Winter 2-2016

Sensitivity of Seven Diverse Species to Blue and Green Light: Interactions With Photon Flux

Michael Chase Snowden
chase.snowden@gmail.com

Kevin R. Cope
kevin.cope@usu.edu

Bruce Bugbee
Utah State University, bruce.bugbee@usu.edu

Sensitivity of seven diverse species to blue and green light:

Interactions with photon flux

M. Chase Snowden*, Kevin R. Cope and Bruce Bugbee

Crop Physiology Laboratory, Department of Plants Soils and Climate, Utah State University

4820 Old Main Hill, Logan, UT 84322-4820

*Corresponding author: chase.snowden@gmail.com

Abstract

The effects of spectral quality on growth, carbon-partitioning and whole-plant net assimilation remain poorly understood. Much of the research data is at light levels less than 10 % of summer sunlight so interactions between light quality and quantity are poorly characterized. Several studies have reported that growth is increased under fluorescent lamps compared to mixtures of wavelengths from LEDs. Conclusions regarding the effect of green light fraction range from detrimental to beneficial. Here we report the effects of eight blue and green light fractions at two photosynthetic photon fluxes (PPF; 200 and 500 µmol m^{-2} s^{-1}; daily light integral, 11.5 and 29 mol m^{-2} d^{-1}) on growth, leaf expansion, stem and petiole elongation, and whole-plant net assimilation of seven diverse species. The treatments included cool, neutral, and warm white LEDs, and combinations of blue, green and red LEDs. At the higher PPF (500), increasing blue light in increments from 11 to 28 % reduced growth (dry mass) in tomato, cucumber, and pepper by 22, 26, and 14 % respectively, but there was no statistically significant effect on radish, soybean, lettuce or wheat. At the lower PPF (200), increasing blue light reduced growth only in tomato (41 %). The effects of blue light on growth were mediated by changes in leaf area and radiation capture, with minimal effects on whole-plant net-assimilation. In contrast to the significant effects of blue light, increasing green light in increments from zero to 30 % had a relatively small effect on growth, leaf area or net assimilation at either low or high PPF. Surprisingly, growth (dry mass) of three of the seven species was not reduced by a treatment with 93 % green light compared to the broad spectrum treatments. Collectively, these results are consistent with a shade avoidance response associated with either low blue or high green light fractions.

Abbreviations

PPF, Photosynthetic Photon Flux
LEDs, Light Emitting Diodes
BL, Blue Light
GL, Green Light
RL, Red Light
RB, Red and Blue
RGB, Red, Green and Blue
DM, Dry Mass
LAI, Leaf Area Index
DLI, daily light integral

Introduction

Effects of blue light on growth and development

Radiation provides energy for photosynthesis and information for photomorphogenesis (Smith, 2013). Although RL efficiently drives photosynthesis, without BL it often induces shade avoidance responses including elongated, stems. Several studies indicate that adding a small amount of BL reduces stem length and promotes a more compact plant shape (Dougher and Bugbee, 2001; Kim et al., 2005). Yorio et al. (2001) found that dry mass of spinach, radish and lettuce increased with the addition on 10 % BL but growth in this treatment was still lower than a cool-white fluorescent control (16 % BL). Goins et al. (1997) used identical light treatments to Yorio et al. (2001) and found that growth of wheat in the RL with 10 % BL was comparable to a white light control (33 % BL).

Too much BL can inhibit growth. Recent studies have examined a range of BL fractions and found optimal growth between about 5 and 15 % BL. Cope and Bugbee (2013) and Cope et al. (2014) found that growth (dry mass) and leaf area decreased above 15 % BL for lettuce, radish and pepper. Hernández and Kubota (2015) reported decreased dry mass and leaf area for cucumber when the BL fraction increased above 10 %. Dougher and Bugbee (2004) described histological effects of BL on development of lettuce and soybean and found that increased BL decreased cell expansion and division in the stems and leaves of soybean. In contrast to these studies, Son and Oh (2013) reported the highest fresh and dry mass of lettuce at 0 % BL, but the plants were chlorotic and etiolated. With this exception, these studies confirm earlier studies by Bula et al. (1991) and Hoenecke et al (1992) who found optimal growth of lettuce when red LEDs were supplemented with blue LEDs.

Chen et al. (2014) studied the addition of red and blue LEDs to a fluorescent light source. Supplementing fluorescent with either red or blue light generally increased growth at the same PPF, but the studies were conducted at a PPF of only 135 μmol m^{-2} s^{-1}, which resulted in unusually slow growth in all treatments.

Effects of light on photosynthesis in single leaves

Single leaves in monochromatic light have a 25 to 35% higher quantum efficiency under red light (RL) (600 to 700 nm) than under blue light (BL) (400 to 500 nm); and red light is 5 to 30 % higher than green light (GL) (500 to 600 nm) (Inada, 1976; McCree, 1972). Studies have often extrapolated from these low-light, short-term, single-leaf measurements to predict whole-plant growth in long-term studies. Extrapolating from measurements under monochromatic light to predict responses under multi-wavelength light should be done with caution because the relative quantum efficiency curves of Inada (1976) and McCree (1972) indicate only photosynthetic efficiency and not the combined effects of photosynthesis and development.

