmental changes early in the life cycle to increase canopy closure .

REFERENCES

- Britz, S.J., and J.C. Sager, Photomorphogenesis and Photoassimilation in Soybean and Sorghum Grown under Broad Spectrum or Blue-Deficient Light Sources, *Plant Physiol.,* 94, 448 (1990).
- Bugbee, B., Steady-State Canopy Gas Exchange: System Design and Operation, *HortScience,* 27, 770 (1992) .
- Downs, R.J., and J.F. Thomas, Morphology and Reproductive Development of Soybeans under Artificial Conditions, *Biotronics,* 19, 19 (1990) .
- Erwin, J., Temperature Effects on Plant Growth, *Proc. of the Twelfth Ann. Conj on Hydroponics,* 1, I (1991).
- Erwin, J., and R.D. Heins, Thermomorphogenic Responses in Stem and Leaf Development, *HortScience,* 30, 940 (1995).
- Gbikpi, P.J., and R.K. Crookston, A Whole-Plant Indicator of Soybean Physiological Maturity, *Crop Sci.,* 21, 469 (1981).
- Pausch, R.C., S.J. Britz, and C.L. Mulchi, Growth and Photosynthesis of Soybean *(Glycine max* (L.) Merr.) in Simulated Vegetation Shade: Influence of the Ratio of Red to Far-Red Radiation, *Plant, Celi and Env.,* 14, 647 (1991).
- Raper, D., and Kramer, Stress Physiology, in *Soybeans: Improvement, Production and Uses,* edited by J.R Wilcox, pp. 590-591, Agronomy Publishers, Madison, WI (1987).
- Tibbitts, T.W., D.C. Morgan, and I.I. Warrington, Growth of Lettuce, Spinach, Mustard, and Wheat Plants under Four Combinations of High-pressure Sodium, Metal Halide, and Tungsten Halogen Lamps at Equal PPFD, *J. Amer. Soc. Hort. Sci.,* 108, 622 (1983).
- Wells, R., J.W. Burton, and T.C. Kilen, Soybean Growth and Light Interception: Response to Differing Leaf and Stem Morphology, *Crop Sci.*, 33, 520 (1993).
- Wheeler, R.M., C.L. Mackowiak, and J.C. Sager, Soybean Stem Growth under High-Pressure Sodium with Supplemental Blue Lighting, *Agron. J.*, 83, 903 (1991).

lamp type	canopy height (cm)	main stem length cm)	longest branch length cm)	seed yield $(g m^{-2} d^{-1})$	photo- synthetic efficiency ¹ $(g \text{ mol}^{-1})$	total biomass $(g m^{-2} d^{-1})$	pods per m ²	seeds per pod	mass per seed (mg)
HPS	46.4	41.2	47.1	4.99	0.257	13.7	1486	1.91	159
MH	33.2	19.9	26.6	4.62	0.238	12.0	1385	1.85	167
p-value	< 0.01	< 0.01	< 0.01	0.24	0.24	0.04	< 0.01	0.10	0.42

Table 3. I. Three plant length measures, seed yield, and yield components of soybeans grown under two lamp types . Each parameter is an average of the five chambers with different temperature regimes.

¹grams of seed per mol of PPF

Table 3.2. Carbon partitioning of soybeans under two lamp types. Measures are a percent of total dry mass. Sum of the five components equals 100%.

lamp type	seed (harvest index)	stem	leaves	pod	root
HPS	36.4	14.7	27.9	2.8	
MH		2.2	28.6	2.2	9.3
p-value	J.18	≤ 0.01	0.14	0.19	0.18

day/night temperature	seed yield $(g m-2 d-1)$	PE [†] $(g \text{ mol}^{-1})$	J_{I} total biomass pods per $(g m^{-2} d^{-1})$	m ²	seeds per pod	mass per seed (mg)	days to first flower	days to harvest	seed fill (days)
29/25	5.46	0.281	13.6	1483b	1.96ab	167	19c	87	68
26/22	5.43	0.280	13.1	1550ab	2.03a	153	24 _b	87	63
24/24	5.02	0.258	12.9	1594a	1.89bc	160	27 _b	90	63
23/19	4.13	0.213	12.6	1321c	1.79cd	167	32a	90	58
21/21	3.98	0.205	11.9	1230d	1.73d	169	33a	90	57
p-value	0.06	0.06	0.46	< 0.01	0.01	0.74	< 0.01	0.67	

Table 3.3. A comparison of yield and yield components for soybeans grown under five temperature regimes. Each parameter is an average of the two chambers of differing lamp type $*$.

 $tPE = photosynthetic efficiency$

*Letters within a column indicate significant differences using least significant difference mean separation test at α =0.05

*Letters within a column indicate significant differences using least significant difference mean separation test at *a=0 .05*

Fig. 3.1. Net photosynthesis $(CO₂$ uptake) of soybean canopies. Endpoints are an average day of harvest. a) Comparison of lamp types. Measurements are an average of the five chambers of different temperatures. b) Comparison of temperatures. Measurements are an average of the two chambers of differing lamp types.

CHAPTER 4

EFFECT OF BLUE LIGHT ON LEAF EXPANSION. STEM ELONGATION, AND GROWTH

Abstract

Blue photons are energetically expensive so the most energy-efficient lamps contain the least blue light. Blue photons are not used efficiently in photosynthesis, but blue light has dramatic effects on plant development. We studied the growth and development of soybean, wheat, and lettuce plants under high pressure sodium and metal halide lamps with yellow filters creating 5 fractions of blue light $(< 0.1\%$, 2% , 6% , 12% , and 26%) at 200 and 500 μ mol m⁻² s⁻¹. The response was species dependent. Soybean responses were attributable to blue light fraction, whereas lettuce responses were attributable to absolute blue light. Lettuce was highly sensitive to blue light fraction between 0% and 6% blue, but results were complicated by sensitivity to lamp type . For all parameters tested, wheat did not respond to blue light. Soybean stem length decreased with increasing blue light fraction and leaf area was greatest at 6% blue, but total dry mass was unchanged. The data suggest that lettuce growth and development requires some added blue light, but soybean and wheat may not.

Introduction

Energy efficiency of electric lamps has always been important in Earth-based research and it plays an even larger role in space-based life support systems. Photons at short wavelengths are more energetically expensive than longer wavelengths so the most efficient lamps have reduced blue light. Recent studies have utilized high pressure sodium (HPS) and red light emitting diodes (LEDs) . This raises the question of how critical blue light is to plant growth and development.

Blue light has been shown to reduce cell expansion (Cosgrove, 1981) and longterm exposure to blue light could thus reduce leaf area and stem elongation, altering canopy architecture. This, in turn, would affect radiation capture, photosynthesis, and, ultimately, yield (Board et al., 1992; Gent, 1995). Indeed, several studies have shown that reducing blue light can have a positive effect. A decrease in blue light can increase specific leaf area (SLA) (Britz and Sager, 1990; Dougher and Bugbee 1997). Soybeans grown under HPS had more biomass yield than those grown under metal halide (MH) (Dougher and Bugbee, 1997). Increase in total dry mass under HPS versus MH has also been reported for potato (Yorio et al., 1995) and lettuce (Wheeler et al., 1994).

Several studies indicate that blue light does not alter growth. Tibbitts et al. (1983) found that lettuce, spinach, and mustard tended to have increased leaf area under lamps with less blue light, but there were no consistent changes in dry mass of these species under different lamp types. Barnes and Bugbee (1992) also found that blue light did not affect dry matter accumulation, but low blue light increased leaf length in wheat. Goins et al. (1997) also found no change in wheat dry matter accumulation when blue fluorescents were added to red LEDs. White clover grown under orange-filtered (<0.1% blue) metallic iodure lamps had similar total biomass as plants under unfiltered lamps (23% blue)

(Gautier et al., 1997). However, non-photosynthetic thermal radiation was not filtered in this experiment.

By the same token, reducing blue light has been shown to have a negative effect. Wheat tended to have decreased leaf area under lamps with less blue light (Tibbitts et al., 1983). A decrease in blue light greatly elongates stems of soybean (Wheeler et al., 1991) and lettuce (Hoenecke et al., 1992). Brown et al. (1995) found that supplementing with blue light increased pepper plant biomass when plants were grown under red LEDs.

Some studies compared lamp types and the authors suggested that differences were due to blue radiation (Sager and McFarlane, 1997). Other studies added blue fluorescents but the treatments did not have identical non-blue wavelengths (Brown et al., 1995; Hoenecke et al., 1992; Yorio et al., 1998). Due to lamp limitations, these studies have only been conducted at low light levels. Our objective was to test multiple blue light fractions at high and low light. We compared six levels of blue light by filtering blue from two lamp types. We also compared unfiltered HPS and filtered MH lamps at the same blue light level to test if other wavelengths alter blue light effects.

Materials and Methods

Lettuce *(Lactuca saliva,* cv. 'Grand Rapids'), soybean *(Glycine max,* cv. 'Hoyt'), and wheat *(Triticum aestivum,* cv. 'USU-Apogee') were grown in six blue light treatments comprising five blue light fractions (Table 4.1) at a photosynthetic photon flux (PPF) of 500 μ mol m⁻² s⁻¹ and 200 μ mol m⁻² s⁻¹. A single growth room was divided into six compartments for the light treatments . Each of the six light banks was filtered with

tempered glass and had a chilled, circulating water barrier to minimize non-photosynthetic thermal radiation. Canary yellow acetate film (Roscolux #312, Oasis Stage Werks, Salt Lake City, UT) was used to reduce the amount of blue light of a given source (transmission curve, Figure 4.1). A comparison of HPS and MH 6% blue was used to determine if any parameter differences were caused by other wavelengths. Spectral output of the lamps was measured with a spectroradiometer (LI-1800, LICOR, Lincoln, NE). All treatments were shaded with neutral density fiberglass screening to obtain the desired PPF.

Definition of blue light

We defined blue light as ranging from 320 to 496 nm. UVA wavelengths (320-400) nm) are present in many lamps and are known to be involved in photomorphogenic responses (Salisbury and Ross, 1992; Munzner and Voigt, 1992; Baskin and Iino, 1987). The UVA wavelengths increase the blue light fraction in HPS lamps by only 0.2% and in MH lamps by 3.6% (Table 4.2). Wavelengths from 496-500 nm were not included in the blue fraction because HPS lamps have a spectral peak from 494-502 nm and photomorphogenic responses rapidly decrease above about 490 nm (Salisbury and Ross, 1992). Including the 496-500 nm wavelengths tends to exaggerate the effective blue from HPS by 20% (Table 4.2).

Phytochrome photoequilibrium differences

Plant morphology and growth are known to respond to the balance of active phytochrome to total phytochrome, measured as phytochrome photoequilibrium (PPE) (Sager and Mcfarlane, 1997; Barnes and Bugbee, 1991). PPE estimates the balance of

active and inactive phytochromes from response curves of the purified phytochrome *in vitro.* There is still controversy as to the *in vivo* role of chlorophyll altering the radiation absorbed and thus PPE (Sager and McFarlane, 1997). However, measuring the phytochrome response *in vivo* has not yet been achieved and researchers rely on the use of PPE as an estimate. The PPE in these experiments, calculated from spectroradiometric data, ranged from 0. 81 at the highest blue light fraction to 0. 86 at the lowest blue light fraction. While phytochrome response cannot entirely be ruled out, the magnitude of the responses observed was likely too large to be elicited by such a small change in PPE.

Plant culture and harvest

Germination. Blue light treatments began at imbibition. Plants were given light 2 h at a PPF of 200 μ mol m⁻² s⁻¹ or 1 h at a PPF of 500 μ mol m⁻² s⁻¹ for the first 4 days. At the end of 4 days, lettuce and wheat were at emergence and with soybean was fit for transplanting. Temperature was maintained at 24/22 °C day/night during the germination period. Lettuce seeds were sown directly in Ethafoam plugs with a diatomaceous earth (Isolite) core. Lettuce plugs were kept in a shallow pan of nutrient solution until emergence, 4 days after planting, and then they were transferred to the aerated hydroponic system. Wheat seeds were stratified at 4°C in moist paper towels for 48 h prior to planting. Wheat seeds were also planted in Ethafoam plugs with an Isolite core . Wheat plugs were placed directly in the system. Soybean seeds were germinated in trays of moist Isolite. Seedlings were transferred to Ethafoam plugs and into the system when the hypocotyl was at least 2 cm long, 4 days after planting.

