Annals of Botany **79**: 273–282, 1997

Anatomical Features of Pepper Plants (*Capsicum annuum* L.) Grown under Red Light-emitting Diodes Supplemented with Blue or Far-red Light

ANDREW C. SCHUERGER*, CHRISTOPHER S. BROWN† and ELIZABETH C. STRYJEWSKI†

** Science and Technology Office, The Land, Epcot, Walt Disney World Co., P.O. Box 10,000, Lake Buena Vista, FL 32830 and † Dynamac Corp., 1910 Sedgwick Rd., Bldg. 100, Durham, NC 27713, USA*

Received: 30 October 1995 Accepted: 11 September 1996

Pepper plants (*Capsicum annuum* L. cv., Hungarian Wax) were grown under metal halide (MH) lamps or light-emitting diode (LED) arrays with different spectra to determine the effects of light quality on plant anatomy of leaves and stems. One LED (660) array supplied 99% red light at 660 nm (25 nm band-width at half-peak height) and 1% far-red light between 700–800 nm. A second LED (660/735) array supplied 83% red light at 660 nm and 17% far-red light at 735 nm (25 nm band-width at half-peak height). A third LED (660/blue) array supplied 98% red light at 660 nm, 1% blue light between 350–550 nm, and 1% far-red light between 700–800 nm. Control plants were grown under broad-spectrum metal halide lamps. Plants were grown at a mean photon flux (300–800 nm) of 330 μmol m^{-2} s^{-1} under a 12 h day-night photoperiod. Significant anatomical changes in stem and leaf morphologies were observed in plants grown under the LED arrays compared to plants grown under the broad-spectrum MH lamp. Cross-sectional areas of pepper stems, thickness of secondary xylem, numbers of intraxylary phloem bundles in the periphery of stem pith tissues, leaf thickness, numbers of chloroplasts per palisade mesophyll cell, and thickness of palisade and spongy mesophyll tissues were greatest in peppers grown under MH lamps, intermediate in plants grown under the 660/blue LED array, and lowest in peppers grown under the 660 or 660/735 LED arrays. Most anatomical features of pepper stems and leaves were similar among plants grown under 660 or 660/735 LED arrays. The effects of spectral quality on anatomical changes in stem and leaf tissues of peppers generally were correlated to the amount of blue light present in the primary light source.

Key words: Controlled ecological life support system, CELSS, bioregenerative life support system, *Capsicum annuum*, leaf anatomy, stem anatomy.

INTRODUCTION

Light-emitting diodes (LEDs) have been proposed as a primary light source for space-based plant research chambers or bioregenerative life support systems (Bula *et al*., 1991; Barta *et al*., 1992). Light-emitting diodes typically have narrow-bandwidth wavelength emissions, small mass and volume, solid-state construction, and long-life, potentially making them an ideal light source for small intensive plant culture systems. Furthermore, the wavelength specificity of LEDs may be used to study plant physiological or plant disease resistance qualities of crops grown in closed plant production systems (Schuerger and Brown, 1994; Brown, Schuerger, and Sager, 1995).

Spectral quality can have dramatic effects on plant growth and development (Hanson, 1917; Crookstone *et al*., 1975; Cui, Vogelmann and Smith, 1991; Barreiro *et al*., 1992; Sims and Pearcy, 1992). High ratios of red to far-red illumination can stimulate phytochrome responses in plants including stem elongation, flowering, and changes in stomatal conductance or plant anatomy (Boardman, 1977; Smith, 1982). Red light is important for the development of the photosynthetic apparatus of plants and may increase starch accumulation in several plant species by inhibiting the translocation of photosynthates out of leaves (Saebo, Krekling and Appelgren, 1995). In contrast, blue light is important in the formation of chlorophyll (Senger, 1982; Pushnik *et al*., 1987), chloroplast development (Akoyunoglou and Anni, 1984), stomatal opening (Zeiger, 1984), enzyme synthesis (Senger, 1982), activation of the circadian rhythm of photosynthesis (Senger, 1982), and photomorphogenesis (Cosgrove, 1981; Senger, 1982; Wheeler, Mackowiak and Sager, 1991). Physiological responses to spectral changes can vary among different plant species (Deutch and Rasmussen, 1974; Boardman, 1977; Senger, 1982), but dicotyledonous plants appear to be more sensitive than moncotyledonous plants to spectral changes (Deutch and Rasmussen, 1974).

Spectral quality also affects anatomical structure of plant leaves (Hanson, 1917; Boardman, 1977). Leaf thinning under shaded conditions appears to be a general phenomenon in many dicotyledonous and monocotyledonous plants (Hanson, 1917; Boardman, 1977; Louwerse and Zweede, 1977). Leaf thinning under shaded conditions has been attributed to an increase in the ratio of red to far-red (R:FR) light (Kasperbauer and Peaslee, 1973; Boardman, 1977; Barreiro *et al*., 1992) but also may result from a decrease of blue light (Pushnik *et al*., 1987) or a decrease in the total photosynthetic photon flux (PPF) (Chabot and Chabot, 1977; Smith, 1982; Sims and Pearcy, 1992).

* For correspondence.

0305-7364/97/030273+10 $25.00/0 bo960341

Furthermore, leaf thinning under shaded conditions occurs as a direct result of a decrease in the thickness of mesophyll parenchyma tissue (Crookston *et al*., 1975; Cui *et al*., 1991; Barreiro *et al*., 1992; Sims and Pearcy, 1992) caused generally by reductions in cell size and cell number in palisade tissue (Hanson, 1917; Sims and Pearcy, 1992). Spectral quality appears to impart the greatest effect on leaf thickness during leaf expansion (Louwerse and Zweerde, 1977; Sims and Pearcy, 1992). No papers were found in the literature that described anatomical changes in plant stem tissues related to changes in spectral quality of the primary light source.

It is clear from the literature that plants exhibit a high degree of physiological, morphological, and anatomical plasticity to changes in spectral quality. The primary objective of this study was to compare anatomical features of leaves and stems of peppers grown under LEDs with different spectral regimes to evaluate the use of LEDs for plant growth in future space-based plant research chambers or bioregenerative life support systems. Spectral qualities of all light sources used in the current study (spectra between 300–1100 nm, weighted photosynthetic values (300–800 nm), yield photon fluxes (YPF), phytochrome photostationary states (ϕ), lamp irradiances (Wm^{-2}) for emissions greater than 700 nm, and red to far-red ratios) and the effects of the spectral regimes used in the current study on growth and photomorphogenesis of peppers have been described (Brown *et al*., 1995).