Effects of blue light on photosynthetic efficiency

In contrast to the monochromatic light studies by Inada (1976) and McCree (1972), several studies have found that an increasing BL fraction can increase photosynthetic capacity and efficiency. Goins et al. (1997) were one of the first studies to report that BL increased net leaf photosynthesis compared to RL alone in wheat. Hogewoning et al. (2010a) found that only 7

% BL doubled the photosynthetic *capacity* (photosynthetic potential in higher PPF) over RL alone, and that photosynthetic capacity continued to increase with increasing BL up to 50 % BL for cucumber. Terfa et al. (2013) compared LEDs with 20 % BL to high pressure sodium lamps with 5 % BL and found that increased BL increased leaf thickness and photosynthetic capacity as the PPF was increased, but it is important to note that there was no effect of BL whole plant dry mass. Wang et al. (2014) compared BL effects in extremely low PPF (50 µmol m^{-2} s^{-1}) and found similar photosynthetic rates in blue and white light, but the white light resulted in the highest dry mass per plant. In contrast to these studies, Ouzounis et al. (2014) and Ouzounis et al. (2015) showed decreased or no effect on photosynthesis with increasing BL for roses, chrysanthemums and campanulas and lettuce. The effect of BL on photosynthesis and growth may vary with species, daily light integral, stage of development, and fraction of BL.

Effects of green light

Chlorophyll has minimal absorption of green light (GL) and there is a widespread perception that GL is not used efficiently in photosynthesis. Older editions of all plant physiology textbooks show chlorophyll a and b absorption curves and imply that green light is significantly less effective than red and blue light in driving photosynthesis. More recent editions of plant physiology textbooks (e.g. Taiz et al. 2015, 6th edition) now include comprehensive lists of photosynthetic pigments, including the green light absorbing pigment phycoerythrobilin. Sepúlveda-Ugarte et al. (2011) showed that this green light absorbing pigment efficiently channeled excitation energy to chlorophyll a in *Gracilaria chilensis*, a red macroalga. It is clear that GL can be as effective as blue and red light in some species.

Green light penetrates deeper into leaves and canopies. Sun et al. (1998) found that RL and BL drive CO_2 fixation primarily in the upper palisade mesophyll while GL drives CO_2

fixation in the lower palisade. Once the upper part of individual leaves and the upper canopy are saturated by RL and BL, additional GL should be beneficial in increasing whole plant photosynthesis (Nishio, 2000). Indeed, Terashima et al. (2009) found that GL increased single leaf photosynthesis more than RL or BL at high PPF. In contrast to measurements of photosynthesis in single leaves, whole-plant photosynthesis could be increased by GL both in upper leaves and by transmission to lower leaf layers.

Some whole plant studies have found that increasing the fraction of GL can improve plant growth. Kim et al. (2004a) reported that increasing GL from zero to 24 % increased growth. Lin et al. (2013) reported that lettuce grown without GL had reduced DM compared to two types of broad spectrum light sources (red + blue + white LEDs and fluorescent lamps) at the same PPF. Johkan et al. (2012) studied lettuce at three PPFs (100, 200, and 300 µmol m^{-2} s^{-1}) using three wavelengths of green LEDs and cool white fluorescent controls at all three PPFs and reported that the growth response to GL was inconsistent. Their results, however, indicate that growth *decreased* as PPF increased, so other unidentified environmental factors likely limited plant response to PPF. Hernández and Kubota (2015) found that increasing the fraction of had no effect on growth of cucumbers.

Other studies suggest that increasing green light (GL) can reduce growth and alter development (Folta and Maruhnich, 2007), and that its role may be especially important in the low light conditions that typically occur below plant canopies (Wang and Folta, 2013). To date, differences among species and among studies make it difficult to make general conclusions regarding GL effects.

The beneficial effects of fluorescent light: direct vs. diffuse light

Several studies have reported increased growth under fluorescent lamps compared to combinations of LEDs at the same PPF (Chen et al., 2014; Goins et al., 1997; Lin et al. 2013; Wang et al., 2014; Yorio et al., 2001). This increased growth may be the result of several factors. Fluorescent lamps have a higher fraction of diffuse light compared to the direct beam light from LEDs. Measurements and models of canopy photosynthesis have shown that diffuse light penetrates canopies better than direct light and that this increased penetration causes increased photosynthesis and growth (Li and Yang, 2015; Sinclair et al., 1992; Tubiello et al., 1996). Fluorescent lamps also have increased long-wave radiation, which warms leaves about 2°C more than LEDs at the same PPF (Nelson and Bugbee, 2015). Warmer leaves can increase leaf expansion rate and thus increase radiation interception. Fluorescent lamps also have some far-red radiation, which can cause a shade avoidance response and increase leaf and petiole expansion and thus radiation capture. For these reasons, it is inappropriate to interpret the increased growth under fluorescent lamps as a blue or green light effect.

Short-term single-leaf photosynthesis vs. long-term whole-plant assimilation

Most studies have used single-leaf techniques (gas-exchange or chlorophyll fluorescence (Genty et al., 1989)) to determine short-term photosynthetic efficiency. An alternative to short term measurements is to determine whole-plant net assimilation rate using crop growth analysis (Lambers, et al. 2008; Leopold and Kriedemann (1975); Hunt (1982; Hunt et al. 2002). Crop growth analysis separates crop growth rate (CGR) into its two components: net assimilation rate (NAR) and leaf area index (LAI):

$$CGR = NAR \text{ X } LAI$$

rearranging yields:

$$NAR = CGR/LAI$$

where CGR is in grams of dry mass m^{-2}; NAR is in grams of dry mass per m^2 of leaf; and LAI is in m^2 of leaf per m^2 of ground. The ratio of crop growth to leaf area index provides a measure of net assimilation integrated over time. When the photon flux is constant, this is a measure of photosynthetic efficiency.