Plant Growth. Plants were grown in an aerated hydroponics system under a 16-h photoperiod. The environment was maintained at $26/22$ °C day/night \pm 0.3/0.2 between sections within a trial and 68% relative humidity. CO_2 was elevated to 860 μ mol mol⁻¹. All sections were connected to a common air conditioning system via a manifold, so carbon dioxide, humidity, and temperature differences between sections was minimal.

Measurements and Harvest. Chlorophyll measurements were made 2 days before harvest with a chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ). Meter readings were in SPAD units, which is based on the ratio of chlorophyll absorbance at 650 nm to nonchlorophyll absorbance at 940 nm. SPAD units are linearly related to measurement made colorimetrically (Monje and Bugbee, 1992). An average of three chlorophyll readings was taken on the middle leaflet of the first trifoliate of soybean, the second true leaf of lettuce, and the second leaf of wheat. Plants were harvested at canopy closure (Appendix B) to test blue light effects without complicating changes in spectral quality caused by canopy closure. Lettuce and soybean were harvested 18 and 17 days after transplanting, respectively. Wheat was harvested 17 days after emergence. Fresh mass and leaf area were taken immediately upon harvest and branch/tiller number and stem length determined. Roots were blotted dry and then weighed for fresh mass. Plant material was dried for 48 hat 80°C and dry mass determined. Specific leaf area was calculated as total leaf area divided by mass of the leaves. Carbon partitioning to each plant part was calculated as dry mass of plant part divided by the total dry mass (times 100 to give percentage). Relative water content (RWC), a potential indicator of cell size, was

calculated for each plant part as fresh mass minus dry mass all divided by fresh mass (times 100 to give percentage).

Statistical procedures

Six plants (pseudo-replicates) of each species were grown under each blue light fraction. Each PPF level was replicated twice in time. Differences between blue light fractions were tested using analysis of variance using a split-plot design with lamp type as the main plot (Appendix D). Mean comparisons were made using LSD at α =0.05 (SAS Institute, NC). To compare relative and absolute blue light, the data were fit using regression analysis (Sigma Plot 4.0, SPSS Inc., Chicago, IL).

Results

Sensitivity to lamp type with constant blue light fraction

MH lamps were filtered to 6% blue to compare with unfiltered HPS lamps at the same blue light fraction. None of the plant responses for wheat and soybean were significantly different between HPS and MH at 6% blue, suggesting that their blue light responses are not affected by the remaining spectral composition (Table 4.3). Wheat and soybean blue light effects were thus considered to be continuous between lamp types over the five blue light fractions.

In contrast to wheat and soybean, lettuce blue light response may be affected by non-blue wavelengths. Chlorophyll concentration, dry mass, leaf area, and specific leaf area of lettuce were significantly different for plants grown under 6% HPS blue and 6%

MH blue (Table 4.3). This indicates that caution must be used in claiming a blue light response when other parts of the spectrum vary. Blue light effects on lettuce chlorophyll concentration, dry mass accumulation, leaf area, and specific leaf area were graphed separately for each lamp type, but other parameters were not significantly different between lamp types and blue light fraction responses were considered continuous.

Dry mass accumulation and partitioning

Wheat dry mass tended to decrease with increasing blue light fraction, but means were not significantly different (Figure 4.2). Wheat carbon partitioning was similar under all blue light treatments (Figure 4.3). Because plants were harvested early, small differences could become larger as the plants matured. However, Barnes and Bugbee (1992) also found no significant difference between 1% and 25% blue light for mature wheat dry mass. Goins et al. (1997) did see a reduction in wheat shoot dry matter between 31% and 0.85% blue, but grain yield was not affected.

Soybean leaf and total dry mass were not responsive to blue light fraction (Figure 4.4a,d) . Stem dry mass decreased with increasing blue light fraction (Figure 4.4b) . Root dry mass was significantly less at high (26%) and low (0%) blue light fraction (Figure 4.4c). Carbon partitioning to stems at lower blue light fraction was mostly at the expense of the roots (Figure 4.5).

Although lettuce dry mass could not be graphed continuously due to significant differences caused by lamp type at 6% blue, there was a trend for increasing dry mass with blue light fraction under each lamp type (Figure 4.6). More carbon was partitioned to the

stem at low blue at the expense of the leaves (Figure 4. 7). Both stem and leaf carbon partitioning changed drastically between 0% and 2% blue. Leaf carbon partitioning recovered by 2% blue, but carbon partitioning to the stem was still significantly higher at 2% blue than 6% blue or above. Some of the carbon partitioning compensation came from the roots, but the change in percent root dry mass with blue light fraction was not statistically significant.

Stem length

Stem length of wheat decreased by only 11% as blue light fraction increased from 0% to 26%, but this response was not significant (Figure 4.8a). Increasing blue light from 0% to 2% decreased soybean stem length only 7%, but a further increase to 6% decreased stem length by 44% (Figure 4.8b). Overall, increasing blue light fraction to 26% decreased soybean stem length 67% from 0% blue. Lettuce stem length decreased 72% between 0% and 2% blue and a further 13% from 2% to 6% blue (Figure 4.8c). Overall, lettuce stem length decreased 88% from 0% to 26% blue.

Leaf area and specific leaf area

Wheat leaf area and SLA were constant under all blue light treatments (Figure 4.9). Soybean leaf area was highest between 2% and 12% blue and decreased at extreme low (0%) and high (26%) blue (Figure 4.10a). Contrary to the findings of Britz and Sager (1990), soybean SLA was not significantly affected by blue light fraction (Figure 4.1 Ob).

Both leaf area and SLA of lettuce were affected by lamp type and could not be drawn as a continuous response curve for blue light fraction. However, there was a

drastic increase in leaf area from 0% to 6% blue under HPS (Figure 4.11a). There was little response to blue light fraction under the MH treatments. SLA decreased with increasing blue light fraction under each lamp type (Figure 4.1 lb). There was a 54% decrease in SLA between 0% and 2% blue. The mean SLA of 152 m^2 kg⁻¹ at 0% blue is extremely high, reflecting the thin, almost transparent, leaves.

Chlorophyll concentration

Wheat chlorophyll was not affected by blue light fraction (Figure 4.12a). Soybean chlorophyll increased 13% between 0% and 2% blue, but was constant from 2% to 26% blue (Figure 4.12b). Although lettuce chlorophyll was significantly different between the two 6% blues, the chlorophyll concentration increased significantly under each lamp type with increasing blue light fraction (Figure 4.12c).

Tillering/branching

Although wheat tiller number tended to decrease with increasing blue light fraction, differences were not significant (Figure 4.13). This is contrary to Barnes and Bugbee (1992), who found that tillering increased 25% between 1% and 25% blue, and Goins et al. (1997), who found no increase in tillering between 0% and 0.85% blue, but a 71% increase between 0.85% and 8.5% blue. Differences in soybean branch numbers were statistically significant (data not shown), but means ranged only from 5.25 to 5.95, which is not physiologically important to light interception and canopy closure.

Relative water content

RWC of wheat leaves, stems, and roots was not affected by blue light fraction (Figure 4.14). RWC of soybean leaves and stems increased significantly with increasing blue light fraction, but root RWC was not significantly affected (Figure 4.15). Lettuce leafRWC decreased with increasing blue light fraction (Figure 4. 16a). Most of the change in leafRWC occurred between 0% and 2% blue. Lettuce stem and root RWC did not change significantly (Figure 4.16b,c).

Discussion

Species differences

Blue light effects were species dependent and the differences may be associated with differences in plant morphology. Wheat, whose meristematic leaves and stems are sheltered from direct light by upper leaves and leaf sheathes, showed no response to blue light. Both lettuce and soybean have exposed meristematic cells in expanding leaves and stems and both responded to blue light fraction. Interestingly, the response of lettuce was more pronounced than soybean (Figure 4.17).

The many planophile species tested have shown a response to blue light (Brown et al., 1995; Hoenecke et al., 1992; Tibbitts et al., 1983; Yorio et al., 1995). Ryegrass, whose growth habit is erectophile and whose expanding tissues are also shielded, leaf area and shoot length did not respond to red- versus blue-biased lamps (Warrington and Mitchell, 1976). Under similar treatment, however, sorghum, also erectophile in growth habit, did respond. Although indicative that some erectophile species may not be blue

light insensitive, Warrington and Mitchell (1976) only tested lamp type and not blue light response. Direct blue light investigation of these and other grass (erectophile) species is needed to test if a generalization can be made between erectophile and planophile plants.

Inconsistencies with other research

Although Barnes and Bugbee (1992) saw a longer wheat leaf length with low blue, only the longest fully extended leaf was measured and there was no significant difference in dry matter accumulation . Contradiction of our tillering data with Barnes and Bugbee (1992) may be genetic differences in cultivars, as their experiments utilized 'Fielder', an extremely high-tillering cultivar compared to 'Apogee'. Goins et al. (1997) used a low tillering cultivar, but tillering differences were also evident and may be due to the shortduration of our experiments, which did not allow time for blue light effects to be manifested. Goins et al. (1997) saw an increase in wheat dry mass with increased blue light, but compared narrow band red LEDs (0% blue) with broad spectrum white light. It is important to point out that our lowest blue treatment was not truly zero blue and our lamp sources also contained far red. However, the phytochrome photoequilbrium of red LEDs is 0.88, similar to our treatments. Therefore, interactions with phytochrome are probably not responsible for the difference seen between our 0.1% blue and red LEDs.

Results for soybean and lettuce were mostly consistent with previous reports, especially under the <0.1% blue treatment compared to red LEDs (0% blue) (Hoenecke et al., 1992). Surprisingly, soybean SLA did not decrease with increasing blue light as seen previously in a comparison of daylight fluorescent and blue-deficient low pressure sodium

(Britz and Sager, 1990). Although blue light between these lamps does vary considerably, non-blue wavelengths also vary.

Consideration of yield photon flux

The most widely used definition of measurement of photosynthetically active radiation (PAR) is the photosynthetic photon flux, which weights each photon between 400 and 700 nm equally. In reality, the photosynthetic efficiency of blue photons is 30% less than that of red photons and the range extends beyond 400-700 nm (McCree, 1972). A more precise definition of PAR is called the yield photon flux (YPF) (Barnes et al., 1993). YPF is a weighting of each photon according to the "average leaf' photosynthetic efficiency curve elucidated by McCree (1972). In these experiments PPF was equivalent between blue light treatments, but YPF declined by as much as 10% as blue light fraction increased (Table 4.4). Considering that photosynthetic efficiency directly affects plant dry mass, caution must be used when claiming blue light effects on dry mass accumulation. YPF is a more theoretically exact definition of the trends for total dry mass in wheat and soybean. The graph of YPF distributes points across quantum flux $(\mu \text{mol m}^2 \text{ s}^{-1})$ more accurately (Figure 4.18) and may explain the response better than blue light fraction does (Figure 4.2, 4.4).

Accurate measurements of YPF can only be made with a spectroradiometer. Under these lamp types, a commercial YPF sensor can have substantial errors (Barnes et al., 1993).

YPF differences do not explain the difference in dry mass between the two 6%

blue treatments in lettuce. Indeed, 6% blue MH has 3% lower YPF than 6% blue HPS. However, the 3% difference in YPF is not enough to account for the 81% difference in total dry mass.

Morphology and carbon partitioning

Although total dry mass is better explained by YPF, the dry mass partitioning of soybean is attributable to the morphological changes caused by altering blue light fraction. The change in stem elongation at low blue came at the expense of the roots and to a lesser extent, the leaves. It is difficult to ascertain whether these shifts in carbon partitioning, although not affecting total biomass yield, would affect seed yield. Where small differences occur early on, large differences in yield could result, especially when dry mass accumulation is affected (Board et al., 1992; Gent, 1995).

Relative versus absolute blue light

Blue light can be described two ways, the absolute amount of blue light or the fraction of blue light relative to the photosynthetically active radiation. It is not clear which definition best describes physiological responses. Hoenecke et al. (1992) found that lettuce hypocotyl extension responded to absolute rather than relative blue light. Similarly, Wheeler et al. (1991) suggested that soybean stem elongation was responsive to absolute rather than relative blue light. We conducted studies using two PPF levels so we could quantify relative versus absolute blue light effects (Table 4.4). If absolute blue light determines plant response, the blue light fraction response curves at the two PPF levels should overlap when graphed on an absolute blue light axis.