MATERIALS AND METHODS

Light-emitting diode arrays were composed of one or more of the following: red LEDs with peak emissions of 660 nm (25 nm band-width at half-peak height) (model 3009A001, Quantum Devices, Inc. Barnveld, WI, USA), far-red LEDs with peak emissions at 735 nm (25 nm band-width at half-peak height) (model 3009A002, Quantum Devices Inc.), and blue fluorescent lamps (model BF6165–12, JKL Components Corp., Paccoima, CA, USA) with a broad level of light emission between 350 and 550 nm. One 400-W metal halide (MH) lamp (model, ETAC-400-MH-CH, Energy Technics, York, PA, USA) was used as a control. Photon flux densities (300–800 nm) were measured with a spectroradiometer (model, LI-1800, Li-Cor, Inc., Lincoln, NE, USA), and photosynthetic photon flux (PPF) densities (400–700 nm) were determined from the broader spectra (300–800 nm). Yield photon fluxes (YPF) (273–289 μmol m^{-2} s^{-1}), YPF:PPF ratios (0·88–0·93), and phytochrome photostationary states (0·84–0·88) were similar among all light treatments used in the current study (Brown *et al*., 1995).

One LED (660) array supplied 99% red light at 660 nm and 1% far-red light between 700–800 nm. A second LED (660/735) array supplied 83% red light at 660 nm and 17% far-red light between 700–800 nm with a peak emission at 735 nm. A third LED (660/blue) array supplied 98% red light at 660 nm, 1% blue light between 350–550 nm, and 1% far-red light between 700–800 nm. Each LED array contained 1344 red LED units in a 0·42 m^2 ventilated enclosure. The 660/735 array was supplemented with 384 far-red LEDs, and the 660/blue array was supplemented with eight, 14-cm-long fluorescent lamps. The design and performance qualities of individual LEDs have been described (Bula *et al*., 1991; Barta *et al*., 1992). The MH lamp had 20% of the PPF at 400 to 500 nm, 56% of the PPF at 500 to 600 nm, and 24% of the PPF at 600 to 700 nm (Brown *et al*., 1995). To reduce thermal infrared irradiation, the MH lamp was housed in a stainless steel luminaire suspended over a 3-cm-deep, deionized water barrier supported by a 5-mm-thick tempered-glass plate. The water temperature in the barrier was maintained at 25 °C by recirculation through a Lauda RMS-20 water chiller (Brinkman Instruments, Westbury, NY, USA). Leaf temperatures among all light treatments were within $\pm$0·5 °C of each other throughout the experiments (data not shown). Plants were grown at a mean photon flux (300–800 nm) of 330 μmol m^{-2} s^{-1} measured at tops of plant canopies, and under a 12 h day-night photoperiod. Each light source was separated from other light sources by constructing opaque plastic barriers around the metal support structures used to suspend light sources over plant growing areas.

Pepper (*Capsicum annuum* L., cv. Hungarian Wax) seedlings were germinated in 2·5 cm^2 rockwool cubes (Grodania A/S, Hedehusene, Denmark) and grown for 21 d under MH lamps at 250 μmol m^{-2} s^{-1} of PPF. Three pepper seedlings were transplanted into four separate 4 l plastic tanks each containing hydroponic nutrient solution (Schuerger and Mitchell, 1992). One tank was placed under a MH lamp, and the three other tanks were placed under separate LED arrays. Nutrient solution in each 4 l plastic tank was aerated continuously, and the hydrogen ion concentration was adjusted daily to pH 5·5 with 0·02 M HNO_3 or 0·02 M KOH. The electrical conductivity of fresh nutrient solution was 1700 μS cm^{-1}. Fresh nutrient solution was added daily to each container to replenish evapotranspirative loss. Metal halide lamps and LED arrays were assembled in a 2×6 m research laboratory equipped with an air-temperature control system. Ambient-air and root temperatures were maintained at 24 °C ($\pm$1·2 °C) and 22 °C ($\pm$1·2 °C), respectively. Ambient relative humidities within the research laboratory fluctuated between 45–65% (mean 55%) RH. A completely randomized experimental design was used in which plants were randomly assigned to each light treatment, and LED arrays were randomly assigned positions in the laboratory. The experiment was conducted three times.

After 21 d of growth under the MH lamp or LED arrays, peppers were destructively sampled; 1-cm-long stem sections from the centres of first and third internodes (measured from the base of the plant), plus 4 mm^2 leaf sections from fully expanded leaves (from fourth or fifth nodes) that included small lateral veins, were excised and fixed for 14 d in a formaldehyde-based fixative containing 50 ml 95% ethanol, 5 ml glacial acetic acid, 10 ml 37% formaldehyde, and 35 ml deionized water. Plant tissues were dehydrated in a graded ethanol series, embedded in paraffin, sectioned, mounted on glass slides, and treated with a safranin and fast-green stain procedure (Clark, 1981). Stained sections of stem and leaf tissues were analysed with an Olympus BH-2

microscope equipped with a Dage-MTI video camera (Dage-MTI, Michigan City, IN, USA). Images were digitized by a Scion Corporation LG-3 frame-grabber board (Scion Corporation, Frederick, MD, USA) and recorded on a Macintosh Quadra 950 PC with the public-domain National Institute of Health (NIH) Image Program (written by Wayne Rasband at the National Institute of Health and available from NTIS, 5285 Port Royal Road, Springfield, VA, USA). Images were concomitantly viewed on a monitor and analysed for morphometric features using the NIH Image Program. Cross-sections of pepper leaves were measured for widths of whole-leaf, palisade mesophyll, spongy mesophyll, and adaxial epidermal tissues. Numbers of chloroplasts per palisade cell were estimated with differential interference contrast microscopy (Leitz Aristoplan, Micro Optics of Florida, Fort Lauderdale, FL, USA). Stem cross-sections were measured for stem area, pith area, stem:pith ratio, width of cortex, width of secondary xylem, number of vessels per mm² of secondary xylem, mean area of vessels, and number of intraxylary phloem bundles embedded within pith tissue.

Plant tissues from peppers grown under the MH lamp or LED arrays were photographed and printed in identical manners so that photographs grouped within individual figures are directly comparable. Statistical analyses were conducted with a PC-based Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA). Data were analysed with analysis of variance (PROC GLM) and protected Fisher's least-squares mean separation tests ($P \leqslant 0{\cdot}05$; $n = 9$).