Growth analysis in field studies typically shows that LAI and radiation capture are more closely related to growth and yield than net assimilation. Poorter and Remkes (1990) used growth analysis to analyze 24 wild species and found that net assimilation rate was relatively constant among species and that LAI better indicated competitive differences. Bullock et al. (1988) studied spacing patterns in corn and found that yield increases were due to higher LAI with minimal change in net assimilation rate. Klassen et al. (2003) showed that daily carbon gain from canopy gas-exchange measurements was determined by LAI and light interception, with a constant NAR. Goins et al. (2001) studied wavelengths of RL at a constant BL level (8-9 % BL) and found that increased radiation capture and LAI caused increased growth, with no effect of leaf photosynthetic rate. Hogewoning et al. (2010b) reported that growth of cucumber was significantly greater under an artificial solar source, which they attributed to increased light interception, not photosynthesis.

We sought to determine the effects of blue and green light on growth, leaf area development and whole-plant net assimilation at two PPF levels for seven diverse species. The arrangement of treatments facilitated the analysis of interactions between light quality and quantity across species.

Materials and methods

Light treatments

The experimental system included 16 chambers with eight spectral treatments at two daily light integrals (DLI; 11.5 and 29 mol m-2 d-1). These DLIs were accomplished by providing a constant PPF of 200 and 500 µmol m^{-2} s^{-1} over a 16-h photoperiod. The treatments were developed using LED arrays, including: warm, neutral, and cool white (Multicomp; Newark, Gaffney, SC), monochromatic green, blue and red, and combinations of red and blue (RB) and red, green, and blue (RGB) (Luxeon Rebel Tri-Star LEDs; Quadica Developments Inc., Ontario, Canada). Measurement of phytochrome photoequilibrium, and the fraction of blue (400 to 500 nm), green (500 to 600 nm) and red (600 to 700 nm) light in in each growth chamber were made using a spectroradiometer (model PS-200; Apogee Instruments, Logan UT) (**Fig. 1**). The spectral trace of each treatment is shown in **Fig. 2**. Photosynthetic photon flux (PPF) was measured with the spectroradiometer, and checked every 3 days using a quantum sensor (LI-188B; LI-COR, Lincoln, NE) calibrated for each treatment against the spectroradiometer. Photosynthetic photon flux was maintained constant at the top of the plant canopy by adjusting the electrical current to each LED array.

Plant material and cultural conditions

Lettuce (Lactuca sativa, cv. ‘Waldmann’s Green’), cucumber (Cucumis sativa, cv. ‘Sweet Slice’), wheat (Triticum aestivium L. cv. ‘USU-Apogee’), tomato (Lycoperscion lycopersicum cv. ‘Early girl’), soybean (Glycine max, cv. Hoyt) and radish (Raphanus sativus, cv. ‘Cherry Belle’) seeds were pre-germinated, selected for uniformity, and eight seeds were transplanted to root modules (15 x 18 x 9 cm, L x W x H; 2430 cm^3), expect wheat in which twelve seeds were transplanted. Root modules were filled with horticultural grade soilless media (1 peat: 1 vermiculite by volume) and 5 g of uniformly-mixed slow-release fertilizer (16N-2.6P-11.2K; Polyon® 1 to 2 month release, 16-6-13). The media was watered to excess with a complete, dilute fertilizer solution (100 ppm N; Scotts® Peat-Lite, 21-5-20; EC = 100 mS per m), and allowed to passively drain. This fertilizer solution was applied as needed to maintain ample root-zone moisture (every 2-3 days). The slow-release fertilizer and nutrient solution maintained a near-optimal leachate electrical conductivity between 100 and 150 mS per m (1.0 to 1.5 millimhos per cm; 1 to 1.5 dS per m). To improve uniformity, root modules in each chamber were rotated 180 ° at each watering event (every two or three days). The photoperiod was 16 h day/8 h night.

Pepper (Capsicum annum, cv. 'California Wonder') seeds were pre-germinated in a germination box for 7 days, and two pre-germinated seeds with emerging radicles were transplanted into 8 x 8 x 7 cm pots (448 cm^3). The pots were filled with soilless media identical to that used for the other species with 1 g of slow-release fertilizer was incorporated into each pot. The pots were watered to excess with the same dilute fertilizer solution and allowed to passively drain. After planting, the pots were placed in a growth chamber (130 x 56 x 108 cm; 0.79 m^3) with a PPF of 300 $\mu mol\ m^{-2}\ s^{-1}$ provided by cool white fluorescent lamps and day/night temperature of 25/20 °C. After 33 days, 48 of the most uniform plants were randomly assigned to 16 groups of four plants and were transplanted into root modules with the same dimensions as used for the other five species.

After planting, each root module was randomly placed into one of the 16 growth chambers (19.5 x 23 x 30 cm; 13455 cm^3) that were lined with high-reflectance Mylar (**Fig. 1**). Seedlings were thinned after emergence to four uniform seedlings, which grew until harvest. Wheat was not thinned and neither were peppers after transplanting.