Indeed, our data for lettuce agree with Hoenecke et al. (1992), where the data better fit on an absolute blue light axis (Figure 4.19a). Soybean stem length, on the other hand, is better fit with a relative blue light axis (Figure 4.19b,c). This discrepancy in the soybean data may lie with the fact that Wheeler et al. (1991) only tested 6% to 26% blue light where stem length is less responsive to blue light.

Because means for wheat response to blue light were not significantly different, it is a moot point to make comparisons between relative and absolute. Because of the complicating factors in other lettuce parameters, we were unable to evaluate the effects of relative versus absolute blue light. For soybean, using stem dry mass and leaf area as typical examples, there is a response to blue light fraction, but the responses are different at the two PPFs (Figure 4.4b, 4.10a). However, graphing the data as absolute blue light does not cause the two PPF levels to overlap (Figure 4.20a,c). The responses do overlap when graphed with blue light fraction and as a percent of maximum (Figure 4.20b,d). This also holds true for leaf, root, and total dry mass and SLA. Although the percent of maximum response can be predicted by blue light fraction, the absolute magnitude of the response is determined by **PPF.**

Other wavelengths affecting lettuce growth

As mentioned previously, we included two 6% blue treatments, one with HPS, one with filtered MH. For soybean and wheat, the means of these treatments were statistically similar, but for lettuce the two 6% blue treatments produced significantly different chlorophyll concentrations, dry masses, leaf areas, and SLAs (Table 4.3). This

phenomenon was apparent in each trial in this experiment and in trials before this experiment (Dougher and Bugbee, 1998). It is extremely unlikely that these differences were caused by differences between compartments because: i) treatments were randomized each time and ii) atmospheric differences between compartments were minimized by the use of a common air conditioning system. Apparently in lettuce, some wavelength(s) acts in conjunction with blue to affect plant growth. Using data for chlorophyll concentration at a PPF of 200 μ mol m⁻² s⁻¹ as an example (Figure 4.21a) (results for the other parameters and PPF of 500 μ mol m⁻² s⁻¹ are similar), I will discuss the other wavelengths we have considered.

Absolute blue light. Absolute blue light could be more important than blue light fraction, but the 6% blue treatments of the two lamp types have the same absolute blue light at each PPF: 12 μ mol m⁻² s⁻¹ blue at 200 μ mol m⁻² s⁻¹ or 30 μ mol m⁻² s⁻¹ blue at 500 μ mol m⁻² s⁻¹ (Table 4.4). Graphically, data points for 6% blue between PPF levels shift apart, but the two data points within PPF levels are still not separated (Figure 4.21b).

Phototropic blue. Considering that our cutoff points for "blue light" may not be accurate, we tried using the blue response curve for phototropism, developed by Baskin and Iino (1987). Weighting our data with this curve yielded blue levels that were also similar as our 6% blue treatments (Table 4.4). So the curves look very similar to the absolute blue light curves (Figure 4.21c).

Absolute UV. Although MH emits much more UV (300-400nm) than HPS lamps, our system had a water and tempered glass barrier that greatly reduced the UV from either source. However, not all UV was filtered out and the MH treatments still had 3 to 4 times

more UV (Table 4.4). While there is a UV difference between the two 6% blue treatments, it only serves to further separate the data (Figure 4.21d).

UV as a percent of blue. We considered UV-A (320-400nm) as part of the blue range, but MH attains more of its "blue light" from the UV range. Indeed at 6% blue UV as a percent of blue is different for HPS and MH (Table 4.4). However, graphing the data this way also separates the data in the wrong direction (Figure 4.2le) .

Phytochrome photoequilibrium. The PPE for all treatments ranged from 0.82 to 0.86 (Table 4.4). This range is too small to elicit a phytochrome effect and also tends to separate the data (Figure 4.2lf).

Blue to red and blue to far red ratios. Other researchers have suggested the blue response could be altered by the blue and red $(B:R)$ or blue and far red $(B:FR)$ interaction (Goins et al., 1997). Once again, there is a difference between 6% blue HPS and MH values for B:R and B:FR (Table 4.4), but these ratios also tend to separate the data (Figure 4.2lg,h).

Red to far-red ratio. Although we should be able to more accurately calculate PPE utilizing the spectroradiometer, perhaps the response is to R:FR but not a phytochrome response. There is a difference in R:FR of the two 6% blue treatments (Table 4.4), but again graphing on a R:FR axis only separates the data (Figure 4.21i).

Yellow-green wavelengths. At a loss for a known physiological response to explain the difference at 6% blue, we graphed the output of the treatments to find which wavelengths could shift the data to fit their significance (Figure 4.22). We were looking for a shift in lamp output that would make the 6% MH roughly equivalent to the 2% HPS,

because their means are not statistically different. At 570-610 nm we see an overlap of the middle range blue treatments $(2, 6, 12\%)$. Using this range, we not only achieved 6% MH equivalent to 2% HPS, but also 12% MR shifts to about 6% HPS (Table 4.4). While these wavelengths fit the response (Figure 4.2 lj), there is no physiological explanation for it. Green light from 545 to 555 nm has been shown to repress growth, but repression was not seen beyond this range (Klein, 1992).

Thermal radiation . Although the thermal radiation emitted by unfiltered HPS and MH lamps is considerable and different for the two lamp types, the thermal radiation in these experiments was filtered out by water barriers.

Predicting cell expansion using relative water content and dry mass

The change in leaf area and stem length with blue light fraction raises the question of which cellular process is being altered by blue light to achieve these changes : cell division, cell expansion, or both. Without directly measuring the cells, we hypothesized that an increase in R WC without a change in dry mass predicts that cell expansion is primarily responsible for the increase in area or length. For lettuce, leaf area increased from 0% to 6% blue, leafRWC decreased, but the leaf dry mass increased. This suggests that the increase in leaf area is primarily caused by an increase in cell division. For soybean, leaf area decreased with increasing blue light fraction from 6% to 26% blue and leaf RWC increased, while leaf dry mass was constant. This would suggest that the decrease in soybean leaf area was also caused by a decrease in cell division. For soybean stems, length decreased with increasing blue light fraction, stem RWC increased, but stem dry mass decreased. This suggests that cell division may be primarily responsible for the change in stem length with blue light fraction.

Conclusions

Blue light effects were species dependent. When considering dry mass and leaf area, soybean and wheat do not benefit from added blue light. However, in an Advanced Life Support system where plant height is important, well-knovm effects on stem elongation (Britz and Sager, 1990; Dougher and Bugbee, 1997; Wheeler et al., 1991) do not have the same optimum blue light fraction. Lettuce dry mass and leaf area, on the other hand, benefitted from as little as 2% blue, which is also beneficial for suppressing excessive stem elongation (Hoenecke et al., 1992). For some plant growth parameters, blue light responses were small, but the crops were harvested early in the life cycle and effects may not have fully manifested. This research indicates that lamps high in blue photons, such as MH, are not only energetically wasteful, but do not benefit and, in some cases reduce, plant growth.

Literature Cited

- Barnes, C. and B.G. Bugbee. 1991. Morphological responses of wheat to changes in phyotchrome photoequilibrium. Plant Physiol. 97:359-365.
- Barnes, C. and B.G . Bugbee. 1992. Morphological responses of wheat to blue light. J. Plant Physiol. 139:339-342.
- Barnes, C., T. Tibbitts, J. Sager, G. Deitzer, D. Bubenheim, G. Koerner, and B. Bugbee . 1993. Accuracy of quantum sensors measuring yield photon flux and photosynthetic photon flux. J. of Amer. Soc. of Hort. Sci. 28:1197-1200.
- Baskin, T.I. and M. Iino. 1987. An action spectrum in the blue and ultraviolet for phototropism in alfalfa. Photochem. and Photobiol. 46:127-136.
- Board, J. E., M. Kamal, and B.G. Harville. 1992. Temporal importance of greater light interception to increased yield in narrow-row soybean. Agronomy Journal 84:575- 579.
- Britz, S. J. and J.C. Sager. 1990. Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad spectrum or blue-deficient light sources. Plant Physiol. 94:448-454.
- Brown, C. S., AC . Schuerger, and J.C. Sager. 1995. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. J. of the Amer. Soc. Hort. Sci. 120:808-813.
- Cosgrove, D. J. 1981. Rapid suppression of growth by blue light. Plant Physiology 67 :584-590.
- Dougher, T.A.O. and B.G. Bugbee. 1998. Is blue light good or bad for plants? Life Support and Biosphere Science. 5:129-136.
- Dougher, T.A.O . and B.G. Bugbee. 1997. Effect oflamp type and temperature on development, carbon partitioning and yield of soybean. Adv. Space Res. 20:1895-1899.
- Gautier, H., C. Varlet-Grancher, and N. Baudry. 1997. Effect of blue light on the vertical colonization of space by white clover and their consequences for dry matter distribution. Annals of Bot. 80:665-671.
- Gent, M. P. N. 1995. Canopy light interception, gas exchange, and biomass in reduced height isolines of winter wheat. Crop Sci. 35:1636-1642.
- Goins, G.D., N.C. Yorio, M.M. Sanwo, and C.S. Brown. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. of Exp. Bot. 48:1407-1413.
- Hoenecke, M. E., R.J. Bula, and T.W. Tibbitts. 1992. Importance of'blue' photon levels for lettuce seedlings grown under red-light-emitting diodes. HortScience 27:427- 430.
- Klein, R.M. 1992. Effect of green light on biological systems. Biol. Rev. of the Cambridge Phil. Soc . 67: 199-284.
- McCree, K.J. 1972. Test of current definitions of photosynthetically active radiation against leaf photosynthesis data. Agric. Meteor. 10:443-453.
- Monje, 0 .A. and B. Bugbee. 1992. Inherent limitations of nondestructive chlorophyll meters: A comparison of two types of meters. HortScience 27:69-71.
- Munzner, P. and J. Voigt. 1992. Blue light regulation of cell division in *Chlamydomonas reinhardtii .* Plant Physiol. 99: 1370-1375.
- Sager, J.C. and J.C. McFarlane. 1997. Radiation, p. 1-29. In: R.W. Langhans and T.W. Tibbitts (eds.). Plant growth chamber handbook. Iowa Agriculture and Home Economics Experiment Station Special Report No. 99. Iowa State Univ., Ames, Iowa.
- Salisbury, F.B. and C.W. Ross. 1992. Plant physiology. 4th ed. Wadsworth Publishing Company, Belmont, CA
- Tibbitts, T. W., D.C. Morgan, and I.J. Warrington. 1983. Growth of lettuce, spinach, mustard, and wheat plants under four combinations of high-pressure sodium, metal halide, and tungsten halogen lamps at equal PPFD. J. of the Amer. Soc. Hort. Sci. l 08 :622 -630 .
- Warrinton, I.J. and K.J. Mitchell. 1976. The influence of blue- and red-biased light spectra on the growth and development of plants. Agricultural Meteorology 16:247-262 .
- \Vheeler, R.M ., C.L. Mackowiak, J.C. Sager, N.C. Yorio, and W.M. Knott. 1994. Growth and gas exchange by lettuce stands in a closed, controlled environment. J. of the Amer. Soc. Hort. Sci. 119:610-615.
- Wheeler, R. M., C.L. Mackowiak, and J.C. Sager. 1991. Soybean stem growth under high-pressure sodium with supplemental blue lighting. Agronomy Journal 83:903-906.
- Yorio, N.C., R.M . Wheeler, G.D. Goins, M.M. Sanwo, C. Mackowiak, C.S. Brown, J.C. Sager, and G.W. Stutte. 1998. Blue light requirements for crop plants used in bioregenerative life support systems. Life Support and Biosphere Science 5:119-128.
- Yorio, N. C., C.L. Mackowiak, R.M. Wheeler, and J.C. Sager. 1995. Vegetative growth of potato under high-pressure sodium, high-pressure sodium SON-Agro, and metal halide lamps. HortScience 30:374-376.

*blue fraction from the lamp filtered only with tempered glass and water

Table 4.2. Blue light fractions (%) calculated for the six blue light treatments with and without UVA (320-400 nm) and with the HPS 496-500 nm spike. The 320-496 nm range was used in this study. Data are based on quantum flux (moles), not energy flux (watts).