RESULTS

Pepper leaf thickness was greatest in plants grown under the MH lamp and lowest in plants grown under 660 or 660/735 nm LED arrays (Table 1). When blue light was combined with red LEDs, leaf thickness was significantly lower than MH-grown plants, but significantly higher than 660 nm-grown or 660/735 nm-grown plants. Leaf thickness differences were the result of a thinning of both the palisade and spongy mesophyll layers in LED-grown plants (Table 1, Fig. 1). Furthermore, the spongy mesophyll in LED-grown plants tended to become disorganized in plants grown under 660 or 660/735 light (Fig. 1). The adaxial epidermal layers of MH- and 660/blue-grown plants were similar, and both were significantly greater than adaxial epidermal layers in peppers grown under LED arrays lacking blue light. Druse crystals of calcium oxalate (Fig. 1) were observed in pepper leaves grown under MH or LED light sources. Numbers of chloroplasts per palisade parenchyma cell were greatest in plants grown under the MH lamp, intermediate in plants grown under the 660/blue light, and lowest in plants grown under the 660 or 660/735 light (Table 1).

Cross-sectional areas of first-internode and third-internode pepper stem sections were greatest in plants grown under the MH lamp compared to plants grown under the LED arrays (Tables 2 and 3). The stellar patterns in both first and third internodes were similar among pepper plants grown under all light treatments. Longitudinal stem ribs composed of angular collenchyma tissues were superficial in first-internode stem sections (Fig. 2), but were prominent in third-internode stem sections (Fig. 3). Differences in cross-sectional areas of first- or third-internode pepper stems were attributed primarily to differences in the thickness of secondary xylem tissues (Tables 2 and 3, Figs 2 and 3).

TABLE 1. *Effects of spectral quality on leaf anatomy in peppers*

Anatomical features†	Lamp*			
	MH	660/BF	660	660/735
Leaf thickness (μm)	221·1[a]‡	168·1[b]	135·4[c]	127·1[c]
Pallisade parenchyma (μm)	71·1[a]	50·7[b]	36·9[c]	30·0[d]
Spongy parenchyma (μm)	118·8[a]	87·4[b]	67·6[c]	69·5[c]
Adaxial epidermis (μm)	18·9[a]	18·3[a]	16·6[b]	15·7[b]
No. of chloroplasts per pallisade cell	44·3[a]	28·7[b]	18·5[c]	19·7[c]

*MH, metal halide lamp; BF, blue fluorescent lamps; 660, red LEDs; 735, far red LEDs.

† Each experimental unit in the data set ($n = 9$) represents the mean of several randomly selected tissues or structures (termed subsamples) measured along a single 1-cm-long section of paraffin-embedded leaf tissue. The numbers of subsamples for each structure were as follows: total leaf thickness (6 subsamples), pallisade parenchyma (6), spongy mesophyll parenchyma (6), adaxial epidermis (6–8), and numbers of chloroplasts per pallisade cell (6). Nine different leaves per treatment were measured.

‡ Treatments in rows followed by different superscript letters were significantly different based on ANOVA and protected Fisher's least-squares mean separation tests ($P \leqslant 0{\cdot}05$).

In first-internode stem cross-sections, no differences were observed in the thickness of the cortical layer nor in the cross-sectional area of pith tissues (Table 2). However, significant differences in vascular tissues among MH- and LED-grown plants were observed. Generally, the thickness of secondary xylem, mean area of vessels, and stem:pith ratios were greatest in peppers grown under MH lamps, intermediate in plants grown under 660/blue light, and lowest in plants grown under LED arrays lacking blue light (Table 2, Fig. 2). The numbers of intraxylary phloem bundles embedded in pith tissues were significantly greater in the MH control plants compared to the LED-grown plants, while the numbers of vessels per mm² of secondary xylem were similar in plants grown under MH, 660/blue, or 660 lights (Table 2). Only peppers grown under 660/735 light exhibited lower numbers of vessels per mm² of secondary xylem in the first internode as compared to peppers grown under the MH lamp.

In third-internode stem cross-sections, stem area, pith area, stem:pith ratios, thickness of cortical tissues, and thickness of secondary xylem tissues were greatest in MH-grown plants and similar among plants grown under 660/blue, 660, or 660/735 LED arrays (Table 3, Fig. 3). Furthermore, numbers of vessels per mm² of xylem, mean areas of vessels, and numbers of phloem bundles embedded in pith tissues were greatest in MH-grown plants, intermediate in 660/blue-grown plants, and lowest in 660- or 660/735 nm-grown plants.

When first- and third-internode stem cross-sections were viewed with polarized light, optical anisotropic qualities of