Chambers were well-ventilated to ensure uniform temperature, CO_2 and relative humidity among the treatments. Average temperature differences among chambers were less than 0.2 °C.

Relative humidity averaged 40 % and varied less than 3 % among chambers; CO_2 averaged 430 µmol mol^{-1} and varied less than 10 µmol mol^{-1} among chambers.

Plant measurements

All species were harvested a few days after canopy closure, which occurred 21 days after emergence for most species. Cucumber and pepper were grown for 16 and 54 days after emergence respectively. Leaf area was measured using a leaf area meter (model LI-3000; LI-COR, Lincoln, NE). Stems and leaves were separated and dried to a constant mass at 80 °C for dry mass (DM) determination. Root mass was not measured. Chlorophyll was measured with an optical chlorophyll meter (model MC-100, Apogee Instruments, Logan, UT) that was calibrated for each species according to the method of Parry et al. (2004).

From the above measurements, leaf area index, specific leaf area, and net assimilation (g of DM per m^2 leaf area) were determined for all species.

Statistical analysis

Three replicate studies were conducted for each species. Regression analysis for BL effects included only the four treatments with comparable red and green light (Cool, Warm, Neutral, RBG) (**Fig. 1**). Variables were separately analyzed for each species at each light intensity. Regression analysis for GL effects included only the three treatments with comparable red and blue light (RB, RGB and warm) (**Fig. 1**). Regression analysis included 12 data points for BL (3 reps x 4 treatments); and 9 data points for GL (3 reps x 3 treatments). Statistical analysis at $p = 0.05$ was conducted with the PROC-REG package in SAS (version 9.3; Cary, NC, USA).

Results

An overhead view of one example replicate study with cucumber showing all eight treatments at both light levels just prior to harvest is provided in **Fig. 3**. Each container with four plants is arranged in order of increasing BL from left to right. The chlorophyll concentration was dramatically reduced in the monochromatic green treatment at the higher light level. It is difficult to visually distinguish differences in leaf area among treatments at harvest because all treatments had reached canopy closure. However, monochromatic blue light at the high light level overall had a visually decreased leaf area, compared to the multi-wavelength treatments (RB, RGB, cool, neutral and warm white). These visual results suggest that differences among treatments were greater at the higher light level. Measurements of chlorophyll and leaf area were consistent with the visual observations.

Selection of comparable treatments

To mitigate confounding factors for the effect of BL, statistical analysis included only the four treatments (RGB, cool, warm and neutral white) that had comparable red and green light (**Fig. 1**). The RB treatment was not included due to the low GL with this treatment (0.71 %) compared to 21.6, 37.8, 29.0 and 34.5 for the RGB, cool, warm and neutral white treatments respectively. The monochromatic treatments (red, blue and green) were not included in the analysis due to the confounding effects of lack of other wavelengths. These treatments, however, were included on all figures to indicate the response to monochromatic light.

To mitigate confounding factors for the effect of GL, statistical analysis only included the three treatments (RB, warm and RGB treatments) with comparable red and blue light (**Fig. 1**). The treatments not included in the regression model were included on all figures to provide a reference to the responses to these treatments.

Dry Mass

Effect of blue light

At the higher light level, dry mass (DM) decreased significantly as BL increased for tomato, cucumber, and pepper among comparable treatments (**Fig. 4**). Dry mass slightly decreased with increasing BL for soybean and wheat at the higher light level, but the effect was not statistically significant. At the lower light level, tomato was the only species for which BL caused a significant decrease in DM.

As expected, DM increased with the 2.5 fold increase in PPF. For tomato, radish, soybean, lettuce and wheat, DM was nearly two and half times greater at high light level, but for cucumber and pepper, DM was only 40 % greater at higher light level.

Overall the highest DM for all species tended to occur in the treatments with 11-15 % BL and the effects of increasing BL were more pronounced at the higher light level.

General comments relating to all Figures

The order of presentation of species in the Figures is based on sensitivity to blue light. Tomato (at the top left in all Figures) was generally the most sensitive and wheat (at the bottom in all Figures) the least sensitive species. The regression line in this and all other Figures includes only the three or four treatments that are directly comparable.

Effect of green light

There were no significant effects of GL on DM at the lower light level and there was minimal change in DM as GL increased from 0 to 30 % at either light level (**Fig. 5**). The only exception was radish, in which DM significantly decreased with increasing GL at the higher light level.

Leaf Area Index

Effect of blue light

Leaf area index (LAI) decreased significantly with increasing BL in tomato, cucumber, radish and pepper at the higher light level (**Fig. 6**). At the lower light level, LAI significantly decreased with increasing BL only in tomato.

As expected, leaf area index increased with PPF. Similar to BL effects on DM, LAI tended to be higher in the treatments with the lower BL for all species at both light levels.

Effect of green light

At the higher light level, leaf area index increased significantly with increasing GL only for cucumber and wheat (**Fig. 7**). Lettuce as the only species at the lower light level that showed a significant decrease in LAI with increasing GL. Leaf area index increased with PPF, except for pepper.

Net Assimilation

Effect of blue light

At the higher light level, net assimilation significantly increased with increasing BL in cucumber, radish, pepper and lettuce (**Fig. 8**). At the lower light level, net assimilation significantly increased with increasing BL only in cucumber and there was no significant effect on the other species. As expected, net assimilation greatly increased with PPF for all species.