		Lamp Type									
Blue Range		HPS High filter Low filter HPS	HPS	Unfiltered	MH High filter Low filter	MH	Unfiltered MH	total range			
	$-$ UVA 400-496	0.49	3.41	5.5	53	15.5	22.7	400-700			
	$+$ UVA 320-496	0.49	3.56	5.7	6.0	18.7	26.3	320-700			
	320-500	0.93	4.52	7 ₁	65	194	27.2	320-700			

	Wheat			Soybean	Lettuce			
Parameter	HPS ^o	MH [†]	HPS	MH [†]	HPS ^o	MH [†]	HPS/MH	
leaf dry weight (g)	0.28	0.23	1.34	1.29	0.52	$0.29*$	1.79	
stem dry weight (g)	0.13	0.10	0.48	0.47	0.028	$0.015*$	1.87	
root dry weight (g)	0.18	0.16	0.49	0.47	0.088	$0.050*$	1.76	
total dry weight (g)	0.59	0.50	2.32	2.22	00.63	$0.35*$	1.80	
leaf as % of total	47.3	46.9	58.0	57.8	81.6	81.2ns	1.00	
stem as % of total	21.6	20.5	20.8	20.9	4.6	4.2ns	1.10	
root as % of total	31.4	32.6	21.2	21.2	13.8	14.6ns	0.95	
leaf area $(cm2)$	67.5	59.0	497	492	258	$181*$	1.43	
specific leaf area $(m^2 \text{ kg}^{-1})$	27.1	27.2	37.7	39.2	54.6	$66.7*$	0.82	
chlorophyll (SPAD)	52.3	52.3	36.3	34.2	7.9	$4.2*$	1.88	
stem length (mm)	74.4	72.5	159	154	18.3	15.6ns	1.17	
leaf RWC $(%)$	84.5	84.2	84.7	85.0	93.6	94.0ns	1.00	
stem RWC $(%)$	89.3	88.9	90.4	90.1	92.1	93.2ns	0.99	
root RWC (%)	92.9	92.8	94.1	94.2	95.3	95.5ns	1.00	

Table 4.3. Effect of lamp type at the same blue light fraction (6%) on dry weight, carbon partitioning, leaf area, specific leaf area, chlorophyll, stem length, and relative water content. Means were tested using an LSD test at α = 0.05. \equiv

 $^{\circ}$ unfiltered High Pressure Sodium lamp (6% blue), [†] Metal Halide lamp filtered with canary yellow acetate film (6% blue)

* significant at α = 0.05, ns = not significant

PPF*	Blue Light Fraction % of total)	Absolute Blue Light* $(320 - 496)$ nm)	Yield Photon $Flux*$ $(300 -$ 800 nm)	Photo- tropic Blue* $(300 - 520)$ nm)	Absolute UV^* $(320 - 400)$ nm)	UV % of PPE blue)	(P_f/P_{total})	R:FR $(600 - 700nm)$ 700-800nm)	B:R $(320 - 496)$ nm $600 - 700$ nm)	B:FR $(320 - 496)$ nm $700 - 800$ nm)	570- 610 nm^* % of total)
	0.1	0.2	194	0.1	0.0005	0.25	0.86	2.80	0.003	0.012	32
200	1.5	$\overline{3}$	192	1.8	0.05	1.7	0.85	2.71	0.033	0.155	26
	6	12	190	6.8	0.22	1.8	0.86	2.86	0.133	0.661	25
	6	12	184	6.3	0.72	6.0	0.84	4.39	0.220	1.306	27
	12	24	182	13	1.99	8.3	0.83	4.13	0.453	2.593	25
	26	52	177	29	3.88	7.5	0.82	5.11	1.209	7.818	19
	0.1	0.5	484	0.3	0.0005	0.1	0.86	2.80	0.003	0.012	32
	1.5	7.5	480	4.1	0.05	0.7	0.85	2.71	0.033	0.155	26
	6	30	474	17	0.22	0.7	0.85	2.86	0.133	0.661	25
500	6	30	458	16	0.71	2.4	0.85	4.39	0.220	1.306	27
	12	60	454	31	1.97	3.3	0.84	4.13	0.453	2.593	25
	26	130	441	71	3.61	2.8	0.82	5.11	1.209	7.818	19

Table 4.4 Ratios of radiation for the blue light fractions in these studies.

* in (μ mo! m⁻² s⁻¹), PPF = Photosynthetic Photon Flux, PPE = Phytochrome Photoequilibrium

Figure 4.1. Transmission curve for canary yellow cellulose acetate film (#312, Roscolux, Oasis Stage Werks, UT) obtained from Roscolux .

Figure 4.2. Effect of blue light fraction on wheat (a) leaf, (b) stem, (c) root, and (d) total dry mass. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and \bullet represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF.

Figure 4.4 . Effect of blue light fraction on soybean (a) leaf, (b) stem, (c) root, and (d) total dry mass. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF .

Figure 4.5. Effect of blue light fraction on soybean (a) leaf, (b) stem, and (c) root dry mass as a percent of total dry mass. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and \bullet represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates) . Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions.

Figure 4.6. Effect of blue light fraction on lettuce (a) leaf, (b) stem, (c) root, and (d) total dry mass. Closed symbols represent blue light fractions created under HPS and open symbols represent blue light fractions created under MH . Triangles represent PPF of 500 μ mol m⁻² s⁻¹ and circles represent PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions.

Figure 4.7. Effect of blue light fraction on lettuce (a) leaf, (b) stem, and (c) root dry mass as a percent of total dry mass. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates) . Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions.

Figure 4.8. Effect of blue light fraction on (a) wheat, (b) soybean, and (c) lettuce stem length. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol $m²$ s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the *a=0.05* level between blue light fractions.

Figure 4.9. Effect of blue light fraction on wheat (a) leaf area and (b) specific leaf area. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and \bullet represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF.

Figure 4.10. Effect of blue light fraction on soybean (a) leaf area and (b) specific leaf area. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and \bullet represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF.

Figure 4.11. Effect of blue light fraction on lettuce (a) leaf area and (b) specific leaf area. Closed symbols represent blue light fractions created under HPS and open symbols represent blue light fractions created under MH. Triangles represent PPF of 500 μ mol m⁻² s⁻¹ and circles represent PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions (within a PPF for figure (a)).

Figure 4.12. Effect of blue light fraction on (a) wheat, (b) soybean, and (c) lettuce chlorophyll concentration. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions (within a PPF for wheat). For wheat and soybeans: Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol m⁻² s⁻¹. For lettuce: Closed symbols represent blue light fractions created under HPS and open symbols represent blue light fractions created under MH. Triangles represent PPF of 500 μ mol m⁻² s⁻¹ and circles represent PPF of 200 μ mol $m^{-2} s^{-1}$.

Figure 4.13. Effect of blue light fraction on wheat tiller number. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and \bullet represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the $\alpha=0.05$ level between blue light fractions within a PPF.

Figure 4.14. Effect of blue light fraction on wheat (a) leaf, (b) stem, and (c) root relative water content. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF .

Figure 4.15. Effect of blue light fraction on soybean (a) leaf, (b) stem, and (c) root relative water content. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions.

Figure 4.16. Effect of blue light fraction on lettuce (a) leaf, (b) stem, and (c) root relative water content. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and • represents PPF of 200 μ mol m⁻² s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions.

Figure 4.19. A comparison of blue light fraction and absolute blue light to describe (a,b) lettuce and (c,d) soybean stem length response to blue light. Δ and ---- represent PPF of 500 μ mol m⁻² s⁻¹, \bullet and — represent PPF of 200 μ mol m⁻² s⁻¹, and represents regression of the combined data. Each point is an average of six plants (pseudo replicates) . Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF.

Figure 4.20. A comparison of absolute blue light and blue light fraction to describe soybean (a) stem dry mass, (b) relative stem dry mass, (c) leaf area, and (d) relative leaf area. Δ represents PPF of 500 μ mol m⁻² s⁻¹ and \bullet represents PPF of 200 μ mol $m²$ s⁻¹. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Error bars represent the least significant difference at the α =0.05 level between blue light fractions within a PPF.

Figure 4.21. A comparison of (a) blue light fraction, (b) absolute blue light, (c) phototropic blue, (d) absolute UV, (e) UV as a percent of blue, (f) phytochrome photoequilibrium, (g) blue to red ratio, (h) blue to far-red ratio, (i) red to far-red ratio, and (j) yellow light as a percent of total, to describe lettuce chlorophyll response to varied light environment. Data are only presented for PPF of 200 *µmol* $m^2 s^1$. \bullet represents treatments created under HPS lamps and \circ represents treatments created under MH lamps. Each point is an average of six plants (pseudo replicates). Replicate data points at each blue light fraction represent replicate trials in time. Lines are linear regressions of the means for each lamp type.

Figure 4.21 . Continued

Figure 4.22 - The light fraction as a percent of total for 20 nm running average increments from 300 to 700 nm for the blue light treatments in our experiment. - represents treatments created under HPS lamps and www. represents treatments created under MH lamps.

CHAPTER 5

BLUE LIGHT EFFECTS ON THE HISTOLOGY OF LEA YES AND STEMS

Abstract

Cell division, cell expansion, or both processes may be affected by blue light. Most studies on changes in cell expansion or division with altered light quality are shortterm, cell-level experiments. Long-term, whole-plant effects are not well characterized. We measured cell size and number for stems of soybean, and leaves of soybean and lettuce, at two blue light fractions. Stem cell expansion is known to be rapidly inhibited by blue light when switched from darkness, but we found that decreased soybean stem length over the long term was caused by an inhibition of cell division. Leaf area, on the other hand, was altered by a change in cell expansion for soybean and by a change in both cell expansion and division for lettuce .

Introduction

Blue light effects on leaf expansion and stem elongation at the cellular level are still controversial as to whether cell division or expansion is primarily altered. Blue lightmediated inhibition of stem elongation is often assumed to be caused by changes in cell expansion. However, three key studies measured only hypocotyl elongation and cell wall properties and did not measure cell size (Shinkle and Jones, 1988; Cosgrove, 1981; Kigel and Cosgrove, 1991). Blue light does appear to regulate cell division in *Ch/amydomonas*

reinhardtii (Munzner and Voigt, 1992; Voigt and Munzner, 1994). In *Phaseo/us vu/garis* leaves, blue light stimulated cell expansion (Van Volkenburgh et al., 1990). However, these studies were short-term hypocotyl or leaf disc experiments . In a longer-term study, Rahim and Fordham (1991) found shade versus sun conditions altered garlic leaf area primarily by changes in cell expansion.

Leaf expansion, however, characteristically has a larger change in cell number than cell size (Dale and Milthorpe, 1983). One would then predict that in response to long-term light exposure, changes in leaf size could be more readily mediated by altered cell division. Based on the current view that the epidermis limits stem and leaf expansion (Dale, 1988; Kutschera, 1992), we measured epidermal cell size and number to account for differences in stem length of soybean and leaf area of soybean and lettuce at two blue light fractions.

Material and Methods

Soybeans and lettuce were cultured in the same system described in Chapter 4. Plants were grown at a photosynthetic photon flux of 500 μ mol m⁻² s⁻¹, 530 μ mol CO₂ mol⁻¹, 25/22 °C day/night temperature, and 73% relative humidity. Plants grown for stem sections were treated with a 16-h photoperiod and plants grown for leaf impressions were treated with a 12-h photoperiod. While these conditions varied from those described in Chapter 4, the blue light response was the same, namely: soybean leaf area decreased between 6% and 26% blue, lettuce leaf area increased between 0% and 6% blue, and soybean stem length decreased between 0% and 26% blue. Blue light treatments were as follows: soybean stem sections 0% (HPS) and 26% (MH), soybean leaf impressions 6%

(HPS) and 26% (MH), and lettuce leaf impressions 0% (HPS) and 6% (HPS) . Six plants were sampled under each blue light fraction and blue light fractions were repeated in another location. Leaf and intemode samples were taken when expansion was complete . Change in expansion was tested by measuring intemode length and leaf length and width every day until three consecutive days of measurements were the same.