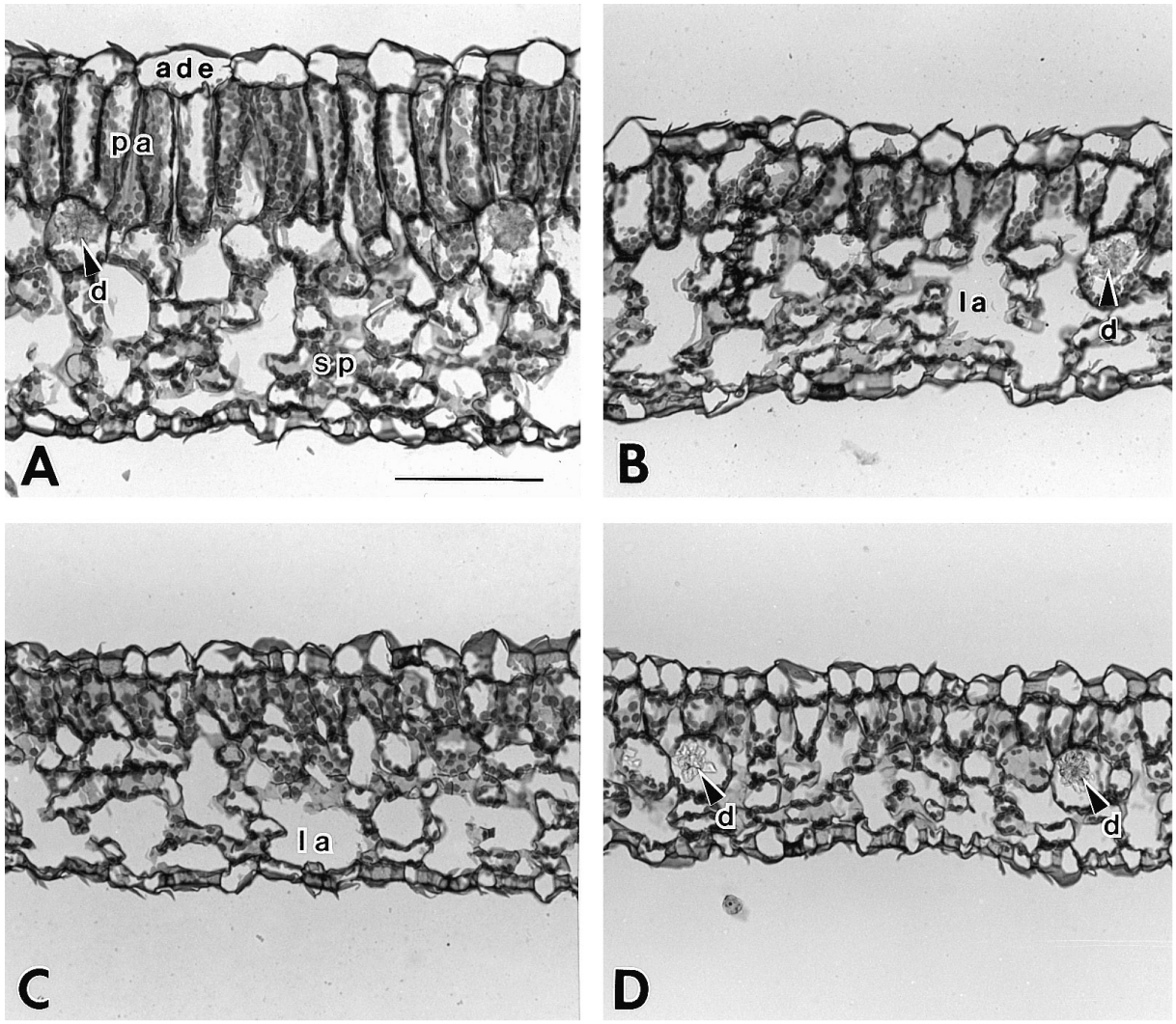

FIG. 1. Cross-sections of pepper (*Capsicum annuum* cv. Hungarian Wax) leaves grown under a metal halide (MH) lamp (A) or under 660/blue (B), 660 (C), or 660/735 (D) light-emitting diode (LED) arrays. Palisade (pa) and spongy (sp) mesophyll tissues were thickest under the MH lamp, intermediate under the 660/blue LED array, and thinnest under the 660 or 660/735 LED arrays. Druse crystals (d) of calcium oxalate were observed in all treatments at relatively similar frequencies. The adaxial epidermal layers (ade) were thicker in plants grown under the MH or 660/blue light as compared to epidermal layers in plants grown under the 660 or 660/735 LED arrays. Lacunae (la) in spongy mesophyll tissues increased in size and frequency in pepper leaves grown under LED arrays as compared to pepper leaves grown under the MH lamp. Bar = 100 μm.

calcium oxalate crystals and lignified vascular tissues were used to determine the distribution of crystals and lignified tissues in pepper stems. Based on visual observations, the occurrence of lignified tissue always coincided with secondary xylem tissue (Fig. 4). Thus, the amount of lignified tissue was greatest in plants grown under MH lamps or 660/blue light and lowest in plants grown under 660 or 660/735 light. Triangular or globoid crystals of calcium oxalate were abundant in the first- and third-internode cross-sections of pepper stems grown under MH or 660/blue light but were nearly absent in cross-sections of stems grown under 660 or 660/735 light (Fig. 4). No differences in the abundance or distribution of druse crystals of calcium oxalate were observed in cross-sections of pepper leaves grown under the MH lamp or LED arrays.

DISCUSSION

Most anatomical features measured in pepper leaves and stems were greatest in plants grown under the broad-spectrum MH lamp, intermediate in plants grown under the 660/blue LED array, and lowest in plants grown under the 660 or 660/735 LED arrays. Differences in measured responses were generally similar among plants grown under 660 or 660/735 LED arrays. Similar trends were observed for photosynthetic rates; leaf chlorophyll levels; numbers of leaves per plant; and leaf, stem, and root dry weight accumulation in pepper plants (cv. Hungarian Wax) grown under identical light sources (Brown and Schuerger, 1992; Brown *et al.*, 1995). Mesophyll tissues in pepper leaves and secondary xylem tissues in stems exhibited the greatest

TABLE 2. *Effects of spectral quality on anatomy of pepper stems within the first internode*

Anatomical features†	Lamp*			
	MH	660/BF	660	660/735
Stem area (mm^2)	$26{\cdot}8^{a}$‡	$23{\cdot}6^{b}$	$20{\cdot}1^{c}$	$22{\cdot}4^{bc}$
Pith area (mm^2)	$5{\cdot}7^{a}$	$6{\cdot}1^{a}$	$6{\cdot}5^{a}$	$6{\cdot}5^{a}$
Stem:pith area ratio	$5{\cdot}1^{a}$	$3{\cdot}9^{b}$	$3{\cdot}1^{c}$	$3{\cdot}5^{bc}$
Cortical layer (μm)	$346{\cdot}7^{a}$	$314{\cdot}4^{a}$	$318{\cdot}9^{a}$	$340{\cdot}0^{a}$
Secondary xylem (μm)	$1165{\cdot}5^{a}$	$957{\cdot}8^{b}$	$715{\cdot}6^{c}$	$811{\cdot}1^{c}$
No. of vessels mm^{-2}	$31{\cdot}2^{a}$	$27{\cdot}5^{ab}$	$31{\cdot}1^{a}$	$24{\cdot}3^{b}$
Mean area of vessels (μm^2)	5014^{a}	4531^{b}	3644^{c}	3161^{d}
No. of phloem bundles in pith tissues	$46{\cdot}1^{a}$	$39{\cdot}4^{b}$	$39{\cdot}7^{b}$	$41{\cdot}6^{b}$

*MH, metal halide lamp; BF, blue fluorescent lamps; 660, red LEDs; 735, far-red LEDs.

†Each experimental unit in the data set ($n = 9$) represents the mean of several randomly selected tissues or structures (termed subsamples) measured in a single cross-section of paraffin-embedded stem tissue. The numbers of subsamples for each datum point were as follows: stem area (1 subsample), pith area (1), cortical layer (8–9), secondary xylem (8–9), number of vessels per measured unit area and mathematically adjusted to equal the number of vessels per square millimeter (3 areas measured), mean area of vessels (20–30 vessels measured), and number of phloem bundles within pith tissues per stem cross section (actual number counted). Nine different stem cross-sections per treatment were measured.