Effect of green light

At the higher light level, there were no significant differences in net assimilation with increasing GL (**Fig. 9**). At the lower light level, net assimilation significantly decreased with increasing GL for cucumber and soybean but there was minimal change for the other species.

Net assimilation greatly increased with PPF for each species. With the exception of pepper, which has an unusually high concentration of chlorophyll in its leaves, the highest net assimilation tended to occur in the treatments with the lowest GL.

Stem Length

Effect of blue light

At the higher light level, increasing BL significantly decreased stem length in tomato, cucumber, and pepper but there was no significant effect on the other species. (Fig 10). At the lower light level, increasing BL significantly decreased stem length for tomato, pepper and soybean but there was no significant effect on the other species.

The higher PPF decreased stem length for pepper, soybean and increased stem length for wheat. Overall the longest stem length tended to occur in the treatments with lower BL.

Effect of green light

At the higher light level, stem length significantly increased with increasing GL only for tomato (**Fig. 11**). At the lower light level, stem length significantly increased with increasing GL for pepper, soybean and lettuce.

Stem length was similar between PPF levels for all species except soybean and wheat. Overall the longest stem length tended to occur in the treatments with lower GL fraction at both light levels.

Petiole Length

Effect of blue light

At the higher light level, petiole length significantly decreased with increasing BL for tomato, cucumber and radish (**Fig. 12**). At the lower light level, petiole length significantly decreased with increasing BL only for cucumber but it tended to decrease for all species.

Petiole length increased at the lower PPF for all species except cucumber. This is a typical shade avoidance response and results in increased radiation capture in low light.

Lettuce and wheat are not included in these results because they do not develop measureable petioles.

Effect of green light

At the higher light level, increasing GL increased petiole length only for radish (**Fig. 13**). At the lower light level, increasing GL increased petiole length for cucumber, pepper and soybean.

Petiole lengths were longer at the lower PPF in all species, likely because of a shade avoidance response. Overall, petioles tended to be longer with increasing GL but the effect was not always statistically significant.

Discussion

Effects of blue light

Although some BL is necessary for normal growth of many species, more BL is not necessarily better. Among the comparable treatments, the highest DM, greatest LAI, and longest stem and petiole length tended to occur with the lowest BL (11 %). These results are consistent with the findings from several other studies on individual species and are consistent with a shade avoidance response in reduced blue light

Hernández and Kubota (2015) found that leaf area and dry mass of cucumber decreased as BL increased up to 75 %. Cope et al. (2014), reported similar effects for lettuce, radish, and pepper, but they analyzed the effects of BL across all treatments (0 to 92 % BL) including treatments that had potentially confounding factors, which might have caused growth effects associated with other variables.

Hoenecke et al. (1992) grew lettuce seedlings for six days under treatments from 0 to 40 % BL at two PPFs (150 and 300 µmol m^{-2} s^{-1}) and reported that hypocotyl length rapidly decreased as BL increased. Brown et al. (1995) reported that pepper stem length decreased as BL increased up to 21 % BL. Dougher and Bugbee (2004) examined the effects of BL on cell size and cell division and found that both were reduced under increased BL. Wargent et al. (2009) found that UV radiation had a powerful effect on leaf expansion in lettuce.

Monocots may have a fundamentally different response than dicot species. Dougher and Bugbee (2001) found that BL had only a small effect on growth and morphology of wheat, and indicated that the response may be associated with the below ground meristem position of monocots during early growth. Our data confirm these results.

All of our spectral treatments were developed with LEDs, and this minimizes the confounding effects that occur when comparisons are made between LEDs and fluorescent lamps. The growth of all species under the three types of white LEDs was similar to the RB and RGB treatments. These results suggest that the beneficial effect of fluorescent light in previous studies was caused by factors other than spectral effects. (Chen et al., 2014; Goins et al., 1997; Wang et al., 2014; Yorio et al., 2001).

Net Assimilation

The ratio of growth to leaf area index provides a measure of net assimilation integrated over time (Lambers, 2008; Leopold and Kriedemann, 1975; Hunt, 1982; Hunt, 2002). Several other studies have found that radiation capture, as predicted by LAI, is the dominant factor in determining growth rates (Bullock et al., 1988; Goins et al., 2001; Hogewoning et al., 2010b; Klassen et al., 2003; Poorter and Remkes, 1990). Because PPF was constant at either 200 or 500 µmol m^{-2} s^{-1}, net assimilation rate provides a relative measure of photosynthetic efficiency. After the monochromatic treatments are excluded, there was a surprisingly small effect of light quality on photosynthetic efficiency in any species at either PPF.

This finding is in contrast to the effect of light quality on photosynthesis based on short-term, single-leaf measurements in several other studies. Hogewoning et al. (2010a), reported that increasing BL increased photosynthetic capacity (photosynthetic potential in higher PPF), but they used an unusually low baseline PPF of only 100 µmol m^{-2} s^{-1}. Terfa et al. (2013) found that increasing blue light from 5 to 20 % increased leaf thickness and increased photosynthetic capacity of single leaves. Hernández and Kubota (2015) found that short-term net photosynthesis of single-leaves increased in cucumber as blue light fraction increased from 10 to 80 %, but whole-plant dry mass of the same plants steadily decreased as blue light increased. These results highlight the often poor relationship between short-term single-leaf measurements and whole plant dry mass gain.