Microscopic examination and measurement. Both stem sections and leaf impressions were viewed under a microscope (Leitz, Laborlux 12 Pol, Wetzal, Germany) and photographed using an attached microcamera (WILD, Heerbrugg, Switzerland). A stage micrometer was photographed separately at the same magnification and used for calibration of the analysis software (Arc View, ESRI, Redlands, CA). The analysis software determined cell areas from tracings of the cells. Stem cell number was determined by dividing the intemode length by the average longitudinal cell length. Leaf cell number was determined by dividing leaf area by the average cell area .

Stem sections. Eighteen days after transplanting, soybean plants were transferred to single bottles, two at a time, one from each blue light treatment, to be moved to the lab. Stems were sectioned in the intemode just above the cotyledonary leaves. The fresh sections were cut on glass slides in water and the epidermis was peeled away from the section. Samples were covered with a cover slip and immediately viewed at 25X and photographed.

Leaf impressions. The middle leaflet of the soybean first trifoliate and the second true leaf of lettuce were sampled in the middle of the leaf(let) 18 and 17 days after transplanting, respectively. A 4% (w:v) solution of Formvar resin in chloroform

(polyvinyl-formaldehyde) was painted in a thin layer on each leaf. The solution was allowed to dry for at least 30 seconds. Samples were then covered with cellophane tape, peeled off the leaf, and mounted on a glass microscope slide. The sampled leaf was then measured with a leaf area meter (LI-COR, Lincoln, NE). Leaf impressions were viewed and photographed at 40X. All sampling, viewing and photographing took place within 2 h (see Appendix C).

Results and Discussion

The effect of blue light treatments on soybean stem epidermal cells was visually apparent in microscope photographs (Figure 5.1). Plant and cell measurement revealed a 4.7-fold increase in soybean stem length (Figure 5.2a) was associated with a 4.5-fold increase in cell number (Figure 5.2c). This suggests that blue light fraction alters cell division to elicit the inhibition of soybean stem length. Blue light suppression of cell expansion in etiolated seedlings (Cosgrove, 1981; Kigel and Cosgrove, 1991) explains dark to light inhibition of growth, but does not explain the blue light effects of light-grown soybeans.

Microscope photographs did not reveal, to the human eye, any difference in soybean leaf epidermal cells between blue light treatments (Figure 5.3). The 23% decrease in soybean leaf area (Figure 5.4a) caused by changing blue light fraction from 6% to 26% was associated with a 15% decrease in cell area and an 11 % decrease in cell number (Figure 5.4b,c). However, only the differences in cell area were statistically significant. This suggests that changes in cell expansion may be the primary driver for

changes in leaf area, but cell division may aid in the change. It is important to note that leaf expansion in soybean is greatest at 6% blue and that blue light enhances leaf expansion from 0% to 6% blue (Chapter 4). According to Van Volkenburgh et al. (1990), blue light is as effective as red light at stimulating cell expansion in P. *vulgaris* leaf discs, but the effectiveness of red light is suppressed by far-red light. The curvilinear response of leaf area to an increase in blue light fraction suggests that this hypothesis may not be true . In our experiments, there were changes in red to far-red ratio with increasing blue light fraction (Table 4.4), but the changes in the ratio do not coincide with the variations in leaf area. More directly, the decrease in cell size with increased blue light fraction between 6% and 26% blue more strongly suggests that, in the long term, blue light at high levels may not be as effective as red light in affecting cell expansion .

An increase in lettuce leaf epidermal cell size was visually apparent in microscope photographs (Figure 5.5). Indeed, the 4.4-fold increase in lettuce leaf area between 0% and 6% blue (Figure 5.6a) was caused by a 3.1-fold increase in cell area and a 1.6-fold increase in cell number (Figure 5.6b,c). The change in lettuce leaf cell expansion, although opposite that of soybean, also raises the question of the effectiveness of blue light compared to red in stimulating cell expansion. For lettuce leaves, both blue and red light may be necessary to trigger the expansion responses. Even so, it appears that blue light is more effective at eliciting the expansion response. These discrepancies in cell expansion may be due to the fact that our plants were started from imbibition under their respective treatments, whereas Van Volkenburgh et al. (1990) used leaf discs from plants that were grown first under white fluorescents and were only treated under red or blue lights for a

short time.

While blue light signal transduction pathways for phototropism have been elucidated (Short and Briggs, 1994), the pathways for blue-light-induced cell expansion and cell division have not, and are likely separate pathways due to the very different kinetics (Short and Briggs, 1994; Liscum et al., 1992). The most likely mechanism suggested for blue light to alter cell expansion is a photoreceptor . A blue light photoreceptor may act to stimulate proton efflux, thus affecting calcium channels (Staal et al., 1994), calcium-calmodulin signaling (Elzenga et al., 1997), and auxin-binding (Jones et al., 1998) .

In cell division, the formation of a new cell wall is mediated by Golgi vesicles, which are guided by microtubules. Two light mechanisms known to alter microtubules may also be mechanisms for blue light to alter cell division. β -tubulin, a building block of microtubules, is known to be downregulated in dark versus light-grown soybean seedlings (Bustos et al., 1989). More specifically, blue light causes microtubules to align longitudinally rather than transversely (Short and Briggs, 1994). The recent elucidation of the HY4 gene, believed to code for a blue-light receptor (Ahmad and Cashmore, 1993), may shed light on how blue light might alter microtubule formation or orientation to affect cell division.

Literature Cited

Ahmad, M. and AR. Cashmore . 1993. HY4 gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366:162-166.

- Bustos, M.M, M.J. Guiltinan, R.J. Cyr, D. Ahdoot, and D.E. Fosket 1989. Light regulation of b-tubulin gene expression during internode development in soybean *(Glycine max (L.) Merr.).* Plant Physiol. 91:1157-1161.
- Cosgrove, D. 1981. Rapid suppression of growth by blue light. Plant Physiol. 67:584- 590.
- Dale, J.E. 1988. The control of leaf expansion. Ann. Rev. Plant Physiol. Mol. Biol. 39:267-295 .
- Dale, J.E. and F.L. Milthorpe. 1983. General features of the production and growth of leaves, pp 151-178 . In: J.E. Dale and F.L . Milthorpe (eds.). The growth and functioning of leaves. Cambridge University Press, Cambridge, U.K.
- Elzenga, J.T.M., M. Staal, and H.B.A. Prins. 1997. Calcium-calmodulin signalling [sic] is involved in light-induced acidification by epidermal leaf cells of pea, *Pisum sativum* (L.) J. of Expt. Bot. 48:2055-2060.
- Jones, A.M., K. Im, M.A. Savka, M. Wu, G. DeWitt, R. Shillito, and A.N. Binns. 1998. Auxin-dependent cell expansion mediated by overexpressed auxin-binding protein. Science 282:1114-1117.
- Kigel, J. and D.J. Cosgrove. 1991. Photoinhibition of stem elongation by blue and red light. Plant Physiol. 95:1049-1056.
- Kutschera, U. 1992. The role of the epidermis in the control of elongation growth in stems and coleoptiles. Bot. Acta. 105:246-252.
- Liscum, E., J.C. Young, K.L. Poff, and R.P. Hangarter. 1992. Genetic separation of phototropism and blue light inhibition of stem elongation. Plant Physiol. 100:267- 271.
- Munzner, **P.** and **J.** Voigt. 1992. Blue light regulation of cell division in *Chlamydomonas reinhardtii.* Plant Physiol. 99: 1370-1375.
- Rahim, M.A. and R. Fordham. 1991. Effect of shade on leaf and cell size and number of epidermal cells in garlic *(Allium sativum).* Annals of Bot. 67: 167-171.
- Shinkle, J.R. and R.L. Jones . 1988. Inhibition of stem elongation in *Cucumis* seedlings by blue light requires calcium. Plant Physiol. 86:960-966.
- Short, T.W. and W.R. Briggs. 1994. The transduction of blue light signals in higher plants. Ann. Rev. Plant Physiol. Mol. Biol. 45:143-171.
- Staal, M., J.T.M. Elzenga, A.G. van Elk, H.B.A. Prins, and E. Van Volkenburgh. 1994. Red and blue light-stimulated proton efflux by epidermal leaf cells of the Argenteum mutant of *Pisum sativum*. J. of Expt. Bot. 45:1213-1218.
- Van Volkenburgh, E., R.E. Cleland, and M. Watanabe. 1990. Light-stimulated cell expansion in bean *(Phaseolus vulgaris L.)* leaves. II Quantity and quality of light required. Planta. 182:77-80.
- Voigt, J. and P. Munzner . 1994. Blue light-induced lethality of a cell wall-deficient mutant of the unicellular green alga *Chlamydomonas reinhardtii .* Plant Cell Physiol. 35:99-106.

Soybean Stem ⁸¹

0% blue

26% blue

Figure 5.2. Effect of 0% and 26% blue light on soybean stem length, stem epidermal cell area, and stem epidermal cell number per internode. Error bars represent the least significant difference at α = 0.05.

Soybean Leaf

6% blue

26% blue

Figure 5 .3. Microscope photographs of soybean leaf epidermal cells at 6% and 26% blue. Photographs were taken at 40X.

Lettuce Leaf

Figure 5.5. Microscope photographs of lettuce leaf epidermal cells at 0% and 6% blue. Photographs were taken at 40X.

Lettuce Leaf

CHAPTER 6

CONCLUSIONS

Growing plants in controlled environments, such as for an Advanced Life Support system, provides an opportunity to manipulate environmental conditions without complicating plant stress factors. Ultimately, all environmental factors could be manipulated to optimize system efficiency. We know that high carbon dioxide (excluding super-elevated carbon dioxide), high relative humidity, and direct nutrient delivery (hydroponics) provide a luxury environment for plant water potential and growth. Soybean canopy height responded minimally to temperature changes under these luxury conditions. A lack of elongation sensitivity means that temperature can be manipulated to optimize other productivity parameters such as yield and harvest index without drastically altering plant height.

The manipulation of light quality under these same luxury conditions causes greater differences in plant height and affects productivity depending on species. Although red light is the least energetically expensive to make, exclusively or narrowrange red light sources greatly enhance stem elongation and, in some cases, compromise productivity. In our experiments, soybeans grown under HPS lamps had more elongated stems, but higher total biomass than under MH lamps. Because phytochrome photoequilibrium was nearly identical for the two light sources, the differences in leaf expansion and stem elongation were hypothesized to be caused by blue light. Specifically examining blue light effects in further experiments, we found the effect of blue light was

very much species dependent. Wheat, which has an erectophile habit where expanding leaves and stems are shaded from light, showed little to no sensitivity to blue light. The small decrease in total dry mass with increasing blue light fraction could better be accounted for by differences in yield photon flux rather than blue light. To this end, wheat can be grown without blue light provided no other life cycle processes are affected by the lack of blue light. Soybean, which has a planophile habit where expanding leaves and stems are exposed to light, was much more sensitive to blue light. Differences in soybean dry mass could not be entirely explained by yield photon flux differences . Our data also indicated that soybean stem elongation responded to blue light fraction rather than absolute blue light. Soybean can be grown without blue light, but where canopy height is a concern, as in Advanced Life Support, some blue light may need to be added to control the height. The general division of species blue light sensitivity by plant growth habit, erectophile versus planophile, requires further investigation using other cultivars and species.

The leafy crop, lettuce, was even more sensitive to blue light fraction and required blue light to develop properly. The fact that lettuce, grown under equivalent phytochrome photoequilibrium and blue light fraction but variable "green" wavelengths, had differing yields suggests lettuce is very sensitive to spectral quality . This raises further questions of which wavelengths also affect lettuce growth and whether other leafy crops have a similar response.

The blue light effect at the cellular level was dependent on both species and plant part. A decrease in soybean stem length was effected by altered cell division while a

decrease in soybean leaf area was effected primarily by altered cell expansion . The fact that blue light affects cell division or expansion, depending on location, suggests sites of perception and/or signal transduction are different for stems and leaves. However, in lettuce leaves, both cell division and expansion were altered by blue light. In some species, the signal perception may not be separate.

Lastly, because we harvested our plants early in the life cycle, our results are only an indication of what might happen to yield. Our early soybean experiments indicated that there may be a change in total biomass and carbon partitioning such that edible yield is not affected. The long-term effects of stem and leaf morphological changes need to be investigated further.