‡Treatments in rows followed by different superscript letters were significantly different based on ANOVA and protected Fisher's least-squares mean separation tests ($P \leqslant 0{\cdot}05$).

TABLE 3. *Effects of spectral quality on anatomy of pepper stems within the third internode*

Anatomical features†	Lamp*			
	MH	660/BF	660	660/735
Stem area (mm^2)	$29{\cdot}6^{a}$‡	$20{\cdot}7^{b}$	$20{\cdot}9^{b}$	$21{\cdot}5^{b}$
Pith area (mm^2)	$12{\cdot}8^{a}$	$10{\cdot}1^{b}$	$10{\cdot}0^{b}$	$10{\cdot}3^{b}$
Stem:pith area ratio	$2{\cdot}3^{a}$	$2{\cdot}0^{b}$	$2{\cdot}1^{b}$	$2{\cdot}1^{b}$
Cortical layer (μm)	$510{\cdot}0^{a}$	$391{\cdot}1^{b}$	$406{\cdot}7^{b}$	$410{\cdot}0^{b}$
Secondary xylem (μm)	$865{\cdot}1^{a}$	$676{\cdot}7^{b}$	$637{\cdot}8^{b}$	$607{\cdot}8^{b}$
No. of vessels mm^{-2}	$68{\cdot}9^{a}$	$53{\cdot}1^{b}$	$44{\cdot}8^{c}$	$31{\cdot}7^{d}$
Mean area of vessels (μm^2)	3914^{a}	2956^{b}	2679^{bc}	2492^{c}
No. of phloem bundles in pith tissues	$67{\cdot}8^{a}$	$57{\cdot}7^{b}$	$57{\cdot}0^{bc}$	$50{\cdot}0^{c}$

*MH, metal halide lamp; BF, blue fluorescent lamps; 660, red LEDs; 735, far-red LEDs.

†Each experimental unit in the data set ($n = 9$) represents the mean of several randomly selected tissues or structures (termed subsamples) measured in a single cross-section of paraffin-embedded stem tissue. The numbers of subsamples for each datum point were as follows: stem area (1 subsample), pith area (1), cortical layer (8–9), secondary xylem (8–9), number of vessels per measured unit area and mathematically adjusted to equal the number of vessels per square millimeter (3 areas measured), mean area of vessels (20–30 vessels measured), and number of phloem bundles per stem cross section (actual number counted). Nine different stem cross-sections per treatment were measured.

‡Treatments in rows followed by different superscript letters were significantly different based on ANOVA and protected Fisher's least-squares mean separation tests ($P \leqslant 0{\cdot}05$).

sensitivity to spectral changes and were apparently most responsive to reductions in blue light. These results have been confirmed in subsequent experiments in which leaf and stem anatomies of pepper (cv. Hungarian Wax) were examined from plants grown continuously under red LED light supplemented with 0, 1, 5, or 10 % blue light; increased levels of blue photons were correlated with increased thickness of secondary xylem and increased thickness leaf mesophyll tissues (Schuerger, unpubl. res.). Furthermore, similar results were described for birch plantlets grown under blue, red, incandescent, or cool white fluorescent light (Saebo *et al.*, 1995); plants grown under blue light showed significantly larger epidermal and mesophyll areas in leaf cross-sections as compared to red or fluorescent light.

Previous workers have shown that leaf thickness, particularly the palisade mesophyll tissues of several plant species, decreased when plants were grown under either low broad-spectrum light levels (Hanson, 1917; Crookstone *et al.*, 1975; Chabot and Chabot, 1977; Barreiro *et al.*, 1992; Sims and Pearcy, 1992), high red to far-red ratios (R:FR) (Kasperbauer and Peaslee, 1973; Barreiro *et al.*, 1992), or low levels of blue light (Pushnik *et al.*, 1987; Saebo *et al.*, 1995). Although complete spectra (300–800 nm) of the experimental light sources often were not characterized, it seems possible that the thinning of leaves reported in some of these studies (Kasperbauer and Peaslee, 1973; Crookstone *et al.*, 1975; Cui *et al.*, 1991; Sims and Pearcy, 1992) may have been due to decreased absolute levels of blue photons and not due exclusively to changes in total PPF or R:FR. If leaf thinning in plants is caused solely by an increase in the R:FR, as suggested by previous studies (Crookston *et al.*, 1975; Barreiro *et al.*, 1992), we should have observed a significant thinning of total leaf thickness in the 660/735 nm-grown pepper plants compared to the 660 nm-grown pepper plants. However, most of the leaf anatomical features (including total leaf thickness) measured in the current study were similar among 660- or 660/735 nm-grown plants; although a slight decrease in the thickness of the palisade mesophyll layer of pepper in 660/735 nm-grown plants was observed. Results from our study suggest that both the absence of blue photons or an increase in the R:FR can reduce the thickness of mesophyll tissues in peppers, but overall, the greatest response was observed when blue light was added to a background of pure red light. These results are consistent with other anatomical studies on the effects of blue light on leaf thinning (Pushnik *et al.*, 1987; Saebo *et al.*, 1995).

Barreiro *et al.* (1992) reported that the effects of PPF and R:FR on leaf area and thickness of bean leaves were independent and additive, thus, a light source with low PPF and high R:FR (a situation encountered within shaded canopies) tended to decrease leaf thickness more than light treatments in which either PPF or R:FR were altered independently. The effects of blue light on plant morphometrics may also be independent of total PPF of the primary light source. Wheeler *et al.* (1991) reported that an increase in blue photons (from 23 to 37 $\mu mol\ m^{-2}\ s^{-1}$) to light emitted from high pressure sodium lamps decreased main stem lengths in soybeans; an apparent maximum threshold of blue light was reached at 30 $\mu mol\ m^{-2}\ s^{-1}$, above which