The relative quantum efficiency of single leaves indicates that RL is 25 to 35 % more efficient than BL and 5 to 30 % more efficient than GL in driving photosynthesis (Inada, 1976; McCree, 1972). However, this curve was measured at a low PPF over short intervals. Our results indicate that it is not appropriate to extrapolate from this curve to whole plants or plant communities grown at high PPF under mixed colors of light.

Effect of self-shading on net assimilation

Net assimilation increased for some crops as BL increased. This is at least partly the result of self-shading. At canopy closure there is a reduction in the average PPF per leaf, which causes a reduction in the average net assimilation per leaf. In sensitive species, the lower BL treatments reached canopy closure sooner and had more self-shading. In contrast, the higher BL treatments reached canopy closure later and had less self-shading.

Effects of green light

All species grew in monochromatic green light and, remarkably, the growth of some species (peppers, wheat, low light cucumbers and soybeans) was equal to the broad spectrum treatments. Although a high fraction of GL can reduce growth in some species (Folta and Maruhnich, 2007), we found only small effects of GL and the direction of the response was often inconsistent between light levels and species. In contrast to the findings of Wang and Folta (2013), GL effects did not decrease as PPF increased. However, harvest in these studies occurred shortly after canopy closure and the effects of GL on plant communities may increase in longer term studies as a greater fraction of the light is filtered to lower leaf layers.

In tomatoes, radish and lettuce, the highest dry mass tended to occur in the lowest green light treatments but the effect was only statistically significant in radish at high light. At the lower light level, increasing GL did not have a statistically significant effect on DM in any species. Consistent with our results, Hernández and Kubota (2015) found that increased GL (up to 28 %) had no effect on cucumber growth. Kim et al. (2004b) reported that supplementing red and blue LEDs with green light (from green fluorescent lamps) increased lettuce growth by up to 48 % at the same PPF but these results may be associated with the increase in diffuse light, or warmer leaf temperature, rather than a direct effect of GL.

Paradiso et al. (2011) measured and modeled photosynthesis of individual rose leaves at 18 wavelengths and found increased utilization of GL in plant communities compared to individual leaves. These findings are consistent with those of Sun et al. (1998) and Terashima et al. (2009). Collectively, these results indicate that measurements of spectral effects on single leaves in low light should not automatically be extrapolated to whole plant communities in higher light.

Increasing green light increased stem length in tomato, pepper, soybean, and lettuce, and increased petiole length in radish and soybean. Since GL is selectively enriched at the bottom of plant canopies, this result is consistent with a shade avoidance response, and the effect was greater at the lower PPF level. Increases in either stem or petiole length are typically associated with increased radiation capture and increased growth so increasing the fraction of green light might be used to enhance early growth in sensitive species. Interestingly, GL increased stem length in tomatoes but had no effect on petiole length.

Shade avoidance responses for specific leaf area and leaf chlorophyll concentration

There were also shade avoidance responses for leaf thickness (specific leaf area, SLA, m^2_{leaf} per kg_{leaf} DM) (supplemental data). Increasing PPF significantly increased leaf thickness (decrease SLA) in all species. Increasing BL tended to increase leaf thickness and the effect was statistically significant in cucumber, radish, pepper, and lettuce (supplemental data).

Increasing GL tended to decrease leaf thickness (increase SLA) and the effect was statistically significant in cucumber, pepper, and soybean (supplemental data). Both blue and green light effects on specific leaf area are consistent with a shade avoidance response where more BL is associated with brighter sunlight, and more GL is associated with deeper shade.

There were shade avoidance responses for leaf chlorophyll concentration. Chlorophyll was significantly increased by higher PPF. Increasing BL also tended to increase leaf chlorophyll concentration and the effect was statistically significant in tomato, cucumber, radish, and pepper (supplemental data). Increasing GL tended to decrease chlorophyll concentration and the effect was statistically significant in tomato, cucumber, pepper and lettuce (supplemental data). Plants in the low light conditions of shade would be expected to adapt by reducing the chlorophyll concentration in their leaves.

Anomaly in RB, low-PPF treatment for wheat

The wheat plants in the RB treatment at the lower light level had higher DM, LAI and net assimilation compared to the other treatments. This response was due to significantly increased tillering (data not shown). The reason for increased tillering is unknown, but it occurred in each of the three replicate studies. There were no unique growth or developmental effects in this treatment for any of the other species. This response of wheat warrants further study.

Conclusions

We have begun to characterize photobiological differences among species for both light quality and quantity. As expected, growth of most species was significantly better in the multiple wavelength treatments than in the monochromatic blue, green, or red light treatments. Among the broad spectrum treatments at the higher PPF, increasing blue light in four increments from 11 to 28 % reduced growth in tomato, cucumber, and pepper by 22, 26, and 14 % respectively, but there was no statistically significant effect on radish, soybean, lettuce and wheat. At the lower PPF, growth was reduced by 41 % in tomato, but the effects of blue light on the other species were less than 6 % and were not statistically significant. Effects on leaf area paralleled effects on dry mass in all species at both PPFs, indicating that the effects of blue light on dry mass were mediated by changes in leaf area. Since LAI determines radiation capture and is highly correlated with dry mass gain, it is apparent that improvements in radiation capture efficiency are responsible for nearly all of the increases in dry mass.