APPENDICES

APPENDIX A. SUPPLEMENTARY EXPERIMENT TO: EFFECT OF LAMP TYPE AND TEMPERATURE ON DEVELOPMENT, CARBON PARTITIONING, AND YIELD OF SOYBEAN

Abstract

Parameters sensitive to lamp type in Chapter 3 retained their sensitivity at higher temperatures. Increasing the temperature to 32/28°C was significantly detrimental to total biomass but did not affect seed yield and photosynthetic efficiency. Lowering root zone temperature did not aid in reducing dark respiration and increasing carbon partitioning to the shoot.

Materials and Methods

Soybeans were grown as described in Chapter 1 except only three day/night temperature regimes were used: 32/28, 29/25, and 26/22°C. Root temperatures were kept constant at the average daily temperature of the shoot: 30, 27, and 24°C, respectively. Two additional shoot chambers were treated at 29/25 and 26/22°C but root zone temperatures were kept constant at 23 and 20°C, respectively. These comprised the five temperature treatments for each lamp type.

Results

Effect of lamp type

The results of this experiment confirm the results of Chapter 3. Even at higher temperatures, a significant difference in canopy height, stem and branch length, and total biomass occur between HPS and MH (Table A. I). The trend of reduced stem mass in MH plants associated with an increase in HI was also apparent, but not significant (Table

A.2) . There was a greater and significant change in partitioning to the leaves. Although a trend for decreased seed yield under MH was apparent, once again this trend was not significant (Table A.1). No single yield parameter could account for the trend. No significant changes between treatments were noted in photosynthetic efficiency . Canopy P_{net} measurements were consistent with yield differences between lamp types (Figure $A.1a$).

Effect of temperature

Extreme high temperature of 32/28°C was detrimental to total biomass production but had a small non-significant effect on seed yield and photosynthetic efficiency (Table A.3). We confirmed that cooler temperatures reduced the seed fill period. The higher temperatures also increased P_{net} early in the life cycle (Fig. A. 1b).

At these temperatures there was no difference in carbon partitioning (Table A.4), but the trend for decreased percent root mass with warm temperatures was apparent again. The day/night temperature scheme did not affect canopy height.

We hypothesized that a cooler root zone would have less dark respiration thus making more carbon available for biomass and seed production. However, there was no significant change in biomass or seed production at either shoot temperature regime (29/25 , 26/22°C) (Table A.3) and carbon partitioning was not significantly affected (Table $A.4$).
lamp type	canopy height (cm)	main stem length (cm)	longest branch length (cm)	seed yield $(g\bullet m^{-2}\bullet d^{-1})$	photo- synthetic efficiency [†] $(g\bullet mol^{-1})$	total biomass $(g\bullet m^{-2}\bullet d^{-1})$	pods per m ²	seed s per pod	mass per seed (mg)
HPS	48.6	42.2	51.2	5.04	0.232	14.8	1402	1.82	158
MH	42.2	22.0	29.2	4.97	0.236	13.0	1410	1.90	152
p-value	0.04	0.02	< 0.01	0.84	0.84	< 0.01	0.93	017	0.34

Table A.1. Supplementary data: Three plant length measures, seed yield, and yield components of soybeans grown under two lamp types. Each parameter is an average of the five chambers with different temperature regimes.

^tgrams of seed per mol of PPF

Table A.2. Supplementary data: Carbon partitioning of soybeans under two lamp types. Measures are a percent of total dry mass. Sum of the five components equals 100% .

lamp type	seed (harvest index)	stem	leaves	pod	root
HPS	34.1	17.6	25.9	13.0	9.4
MH	38.2	15.4	22.5	14.2	9.8
p-value	0.14	0.12	0.03	0.19	0.70

day/night temperature	seed yield $(g m-2 d-1)$	PE^{\dagger} $(g \text{ mol}^{-1})$	total biomass $(g m^{-2} d^{-1})$	pods per $m2$	seeds per pod	mass per seed (mg)	days to first flower	days to harvest	seed fill (days)
32/28	4.73	0.219	12.9c	1520	1.85	135	19d	81	62
29/25	5.20	0.244	14.4a	1441	1.90	154	19d	81	62
$29/25*$	5.22	0.247	14.3a	1473	1.83	158	21c	81	60
26/22	5.16	0.240	13.6b	1344	1.90	164	23 _b	81	58
$26/22*$	4.70	0.221	14.2a	1253	1.84	164	26a	81	55
p-value	0.73	0.69	< 0.01	0.42	0.72	0.15	< 0.01	1.00	

Table A.3. Supplementary data: A comparison of yield and yield components for soybeans grown under five temperature regimes. Each parameter is an average of the two chambers of differing lamp type.²

 $tPE = photosynthetic efficiency$ *reduced root zone temperature

2 different letters within a column indicate significant differences using a mean separation test of LSD at *a=0.05*

Table A.4. Supplementary data: Carbon partitioning of soybeans under five temperature regimes. Data are a percent of total dry mass. Sum of the five components equals 100%.

day/night temperature	seed (harvest index)	stem	leaves	pod	root
32/28	36.7	17.4	24.2	13.9	7.8
29/25	36.1	18.2	24.6	12.6	8.6
$29/25*$	36.5	15.9	23.7	14.2	9.8
26/22	38.1	14.1	24.6	13.5	9.8
$26/22*$	33.4	17.0	23.8	13.9	11.9
p-value	0.77	0.33	0.96	0.66	0.25

*reduced root zone temperature

Fig. A.1. Supplementary data: Net photosynthesis $(CO₂$ uptake) of soybean canopies. a) Comparison of lamp types. Measurements are an average of the five chambers of different temperatures. b) Comparison of temperatures. Measurements are an average of the two chambers of differing lamp types.

APPENDIX B. A COMPARISON OF SEQUENTIAL HARVEST OF

SOYBEAN UNDER TWO LAMP TYPES

Purpose

The objective of this experiment was to gain an understanding of when morphological differences between plants grown under high pressure sodium and metal halide become apparent.

Materials and Methods

Soybeans were grown at a density of 36 plants $m²$ in ten Plexiglas chambers (see system description in Chapter 3). HPS or MH lamps were placed over five chambers each. Mylar sheets around the chambers were maintained at canopy height to minimize edge effect. Plants were grown in aerated nutrient solution (pH=5.6, EC=70 mS m⁻¹). replenished as necessary to maintain solution level. Shoot (26/22°C) and root-zone (24°C) temperatures were measured with thermocouples and maintained by computercontrolled heaters. A photosynthetic photon flux (PPF) of $550 \pm 15 \ \mu \text{mol m}^{-2} \text{ s}^{-1}$ was maintained at the top of the canopy. This supplied 49 and 170 μ mol m⁻² s⁻¹ of blue light (400-500nm) from HPS and MH lamps, respectively. Photoperiod was 12-h. Carbon dioxide concentration was enriched to $1100 \ \mu$ mol mol⁻¹. Canopy height, percent PPF absorption, and top of the canopy PPF were measured every other day.

Absorption was measured with a line sensor integrating 10 quantum sensors. Light was measured above the canopy (incident), below the canopy (transmitted), upside down above the canopy (reflected), and upside down below the canopy (reflectedtransmitted). Absorption was then calculated as incident minus transmitted plus reflected plus reflected-transmitted all divided by incident.

One chamber from each lamp type was harvested every six days starting 7 days after transplanting. The following were measured at harvest: node, branch, and leaf number; main stem, branch and internode lengths; and leaf area. Plants were separated into leaves, stems, and roots, dried at 80°C for 48 hours, and weighed. Growth rates were calculated as the first derivative of length measurements versus time .

Results

Stem elongation. HPS stems elongate rapidly so the canopy quickly becomes taller than the MH canopy (Figure B.1a). The growth rate under HPS increased quickly to a maximum of 1.5 cm d^{-1} by day 17 (Figure B.1b). MH had a shallower but broader peak at 0.5 cm d⁻¹. The calculated ratio of main stem length: branch length is an indicator of apical dominance . A phytochrome-mediated response, such as elongation due to competition, would have a high apical dominance ratio $(>=1)$ (Ballare et al., 1995). The apical dominance of plants under HPS and MH lamps were not significantly different from one (Figure B.2). This suggests differences in elongation were not phytochrome related . Individual internode lengths were consistently 2-3 times greater under HPS than MH lamps (Figure B.3).

Leaf expansion. More rapid leaf expansion under HPS than MH was apparent by day 13 (Figure B.4a). However, by day 18 HPS leaf growth was only 10% greater than MH (Figure B.4b). Increased leaf area was not caused by an increase in leaf number (Figure B.5). A more rapid leaf expansion allowed for faster canopy closure and increased

light capture (Figure B.6). A 5.4% increase in absorbed light under HPS corresponded to a 5.2% increase in total dry mass as compared to MH (Figure B. 7a). Carbon partitioning to the stems accounted for most of the difference in total dry mass with a disparity of 32.5% between lamp types (Figure B.7b).

Literature Cited

Ballare, C.L., A.L. Scopel, and R.A. Sanchez. 1995. Plant photomorphogenesis in canopies, crop growth, and yield. HortScience 30: 1172-118 I.

Figure B.1 - The change in (a) canopy height and (b) main stem growth rate with time for soybeans grown under HPS and MH lamps. Each point is a chamber.

Figure B.2 - The apical dominance ratio (main stem length:branch length) for soybeans grown under HPS and MH lamps.

Figure B.3 - The change in the ratio of HPS to MH soybean internode length with time. Each point is an average of six plants. Numbers in the upper left of each graph indicate the internode number starting from the internode above the cotyledons.

Figure B.4 - The change in (a) total leaf area and (b) ratio of HPS to MH leaf area with time. Each point is an average of six plants.

Figure B.6 - The change in percent light absorption with time for soybeans grown under HPS and MH lamps. Each point is a chamber.

Figure B.7 - The change in (a) total dry mass and (b) carbon partitioning with time for soybeans grown under HPS and MH lamps. Each point is a plant.

APPENDIX C. A COMPARISON OF FOUR LEAF IMPRESSION

METHODS

Introduction

Leaf impressions have been used to measure cell size and cell number in grasses (Hilu and Randall, 1984; Rahim and Fordham, 1991) and pea leaves (Lecoeur *et al,* 1995). Cellulose acetate has long been used as the casting compound. However, these impressions tend to shrink and cloud making them useless for measuring cell size (Hilu and Randall, 1984; Rahim and Fordham, 1991). Hilu and Randall (1984) used nail polish as the casting compound to prevent shrinkage. A film of clear nail polish is brushed on the leaves, dried for 2 to 4 hours, and then carefully removed. This impression provides an outline of individual cells because it dissolves some of the waxes that may obscure cell outlines. Tests indicate a commercially available casting compound, Formvar (polyvinylformaldehyde), may work better (Schaefer and Harter, 1942). A simpler method, similar to cellulose acetate, using cellulose triacetate film is available but has not been previously referenced. Four methods, nail polish, Formvar, Hartopane (a butyrate film), and cellulose triacetate film, were compared to test for durability and ease of use.

Materials and Methods

Soybeans were grown under high pressure sodium lamps in conditions described in Chapter 4. Eighteen days after transplanting, four leaf impression methods were tested : nail polish (Strong Nail, Cutex, New York, NY), Formvar (Ted Pella Inc., Redding, CA), Hartopane (Hartwig-Hartoglass, Woodstock, IL), and cellulose triacetate. All four methods were tested simultaneously on the middle leaflet of the first trifoliate of 12

soybean plants. The middle region of the leaf was sampled to obtain a distribution of cell size closest to the mean cell size of the entire leaf (Wenzel *et al,* 1997). Nail polish and Formvar (4% Formvar resin in chloroform) were painted on the leaf and allowed to dry. Nail polish took at least 30 seconds to dry and Formvar took only 10-20 seconds to dry. Samples were then peeled off with cellophane tape and mounted on a glass microscope slide. Immediately after the leaf was treated with a drop of acetone, pieces $(1 \times 2 \text{ cm})$ cellulose triacetate and Hartopane were pressed into the leaf for 10 seconds . The pieces were then taped to a glass microscope slide. All four method samples were immediately viewed under a microscope at 40X and photographed. Cells were traced and areas calculated in Arc View (ESRI, Redlands, CA) calibrated with a photograph of a stage micrometer at 40X. The same cells were photographed and measured again after 6 days to determine if sample shrinkage had occurred.