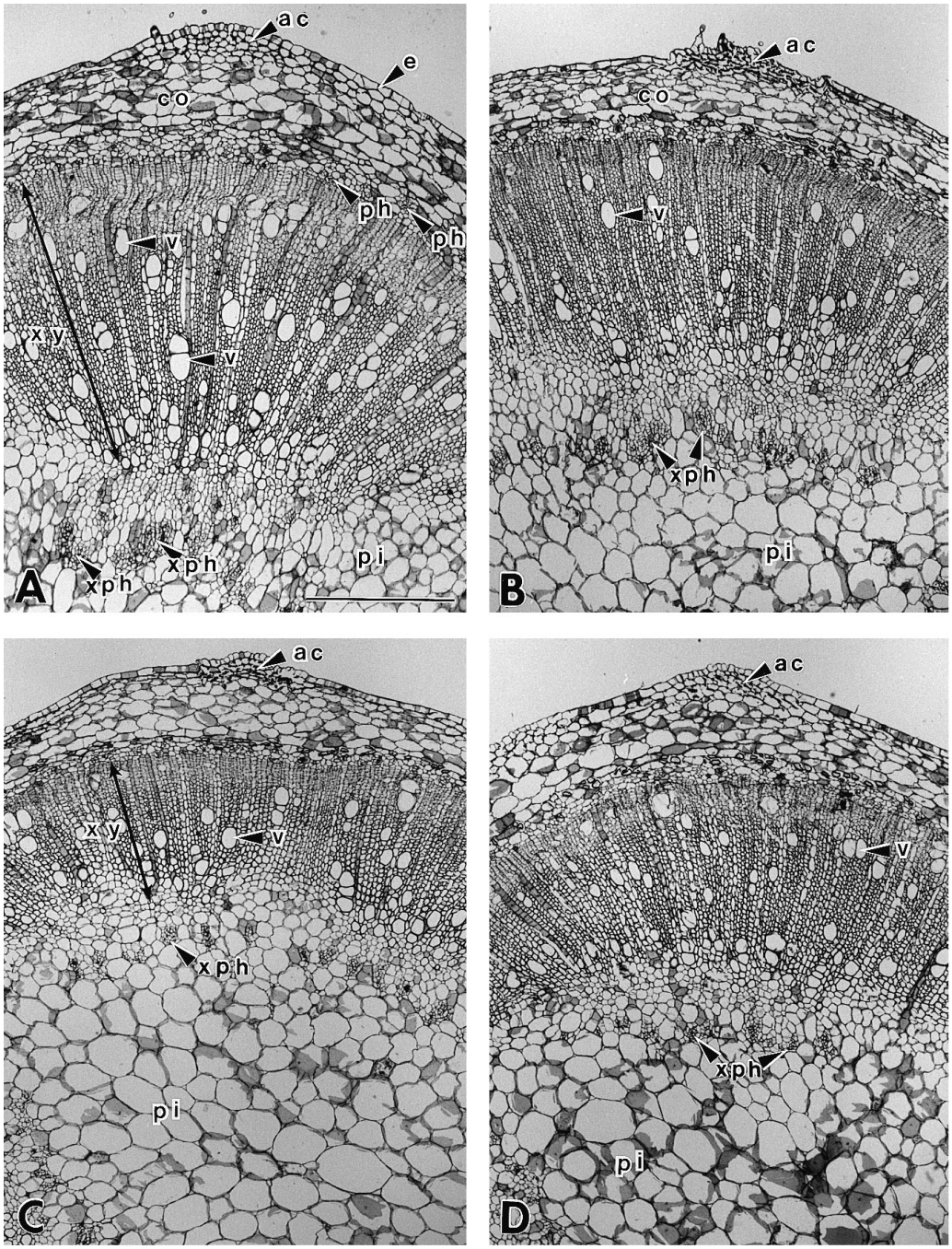

FIG. 2. Stem cross-sections of first internodes of pepper (*Capsicum annuum* cv. Hungarian Wax) plants grown under a metal halide (MH) lamp (A) or under 660/blue (B), 660 (C), or 660/735 (D) light-emitting diode (LED) arrays. Pith (pi) and cortical (co) tissues were similar in plants grown under all light sources. Secondary xylem (xy) was thickest in MH-grown plants, intermediate in the 660/blue-grown plants, and thinnest in the 660- or 660/735 nm-grown plants. Numbers of vessels (v) per unit area were similar among plants grown under the MH, 660/blue, or 660 light sources but were significantly lower in plants grown under the 660/735 LED array. However, the mean area of xylem vessels decreased in plants in the following order: MH > 660/blue > 660 > 660/735 nm. Numbers of intraxylary phloem (xph) bundles present in the periphery of pith tissues were highest in metal halide-grown plants and significantly lower in all LED-grown plants. Phloem bundles (ph) in the inner cortex of plants were not counted. Longitudinal ribs of angular collenchyma (ac) were similar in size and shape among plants grown under all light sources. ↔ indicates the measured dimensions of secondary xylem; e = epidermis. Bar = 300 μm.

no further stem shortening was observed. Blue light effects on soybean stem length appeared to be independent of the total PPF (Wheeler *et al.*, 1991). In addition, hypocotyl length in lettuce grown under red LEDs increased as the amount of blue light was added (from 0 to 60 μmol m^{-2} s^{-1}), thus, lettuce seedlings also responded to a specific number