In contrast to the significant effect of blue light on dry mass and leaf area, increasing green light fraction from zero to 30 % resulted in few significant differences on DM, LAI or net assimilation, and there was no consistent direction among species or PPF levels. Increasing GL increased stem and petiole length in several species, which is consistent with a shade avoidance response. These results indicate that GL had little effect on dry mass, but its importance may increase over time as a dense canopy forms.

Historically, studies to understand spectral effects on plant growth have focused on single leaf photosynthetic efficiency over short time intervals. The results of this study indicate that short-term measurements of photosynthesis can be misleading in the prediction of light quality effects on plant growth in long-term studies.

Collectively, these results indicate that:

1) the effect of blue light on growth is primarily determined by changes in radiation capture and not by a direct effect on photosynthesis,

2) the effects of blue light fraction are greater at higher PPF,

3) there is a wide range in species sensitivity to blue light,

4) the effects on leaf thickness and chlorophyll concentration in response to blue and green light fractions can be interpreted as a shade avoidance response

5) in some species, light quantity has a bigger effect on plant shape than light quality.

Acknowledgments

This research was supported by the Utah Agriculture Experiment Station. We thank Ty Weaver, Alec Hay, Saundra Rhoades and Boston Swan for conscientious technical support. Mention of trade names is for information only and does not constitute an endorsement by the authors.

Literature cited

Barta D, Tibbitts T, Bula R, Morrow R. Evaluation of light emitting diode characteristics for a space-based plant irradiation source. Adv Space Res. 1992;12:141-49.

Brown CS, Schuerger A, Sager JC. Growth and photomorphogenesis of pepper plants under red LEDs with supplemental blue or far-red lighting. J Amer Soc Hort Sci. 1995;120:808-13.

Bula R, Morrow R, Tibbitts T, Barta D, Ignatius R, Martin T. Light-emitting diodes as a radiation source for plants. HortSci. 1991;26:203-05.

Bullock D, Nielsen R, Nyquist W. A growth analysis comparison of corn grown in conventional and equidistant plant spacing. Crop Sci. 1988;28:254-58.

Chen X-l, Guo W-z, Xue X-z, Wang L-c, Qiao X-j. Growth and quality responses of ‘Green Oak Leaf’ lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Sciencia Hortic. 2014;172:168-75.

Cope K, Snowden MC, Bugbee B. Photobiological Interactions of Blue Light and Photosynthetic Photon Flux: Effects of Monochromatic and Broad-Spectrum Light Sources. Photochem Photobiol. 2014;90:574-84.

Cope KR, Bugbee B. Spectral effects of three types of white light-emitting diodes on plant growth and development: absolute versus relative amounts of blue light. HortSci. 2013;48:504-09.

Dougher TAO, Bugbee B. Differences in the response of wheat, soybean, and lettuce to reduced blue radiation. Photochem Photobiol. 2001;73:199-207.

Dougher TAO, Bugbee B. Long-term blue light effects on the histology of lettuce and soybean leaves and stems. J Amer Soc Hort Sci. 2004;129:467-72.

Folta KM, Maruhnich SA. Green Light: a signal to slow down or stop. J Exp Bot. 2007;58:3099-111.

Genty B, Briantais J-M, Baker NR. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA) - General Subjects. 1989;990:87-92.

Goins G, Yorio N, Sanwo M, Brown C. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J Exp Bot. 1997;48:1407-13.

Goins GD, Ruffe LM, Cranston NA, Yorio NC, Wheeler RM, Sager JC. Salad crop production under different wavelengths of red light-emitting diodes (LEDs). SAE Tech Paper. 2001.

Hernández R, Kubota C. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ Exp Bot. 2015;DOI: 10.1016/j.envexpbot.2015.04.001.

Hoenecke ME, Bula RJ, Tibbitts TW. Importance of 'blue' photon levels for lettuce seedlings grown under red-light-emitting diodes. HortSci. 1992;27:427.

Hogewoning SW, Douwstra P, Trouwborst G, Van Ieperen W, Harbinson J. An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra. J Exp Bot. 2010b;61:1267-76.

Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, van Ieperen W, Harbinson J. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. J Exp Bot. 2010a;61:3107-17.

Hunt R. Plant growth analysis: Institute of Terrestrial Ecology; 1982.

HUNT, R.; D. R. CAUSTON; B. SHIPLEY; A. P. ASKEW. 2002. A Modern Tool for Classical Plant Growth Analysis. Annals of Botany 90, 485-488.

Inada K. Action spectra for photosynthesis in higher plants. Plant Cell Physiol. 1976;17:355-65.

Johkan M, Shoji K, Goto F, Hahida S, Yoshihara T. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. Environ Exp Bot. 2012;75:128-33.

Kim H-H, Goins GD, Wheeler RM, Sager JC. Stomatal conductance of lettuce grown under or exposed to different light qualities. Ann Bot. 2004a;94:691-97.

Kim HH, Goins G, Wheeler R, Sager JC, Yorio NC. Green-light supplementation for enhanced lettuce growth under red and blue LEDs. HortSci. 2004b;39:1616-22.

Kim HH, Wheeler R, Sager JC, Yorio NC, Goins G. Light-emitting diodes as an illumination sources for plants: A review of research at Kennedy Space Center. Habitation. 2005;10:71-78.

Klassen S, Ritchie G, Frantz J, Pinnock D, Bugbee B. Real-time imaging of ground cover: Relationships with radiation capture, canopy photosynthesis, and daily growth rate. Digital imaging and spectral techniques: applications to precision agriculture and crop physiology. 2003:3-14.