Results

Both cellulose triacetate and Hartopane curled severely in six days, compromising the plane of focus. Samples had to be re-taped in order to be photographed again after 6 days. Nail polish impressions were easy to view provided the polish was spread thinly enough, which was difficult to do. Formvar was the easiest method to use and produced the best impressions, but the active ingredient (polyvinylformaldehyde) is a relatively hazardous chemical. None of the methods tested appeared to cloud within 6 days. All methods shrank significantly in six days, except Formvar (Figure C. I). However, the variance in shrinkage was much less than the variance caused by differences in sample cell

size. Because of the simplicity of its use and minimal shrinkage, Formvar was used for all leaf impressions.

Literature Cited

- Hilu, K. W., and J. L. Randall. 1984. Convenient method for studying grass leaf epidermis. Taxon 33:413-415.
- Lecoeur, J., et al. 1995. Expansion of pea leaves subjected to short water deficit: cell number and cell size are sensitive to stress at different periods of leaf development. J. of Exp. Bot. 46:1093-1101.
- Rahim, M. A. and R. Fordham. 1991. Effect of shade on leaf and cell size and number of epidermal cells in garlic *(Allium sativum)*. Annals of Bot. 67:167-171.
- Schaefer, V.J. and D. Harker. 1942. Surface replicas for use in the electron microscope . J. of Applied Physics 13:427-433 .
- Wenzel, C.L., P.M. Chandler, R.B. Cunningham, and J. B. Passioura. 1997. Comparative leaf epidermal anatomy of mutants of barley *(Hordeum vulgare* L. 'Himalaya') which differ in leaf length. Annals of Bot. 79:47-52.

Figure C.1 - Shrinkage over time of four leaf impression methods. Each bar is an average of 12 leaves. Asterisks indicate significant differences at *a=0.05.*

APPENDIX D. ANOVA TABLES

Table D.1 - ANOVA tables for parameters cited in Chapter 3.

Table D.2 - General linear model tables for lettuce parameters in Chapter 4.

Analysis of Variance Procedure Dependent Variable: Chlorophyll

Source DF Sum of Source Source DF Sum of Squares Mean Square
Model 23 2029 7675 88.2507 Model 23 2029.7675 88.2507
Error 120 779.2100 6.4934 120 779.2100 6.4934
143 2808 9775 Corrected Total 143 2808.9775 R-Square C.V. Root MSE
0.7226 7.3656 2.5482 0.7226 7.3656 2.5482 Source DF Anova SS Mean Square

REP 1 39,9002 39,9002 REP 1 39.9002 39.9003 PPF 1 594.5469 594.5469 LAMP 1 1.6044 1.6044 PPF*LAMP 1 0.0100 0.0100
BLF(LAMP) 4 311.5372 77.9942 BLF(LAMP) 4 311.5372 77.8843
PPF*BLF(LAMP) 4 27.8706 6.9676 PPF*BLF(LAMP) 4 27.8706 6.9676
REP*PPF 1 957.9025 957.9025 $\begin{array}{cccc} 1 & 957.9025 & 957.9025 \\ 2 & 10.2444 & 5.1222 \end{array}$ REP*PPF*LAMP 2 10.2444 5.1222 REP*PPF*BLF(LAMP) 8 86.1511 10.7689 Tests of Hypotheses using the Anova MS for REP*PPF as an error term
Source DF Anova SS Mean Square Source DF Anova SS Mean Square
PPF 1 504,5460 504,5460 PPF 1 594.5469 594.5469 F Value 13.59 Mean 34.5958 F Value 6.14 91.56 0.25 0.00 11.99 1.07 147.52 0.79 1.66 F Value 0.62 Tests of Hypotheses using the Anova MS for REP*PPF*LAMP as an error term Source DF Anova SS Mean Square F Value
LAMP 1 16044 16044 031 LAMP 1.6044 1.6044 0.31 Tests of Hypotheses using the Anova MS for REP*PPF*LAMP as an error term Source DF Anova SS Mean Square F Value
PPF*LAMP 1 0.0100 0.0100 0.000 PPF*LAMP 1 0.0100 0.0100 0.00 Tests of Hypotheses using the Anova MS for REP*PPF*BLF(LAMP) as an error term Source DF Anova SS Mean Square F Value BLF(LAMP) 4 311.5372 77.8843 7.23 Tests of Hypotheses using the Anova MS for REP*PPF*BLF(LAMP) as an error term Source DF Anova SS Mean Square F Value PPF*BLF(LAMP) 4 27.8706 6.9676 0.65 Pr 0.0001 Pr > F 0.0146 0.0001 0.6200 0.9688 0.0001 0.3730 0.0001 0.4567 0.1156 Pr 0.5752 Pr 0.6320 Pr 0.9688 Pr 0.0091 $Pr > F$ 0.6446

Table D.3 - ANOVA tables for soybean parameters in Chapter 4

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Table D.4 - General linear model tables for wheat parameters in Chapter 4.

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General Linear Models Procedure Dependent Variable: Leaf Area

General Linear Models Procedure

Dependent Variable: Leaf Dry Mass

Tests of Hypotheses using the Type III MS for REP*PPF as an error term Source DF Type III SS Mean Square F Value Pr > F PPF 1 2.0400 2.0400 110.57 0.0604 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source **DF** Type III SS Mean Square F Value Pr > F LAMP 1 0.0557 0.0557 11.09 0.0795 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*LAMP 1 0.0254 0.0254 5.07 0.1532 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP)as an error term Source DF Type III SS Mean Square F Value Pr > F BLF(LAMP) 4 0.0219 0.0055 0.45 0.7684 Tests of Hypotheses using the Type Ill MS for REP*PPF*BLF(LAMP) as an error term Source **DF** Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 0.0049 0.0012 0.10 0.9792

General Linear Models Procedure Dependent Variable: Stem Dry Mass

General Linear Models Procedure

Dependent Variable: Root Dry Mass

Tests of Hypotheses using the Type III MS for REP*PPF as an error term Source DF Type III SS Mean Square F Value Pr > F
PPF 1 1.0550 1.0550 69.84 0.0758 1 1.0550 1.0550 69.84 0.0758 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F LAMP 1 0.0295 0.0295 22.74 0.0413 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F
PPF*LAMP 1 0.0116 0.0116 8.92 0.0962 1 0.0116 0.0116 8.92 0.0962 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term
Source DF Type III SS Mean Square F Value Pr > F Source DF Type III SS Mean Square F Value Pr > F
BLF(LAMP) 4 0.0029 0.0007 0.07 0.9885 0.0029 0.0007 0.07 0.9885 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 0.0059 0.0015 0.15 0.9585

General Linear Models Procedure

Dependent Variable: Total Dry Mass

Tests of Hypotheses using the Type III MS for REP*PPF as an error term Source DF Type III SS Mean Square F Value Pr > F
PPF 1 9 8813 9 8813 109 06 0 0608 1 9.8813 9.8813 109.06 0.0608 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term
Source DF Type III SS Mean Square F Value P Source DF Type III SS Mean Square F Value Pr > F
LAMP 1 0 3149 0 3149 18 49 0 0501 1 0.3149 0.3149 18.49 0.0501 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F
PPF*LAMP 1 0 1439 0 1439 8 45 0 1008 PPF*LAMP 0.1439 0.1439 8.45 0.1008 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP)as an error term Source DF Type III SS Mean Square F Value Pr > F BLF(LAMP) 4 0.0721 0.0180 0.28 0.8851 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 0.0216 0.0054 0.08 0.9854

General Linear Models Procedure Dependent Variable: Percent Leaf

Tests of Hypotheses using the Type III MS for REP*PPF as an error term Source DF Type III SS Mean Square F Value Pr > F
PPF 1 88.7210 88.7210 0.80 0.5363 PPF 1 88.7210 88.7210 0.80 0.5363 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F LAMP 1 27.3764 27.3764 18.60 0.0498 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*LAMP 1.3411 1.3411 0.91 0.4405 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP)as an error term Source DF Type III SS Mean Square F Value Pr > F BLF(LAMP) 4 74.2083 18.5521 1.26 0.3617 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source **DF** Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 39.2121 9.8030 0.66 0.6343

General Linear Models Procedure

Dependent Variable: Percent Stem

Tests of Hypotheses using the Type III MS for REP*PPF as an error term
Source DF Type III SS Mean Square F V Source DF Type III SS Mean Square F Value Pr > F
PPF 1 40.6299 40.6299 10.34 0.1919 40.6299 40.6299 10.34 0.1919 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term
Source DF Type III SS Mean Square F Value P Source DF Type III SS Mean Square F Value Pr > F
LAMP 1 32 6308 32 6308 13 26 0 0678 1 32.6308 32.6308 13.26 0.0678 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term
Source DF Type III SS Mean Square F Value P Source DF Type III SS Mean Square F Value Pr > F
PPF*LAMP 1 10 2001 10 2001 4 15 0 1787 PPF*LAMP 1 10.2001 10.2001 4.15 0.1787 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP)as an error term Source DF Type III SS Mean Square F Value Pr > F BLF(LAMP) 4 37.9782 9.4946 1.66 0.2503 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 22.1161 5.5290 0.97 0.4750

General Linear Models Procedure

Tests of Hypotheses using the Type III MS for REP*PPF as an error term Source DF Type III SS Mean Square F Value Pr > F
PPF 1 9.2722 9.2722 0.13 0.7829 1 9.2722 9.2722 0.13 0.7829 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F LAMP 1 0.2305 0.2305 1.83 0.3092 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*LAMP 1 18.9382 18.9382 149.99 0.0066 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP)as an error term Source DF Type III SS Mean Square F Value Pr > F BLF(LAMP) 4 120.0547 30.0137 0.94 0.4879 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 71.6636 17.9159 0.56 0.6977

General Linear Models Procedure
Dependent Variable:

Specific Leaf Area

General Linear Models Procedure Dependent Variable: Stem Length

Tests of Hypotheses using the Type III MS for REP*PPF as an error term
Source DF Type III SS Mean Square F.N. Source DF Type III SS Mean Square F Value $Pr > F$
PPF 1874 0148 1874 0148 152 0.4225 PPF 1 1874.0148 1874.0148 1.52 0.4335 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value Pr > F
LAMP 1 840 8165 840 8165 13.12 0.0685 LAIVIP 1 840.8165 840.8165 13.12 0.0685 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source DF Type III SS Mean Square F Value $Pr > F$
PPF*LAMP 1 34.0148 34.0148 0.52 0.5431 PPF*LAMP 1 34.0148 34.0148 0.53 0.5421 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP)as an error term Source DF Type III SS Mean Square F Value $Pr > F$
BLF(LAMP) 4 498 8828 124 7207 1.81 0.2306 BLF(LAMP) 4 498.8828 124.7207 1.81 0.2206 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source DF Type III SS Mean Square F Value $Pr > F$
PPF*BLF(LAMP) 4 214 2684 53.5671 0.78 0.5704 PPF*BLF(LAMP) 4 214.2684 53.5671 0.78 0.5704

General Linear Models Procedure Dependent Variable: Tiller Number

Tests of Hypotheses using the Type III MS for REP*PPF as an error term
Source DF Type III SS Mean Square F V Source **DF** Type III SS Mean Square F Value Pr > F
PPF 1 619.9029 619.9029 321.66 0.0355 1 619.9029 619.9029 321.66 0.0355 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term Source **DF** Type III SS Mean Square F Value Pr > F
LAMP 1 36.8503 36.8503 526.42 0.0019 1 36.8503 36.8503 526.42 0.0019 Tests of Hypotheses using the Type III MS for REP*PPF*LAMP as an error term
Source DF Type III SS Mean Square F Value P Source DF Type III SS Mean Square F Value Pr > F
PPF*LAMP 1 13 1229 13 1229 187 46 0 0053 1 13.1229 13.1229 187.46 0.0053 Tests of Hypotheses using the Type III MS for $REP^*PPF^*BLF(LAMP)$ as an error term
Source DF Type III SS Mean Square F Value $Pr > F$ DF Type III SS Mean Square F Value $Pr > F$ BLF(LAMP) 4 11.8803 2.9701 0.63 0.6565 Tests of Hypotheses using the Type III MS for REP*PPF*BLF(LAMP) as an error term Source DF Type III SS Mean Square F Value Pr > F PPF*BLF(LAMP) 4 8.0538 2.0134 0.43 0.7868

General Linear Models Procedure
Dependent Variable:

General Linear Models Procedure Dependent Variable: Stem Relative

General Linear Models Procedure Dependent Variable: Root Relative

Table D.5 - General linear model tables for lettuce leaf parameters in Chapter 5.