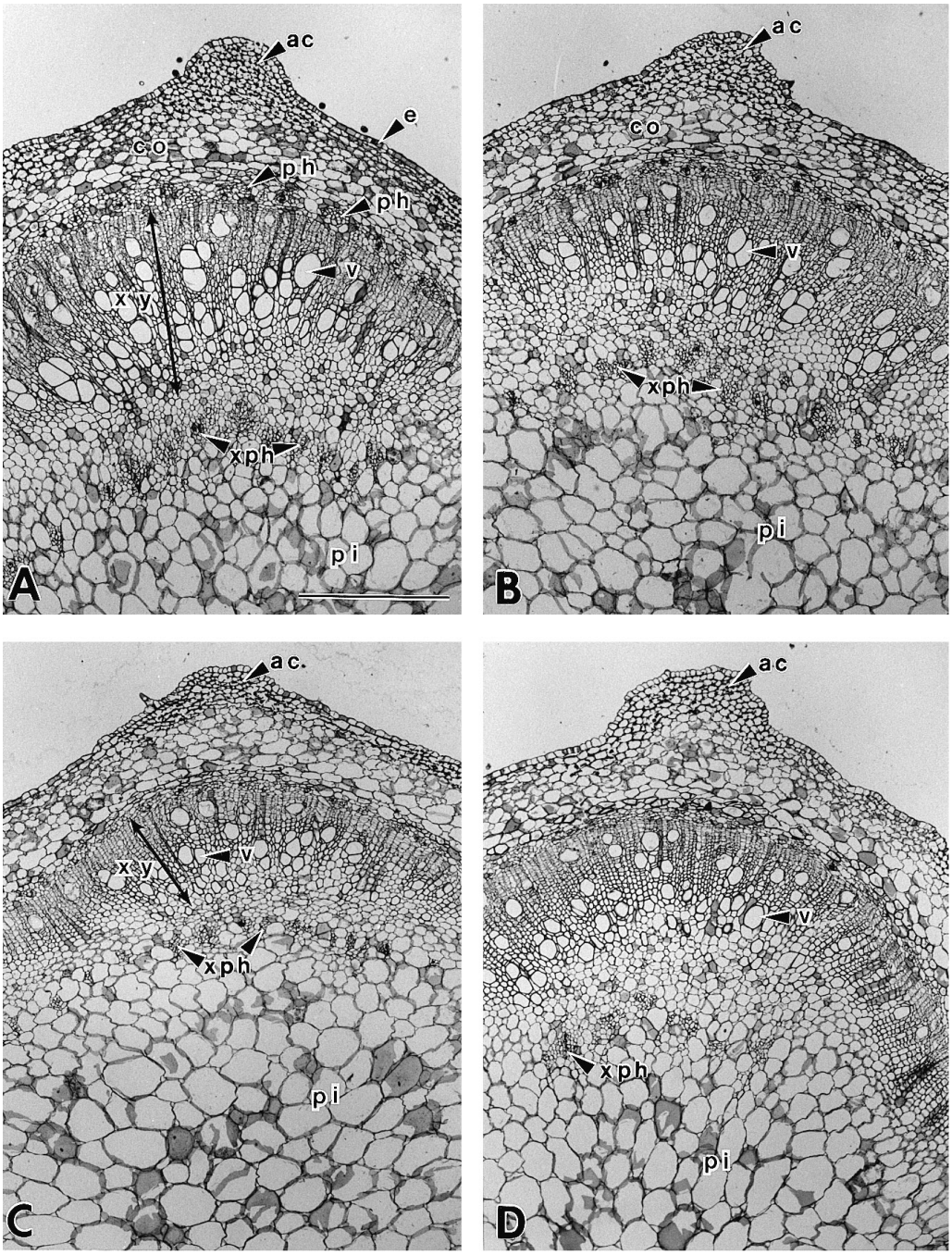

FIG. 3. Stem cross-sections of third internodes of pepper (*Capsicum annuum* cv. Hungarian Wax) plants grown under a metal halide (MH) lamp (A) or under 660/blue (B), 660 (C), or 660/735 (D) light-emitting diode (LED) arrays. Pith (pi), cortical (co), and secondary xylem (xy) tissues were thickest in plants grown under the MH lamp and significantly thinner in plants grown under the LED arrays. The number of vessels (v) per unit area decreased in plants in the following order: MH > 660/blue > 660 > 660/735 nm. Mean areas of xylem vessels decreased in plants in the following order: MH > 660/blue $\geqslant$ 660 = 660/735 nm. Numbers of intraxylary phloem (xph) bundles present in the periphery of pith tissues were highest in metal halide-grown plants, significantly lower in 660/blue- or 660 nm-grown plants, and lowest in 660/735 nm-grown plants. Phloem bundles (ph) in the inner cortex of plants were not counted. Longitudinal ribs of angular collenchyma (ac) were similar in size and shape among plants grown under all light sources. $\leftrightarrow$, indicates the measured dimensions of secondary xylem; e = epidermis. Bar = 300 μm.

of blue photons rather than to a specific PPF (Hoenecke, Bula and Tibbitts, 1992). Considering the results from the current study and from a parallel study (Brown *et al.*, 1995), increased amounts of blue light appeared to concomitantly decrease stem length and increase leaf thickness in peppers. Furthermore, results from the current study indicate that a low level of blue light (4 μmol m^{-2} s^{-1} supplied by the fluorescent lamps in the 660/blue LED array) was sufficient

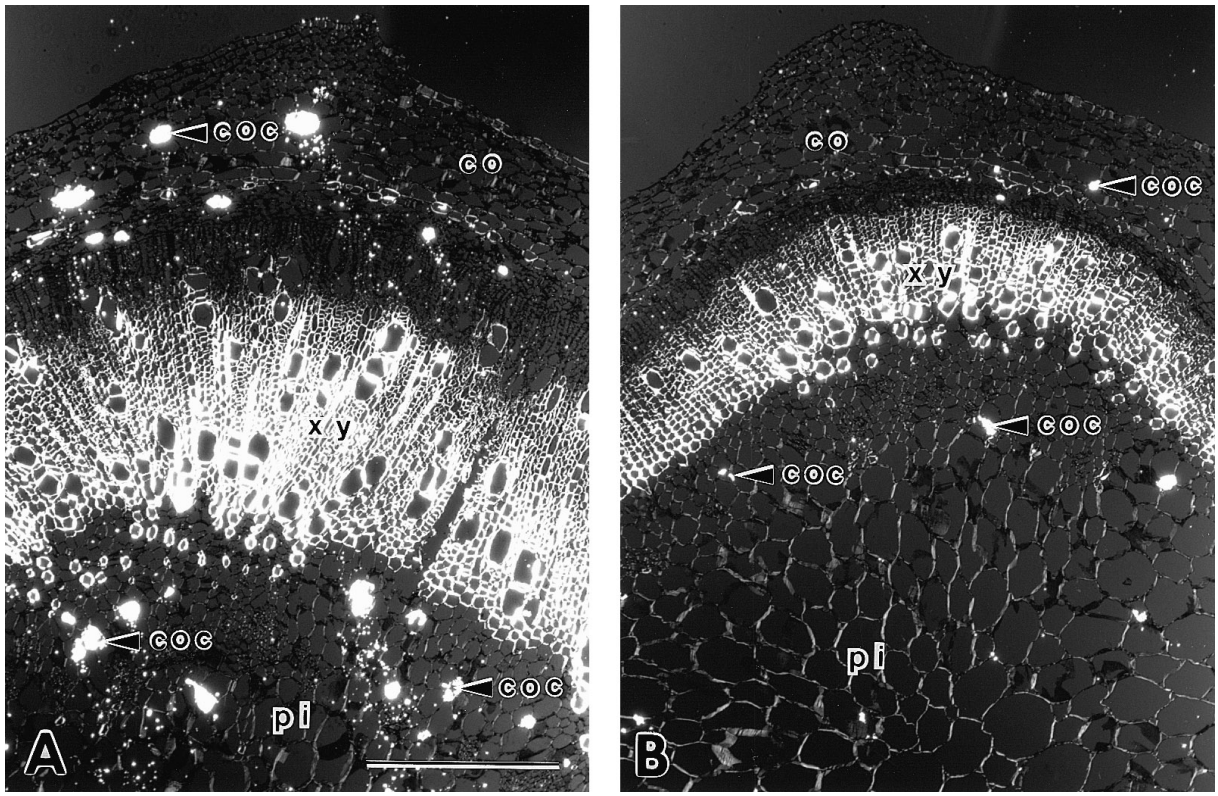

FIG. 4. Stem cross-sections of first internodes of pepper (*Capsicum annuum* cv. Hungarian Wax) plants viewed with polarized light and grown under a metal halide (MH) lamp (A) or under a 660 LED array (B). Optical anisotropic qualities of lignified xylem (xy) tissues and calcium oxalate crystals (coc) indicate that both lignified xylem and calcium oxalate crystals were greater in the MH-grown plants and significantly lower in the 660-grown plants. The numbers and distribution of calcium oxalate crystals were similar among plants grown under the MH or 660/blue lights, and similiar among plants grown under the 660 or 660/735 LED light. Bar = 300 μm.

to mitigate most of the plant growth differences, including leaf thickness, observed in peppers grown under LEDs lacking blue photons compared to plants grown under the MH lamp. Although neither minimum nor maximum thresholds of blue light were established for normal development of pepper, our results do support the conclusion that small amounts of blue light can influence cellular differentiation and maturation of secondary xylem and leaf mesophyll tissues in pepper. Several studies have demonstrated that the addition of small amounts of blue photons to HPS or red light can dramatically alter plant morphometrics (Hoenecke *et al*., 1992; Brown *et al*., 1995; Saebo *et al*., 1995). Furthermore, Holmes and Schafer (1981) reported that as little as 10^{-4} μmol m^{-2} s^{-1} of blue light (at 446 nm) was needed to inhibit hypocotyl elongation in *Sinapis alba* in the absence of other photosynthetic radiation. Our results support the conclusions that (*a*) the effects of blue light should be isolated from total PPF and R:FR when studying the effects of spectral quality on plant anatomy, and (*b*) low levels of blue light can induce dramatic changes in plant anatomy of pepper.

The mechanism responsible for leaf thinning in pepper under a low blue-light fluence rate is not known. Both direct and indirect effects of blue light have been suggested for hypocotyl elongation, regulation and synthesis of enzymes, synthesis of chlorophyll and carotenoids, stomatal opening, maturation of chloroplasts, activation of circadian rhythm of photosynthesis, and photomorphogenesis (Thomas and Dickinson, 1979; Cosgrove, 1981; Schmidt, 1984; Senger, 1982; Zeiger, 1984), but no evidence was found in the literature that directly correlated blue photoreceptors to leaf thinning. A direct effect of phytochrome on leaf thinning has been suggested for high R:FR and low PPF lighting conditions (Boardman, 1977; Smith, 1982), but whether phytochrome was involved with leaf thinning in pepper under low blue-light fluence rates is not known. In contrast, we suspect that the effects of blue light on secondary xylem development are more likely due to indirect effects of reduced photosynthate synthesis or translocation from leaves. This suggestion is based on the observations of lower numbers of chloroplasts per palisade cell (Table 1), lower leaf areas and leaf numbers per plant (Brown *et al*., 1995), and lower photosynthetic rates and chlorophyll levels (Brown and Schuerger, 1992) of peppers grown under blue-deficient LED arrays (660 or 660/735 nm). All of these factors would contribute to a reduction in photosynthesis in leaves grown under blue-deficient light sources. Saebo *et al*. (1995) noted that leaf thickness (reported as tissue area in leaf cross-sections), chlorophyll content, chloroplast development, and photosynthetic rates in birch plantlets were all greatest under blue light and lowest under red light. Saebo *et al*. (1995) concluded that blue light affects

photosynthesis in birch both through effects on the composition of the photosynthetic apparatus and on translocation of carbohydrates from chloroplasts. Although direct effects of blue light on hypocotyl growth have been reported (Thomas and Dickinson, 1979; Cosgrove, 1981), we suspect that the embedded nature of the vascular tissues in older pepper stems (cortical tissues in pepper stems were 300–500 μm thick, Tables 2 and 3) makes it unlikely that a direct blue sensitive photoreceptor in the vascular tissue was responsible for the developmental differences of the secondary xylem observed in peppers grown under the MH or LED light treatments. However, Holmes and Schafer (1981) reported that as little as 10^{-4} μmol m^{-2} s^{-1} of blue light (at 446 nm) was needed to inhibit hypocotyl elongation in *S. alba* in the absence of other photosynthetic radiation. Thus, a direct blue photoreceptor in vascular tissue of pepper cannot be ruled out. The role of blue light on deposition of calcium oxalate crystals in pepper stems also is not known, but may be due to an indirect effect of blue light on photosynthesis. However, no information was found in the literature on the effects of blue light on calcium oxalate biosynthesis or deposition.

Many diverse physiological responses in plants appear to be controlled by blue light (Cosgrove, 1981; Senger, 1982; Schmidt, 1984; Zeiger, 1984; Saebo *et al.*, 1995). Light-emitting diode technologies may be an effective method to precisely manipulate different light spectra because LED lighting systems can be designed such that each spectral range is controlled independently. Recent development of high output blue LEDs (R W Ignatius, Quantum Devices, Inc., Barneveld, WI, USA, pers. com.) will permit tighter control of blue wavelengths in future photobiology studies. Furthermore, light-emitting diode technologies may be useful in space-based plant research chambers or bioregenerative life support systems because of their narrow wavelength emissions, small mass and volume, solid-state construction, and long-life (Bula *et al.*, 1991; Barta *et al.*, 1992). Several plant species have been successfully grown under LEDs (Bula *et al.*, 1991; Hoenecke *et al.*, 1992; Schuerger and Brown, 1994; Brown *et al.*, 1995) proving their utility as an illumination source for plant growth; but based on our study, it appears that at least a mixture of red and blue photons will be necessary for normal plant development in peppers. Other wavelengths of light (ultraviolet, yellow, green, or far-red) also might be needed for normal plant growth in space-based plant research chambers, but little information is available on the interactive effects of multiple narrow-bandwidth spectral regimes on plant growth and development.

ACKNOWLEDGEMENTS

This project was jointly supported by the Walt Disney World, Co., Lake Buena Vista, FL; the National Aeronautical and Space Administration (NASA), Biological Operations and Research Office, Kennedy Space Center, FL; and NASA/Ames Research Center, Space Exploration Projects Office, Moffet Field, CA. Mention of a brand name does not imply endorsement of the product by Walt Disney World, Co. or NASA, nor imply its approval to the exclusion of other products that may be suitable.

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