Analysis of Variance Procedure					
Dependent Variable:		Cell Area			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	0.1625687	0.0090316	0.95	0.5842
Error	5	0.0477351	0.0095470		
Corrected Total	23	0.2103038			
		R-SquareC.V.	Root MSE	Mean	
		0.77301815.63488	0.0977	0.6249	
Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	5	0.0333438	0.0066688	0.70	0.6483
BLUE	1	0.0581938	0.0581938	6.10	0.0566
BLUE*REP	5	0.0329357	0.0065871	0.69	0.6531
LOCALE	1	0.0102424	0.0102424	1.07	0.3478
LOCALE*REP	5	0.0165082	0.0033016	0.35	0.8656
BLUE*LOCALE	1	0.0113448	0.0113448	1.19	0.3254
		Tests of Hypotheses using the Anova MS for BLUE*REP as an error term			
Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLUE	1	0.0581938	0.0581938	8.83	0.0311
		Tests of Hypotheses using the Anova MS for BLUE*REP as an error term			
Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	5	0.0333438	0.0066688	1.01	0.4948
		Tests of Hypotheses using the Anova MS for LOCALE*REP as an error term			
Source	DF	Anova SS	Mean Square	F Value	Pr > F
LOCALE	1	0.0102424	0.0102424	3.10	0.1385
Analysis of Variance Procedure					
Dependent Variable:		Leaf Area			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	806.33000	44.79611	0.99	0.5610
Error	5	226.79000	45.35800		
Corrected Total	23	1033.12000			
		R-SquareC.V.	Root MSE	Mean	
		0.78048021.79558	6.7348	30.900	
Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	5	111.44000	22.28800	0.49	0.7729
BLUE	1	396.90667	396.90667	8.75	0.0316
BLUE*REP	5	207.13333	41.42667	0.91	0.5384
LOCALE	$\mathbf{1}$	6.82667	6.82667	0.15	0.7140
LOCALE*REP	5	75.38333	15.07667	0.33	0.8740
BLUE*LOCALE	1	8.64000	8.64000	0.19	0.6807
		Tests of Hypotheses using the Anova MS for BLUE*REP as an error term			
Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLUE	1	396.90667	396.90667	9.58	0.0270
		Tests of Hypotheses using the Anova MS for BLUE*REP as an error term			
Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	5	111.44000	22.28800	0.54	0.7436
		Tests of Hypotheses using the Anova MS for LOCALE*REP as an error term			
Source LOCALE	DF 1	Anova SS 6.8266667	Mean Square 6.8266667	F Value 0.45	Pr > F 0.5309

Table D.6 - ANOVA tables for soybean leaf parameters in Chapter 5.

Analysis of Variance Procedure						
Dependent Variable:			Cell Area			
Source	DF		Sum of Squares Mean Square		F Value	Pr > F
Model	18		0.0375165	0.0020843	1.01	0.5513
Error	5		0.0103633	0.0020727		
Corrected Total	23		0.0478798			
		R-Square	C.V.	Root MSE	Mean	
		0.783555	14.51817	0.0455	0.3136	
Source					F Value	
	DF		Anova SS	Mean Square		Pr > F
REP	5		0.0133808	0.0026762	1.29	0.3930
BLUE	ı		0.0024402	0.0024402	1.18	0.3274
BLUE*REP	5		0.0072888	0.0014578	0.70	0.6456
LOCALE	1		0.0001127	0.0001127	0.05	0.8249
LOCALE*REP	5		0.0118133	0.0023627	1.14	0.4446
BLUE*LOCALE	1		0.0024807	0.0024807	1.20	0.3238
Tests of Hypotheses using the Anova MS for BLUE*REP as an error term						
Source	DF		Anova SS		F Value	Pr > F
				Mean Square		
BLUE	1		0.0024402	0.0024402	1.67	0.2523
Tests of Hypotheses using the Anova MS for BLUE*REP as an error term						
Source	DF		Anova SS	Mean Square	F Value	Pr > F
REP	5		0.0133808	0.0026762	1.84	0.2605
Tests of Hypotheses using the Anova MS for LOCALE*REP as an error term						
Source	DF		Anova SS	Mean Square	F Value	Pr > F
LOCALE	1		0.0001127	0.0001127	0.05	0.8358
Analysis of Variance Procedure						
Dependent Variable:			Number of Cells			
Source	DF		Sum of Squares Mean Square		F Value	Pr > F
Model	18		26179466	1454415	4.91	0.0432
Error	5		1480135	296027		
Corrected Total	23		27659601			
		R-Square	C.V.	Root MSE	Mean	
		0.946487	39.56133	544.08	1375.3	
Source	DF		Anova SS	Mean Square	F Value	Pr > F
REP	5		1736887	347377	1.17	0.4325
BLUE	1		18474885	18474885	62.41	0.0005
BLUE*REP	5		2049327	409865	1.38	0.3649
LOCALE	l		1249897	1249897	4.22	0.0951
LOCALE*REP	5		1540502	308100	1.04	0.4830
BLUE*LOCALE	1		1127967	1127967	3.81	0.1084
Tests of Hypotheses using the Anova MS for BLUE*REP as an error term						
Source	DF		Anova SS	Mean Square	F Value	Pr > F
BLUE	ı		18474885	18474885	45.08	0.0011
Tests of Hypotheses using the Anova MS for BLUE*REP as an error term						
Source	DF		Anova SS	Mean Square	F Value	Pr > F
REP	5		1736887.2	347377.4	0.85	0.5698
Tests of Hypotheses using the Anova MS for LOCALE*REP as an error term						
Source	DF		Anova SS	Mean Square	F Value	Pr > F

Table D.7 -ANOVA tables of soybean stem parameters in Chapter 5.

169

Analysis of Variance Procedure Dependent Variable: Main Stem Length

Source DF Sum of Squares Source DF Sum of Squares Mean Square F Value Pr > F
Model 3 560 5000 186 8333 10.83 0.0217 Model 3 560.5000 186.8333 10.83 0.0217 Error 4 69.0000 17.2500 Corrected Total 7 629.5000 R-Square C.V. Root MSE Mean 0.890389 13.7300 4.1533 30.2500 Source DF Anova SS Mean Square F Value Pr > F LIGHT 1 512.0000 512.0000 29.68 0.0055 TEMP 1 40.5000 40.5000 2.35 0.2002 LIGHT*TEMP 1 8.0000 8.0000 0.46 0.5333 Analysis of Variance Procedure Dependent Variable: Longest Branch Length

Source DF Sum of Souares Mean S Source DF Sum of Squares Mean Square F Value Pr > F
Model 3 773 0000 257 6667 14 12 0 0136 Model 3 773.0000 257.6667 14.12 0.0136 Error 4 73.0000 18.2500 Corrected Total 7 846.0000 R-Square C.V. Root MSE Mean 0.913712 10.9539 4.2720 39.0000 Source DF Anova SS Mean Square F Value Pr > F LIGHT 1 760.5000 760.5000 41.67 0.0030 TEMP 1 12.5000 12.5000 0.68 0.4544 LIGHT*TEMP 1 0.0000 0.0000 0.000 1.0000 Analysis of Variance Procedure Dependent Variable:

Percent Leaf

Sun of Squares Sum of Squares Mean Square F Value $Pr > F$ Model 3 33.5830 11.1943 7.13 0.0441 Error 4 6.2837 1.5709 Corrected Total 7 39.8667 R-Square C.V. Root MSE Mean 0.842383 5.1880 1.2534 24.1588 Source DF Anova SS Mean Square F Value $Pr > F$ LIGHT 1 28.9941 28.9941 18.46 0.0127 TEMP 1 0.0136 0.0136 0.01 0.9303 LIGHT*TEMP 1 4.5753 4.5753 2.91 0.1631

Table D.8 - ANOVA tables for parameters in Appendix A.

Table D.9 - ANOVA table for Appendix C.

TIME 1 0.0046802 0.0046802 43.86 0.0001

Mean Square F Value $Pr > F$

176

APPENDIX E. COPYRIGHT AND PERMISSION INFORMATION

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To Permissions Editor

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CURRICULUM VITAE

180

- Sp 1993 Plant Propagation Laboratory Instructor, Purdue University, Organized lab equipment, prepared and gave lectures, wrote and graded quizzes.
- F 90, Sp 91 **Remedial Algebra Instructor,** SIU-C, Wrote and graded quizzes and tests, organized and gave lectures, and provided individual tutoring.

Academic Achievements

Professional Memberships

Activities

Publications

- **T.A.O. Dougher** and B.G. Bugbee (1998) Is blue light good or bad for plants? *Life Support and Biosphere Science* 5(2): 129-136.
- J. Cavazzoni, T. Volk, B. Bugbee, and **T. Dougher** *(submitted)* An appraisal of phasic temperature and photoperiod control for soybean using a modified Cropgro model. *Life Support and Biosphere Science.*
- **T.A.O. Dougher** and B.G. Bugbee (1997) Effect of lamp type and temperature on development, carbon partitioning and yield in soybean . *Advances in Space Research* 20(10) :1895-1899 .
- S.S. Nielsen, **T.A. Ohler,** and C.A. Mitchell (1997) Cowpea leaves for human consumption: production, utilization, and nutrient composition. *in* Advances in Cowpea Research . B.B. Singh, D .R. Mohan Raj, K.E. Dashiell, and L.E.N . Jackai eds. International Institute of Tropical Agriculture, Ibadan, Nigeria and Japan International Research Center for Agriculture Sciences, Tsukuba, Ibaraki, Japan. 326-332.
- C.A. Mitchell, M. Belury, **T.A. Ohler, S.S.** Nielsen, and R.A. Wheeler (1996) Costs of providing edible biomass for a balanced vegetarian diet in a controlled ecological life-support system. *in* Plants in Space Biology. H. Suge ed. Tohoku University Press, Sendai, Japan. 245-254.
- **T.A. Ohler** and C.A. Mitchell (1996) Identification of yield-optimizing environments for two cowpea breeding lines by manipulating photoperiod and harvest scenario. *Journal of the American Society for Horticultural Science* 121(3) :576-581.
- **T.A. Ohler,** S.S . Nielsen, and C.A. Mitchell (1996) Manipulation of plant density and harvest time to optimize vegetative yield and proximate composition of cowpea leaves . *HortScience* 31(2):193-197.
- **T.A. Ohler** and C.A. Mitchell (1995) Effect of carbon dioxide level and plant density on cowpea canopy photosynthesis and yield in a controlled environment. *Life Support and Biosphere Science* 2:3-9.

Presentations

- T.A.O. Dougher and B.G . Bugbee (1998) Toward an Understand of Blue Light Effects on Diverse Species. Annual meeting of the American Society of Agronomy (oral presentation).
- **T.A.O.** Dougher and B.G. Bugbee (1998) Is blue light good or bad for plants? Third International Conference on Life Support and Biosphere Science (oral presentation).
- T.A.O. Dougher and B.G. Bugbee (1997) Effect of Blue Light on Leaf Expansion and Stem Elongation. Annual meeting of the American Society of Agronomy (oral presentation).
- T.A.O. Dougher and B.G. Bugbee (1997) Blue light inhibits internode elongation, growth, and yield in soybeans. Annual meeting of COSPAR (poster presentation).
- **T.A.O.** Dougher and B.G . Bugbee (1995) Blue light dose response of soybean growth and development. Annual meeting of the American Society of Agronomy (poster presentation).
- T.A. Ohler and C.A. Mitchell (1994) Effect of CO₂ level on cowpea canopy photosynthesis and growth . Annual meeting of the American Society of Horticultural Sciences (poster presentation).
- T.A. Ohler and C.A. Mitchell (1993) Manipulation of harvest parameters to optimize yield rate and harvest index of cowpea for use in a bioregenerative life-support system. Annual meeting of the American Society of Gravitational and Space Biology (poster presentation).
- T.A. Ohler and C.A. Mitchell (1993) Evaluation of harvest scenarios for cowpea *(Vigna unguiculata* L. Walp), a candidate species for controlled ecological life-support systems. Annual meeting of the American Society of Horticultural Sciences (poster presentation) .
- T.A. Ohler and C.A. Mitchell (1992) Evaluation of cowpea *(Vigna unguiculata* L. Walp) as a candidate species for inclusion in bioregenerative life-support systems (oral presentation).