Lambers, Hans; Chapin III, F.; Stuart, Pons; Thijs L., Chapter 7: Growth and allocation. Plant Physiological Ecology, Second edition. Springer-Verlag, NY. 2008

Leopold AC, Kriedemann PE. Plant growth and development. 2nd ed: McGraw-Hill, Inc.; 1975.

Li, T, and Q Yang. Advantages of diffuse light for horticultural production and perspectives for further research. Frontiers in Plant Sci. 2015; 6: 704.

Lin KH, Huang MY, Hauang WD, Hsu MH, Yang ZW, Yang CM. The effects of red, blue, and white light-emitting diodes on the growth, dvelopment, and edible quality of hydroponiclly grown lettuce (*Lactuca sativa* L. var. capitata). Sciencia Hortic. 2013;150.

McCree KJ. The action spectrum absorptance and quantum yeild of photosynthesis in criop plants. Agric Meteorol. 1972;9:191-216.

Nelson JA, Bugbee B. Analysis of Environmental Effects on Leaf Temperature under Sunlight, High Pressure Sodium and Light Emitting Diodes. PloS one. 2015;10:e0138930.

Nishio JN. Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. Plant Cell Environ. 2000;23:539-48.

Ouzounis T, Frette X, Rosenqvist E, Ottosen C. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and capanulas. J Plant Physiol. 2014;171:1491-99.

Ouzounis T, Parjikolaei BR, Fretté X, Rosenqvist E, Ottosen C-O. Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids, and pigments in Lactuca sativa. Front Plant Sci. 2015;6.

Paradiso R, Meinen E, Snel JF, De visser P, Van Ieperen W, Hogewoning SW, et al. Spectral dependence of photosynthesis and light absorpance in single leaves and canopy in rose. Sciencia Hortic. 2011;127:548-54.

Parry, C., Blonquist, M. and Bugbee, B. 2014. In situ measurement of leaf chlorophyll concentration: Analysis of the optical/absolute relationship. Plant Cell and Environ. 37(11): 2508–2520.

Poorter H, Remkes C. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia. 1990;83:553-59.

Sepúlveda-Ugarte J, Brunet JE, Matamala AR, Martínez-Oyanedel J, Bunster M. Spectroscopic parameters of phycoerythrobilin and phycourobilin on phycoerythrin from Gracilaria chilensis. J Photochem Photobiol A Chem. 2011;219:211-16.

Sinclair TR, Shiraiwa T, Hammer GL. Variation in crop radiation-use efficiency with increased diffuse radiation. Crop Sci. 1992;32:1281-84.

Smith K. The science of photobiology: Springer Science & Business Media; 2013.

Son K-H, Oh M-M. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortSci. 2013;48:988-95.

Sun J, Nishio JN, Vogelmann TC. Green light drives CO_2 fixation deep within leaves. Plant Cell Physiol. 1998;39:1020-26.

Taiz, L., Zeiger, E, Moller IM, Murphy A. Plant Physiology and Development. 6th edition. Sinauer Associates, Inc. 2015.

Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R. Green light drives leafe photosynthesis more efficiently thatn red light i strong white light: revisiting the enigmatic question of why leaves are geen. Plant Cell Physiol. 2009;50:684-89.

Terfa MT, Solhaug KA, Gislerød HR, Olsen JE, Torre S. A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of Rosa× hybrida but does not affect time to flower opening. Physiol Plant. 2013;148:146-59.

Tubiello F, Volk T, Bugbee B. Diffuse light and wheat radiation-use efficiency in a controlled environment. Life Support Biosph Sci. 1996;4:77-85.

Wang XY, Xu XM, Cui J. The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of Cucumis sativus grown under weak light. Photosynthetica. 2014:1-10.

Wang Y, Folta KM. Contribution of green light to plant growth and development. Am J Bot. 2013; 100:70-78.

Wargent, JJ. et al. Ultraviolet radiation as as limiting factor in leaf expansion and development. Photochem. and Photobiology 2009: 85:279-286.

Yorio NC, Goins G, Kagie H, Wheeler R, Sager JC. Improving spinach, radish, and lettuce growth under red LEDs with blue light supplementation. HortSci. 2001;36:380-83.

Radish in Chambers Lined With Mylar

PPF
500
200

Parameter	Red	Green	Warm*†	RB†	RGB*†	Neutral*	Cool*	Blue
% UV-A	0.03	0.17	0.09	0.01	0.11	0.13	0.17	0.18
% Blue	0.27	6.52	10.8	12.0	13.7	19.4	27.5	92.0
% Green	1.63	92.5	41.0	1.70	22.9	45.6	48.0	7.77
% Red	98.1	0.96	48.2	86.3	63.4	35.0	24.5	0.22
PPE	0.89	0.83	0.84	0.89	0.89	0.84	0.83	0.58

*To minimize confounding spectral effects, only these four RGB, cool, warm and neutral white treatments were used in the blue light analysis.

†To minimize confounding spectral effects, only these three RGB, cool, warm and neutral white treatments were used in the green light analysis.

Fig. 1. The eight spectral treatments and characteristics. Blue, green and red values are percent of total PPF (400 to 700 nm). UV-A is percent of total PPF. Phytochrome photoequilibrium (PPE) was determined as described by Sager et al. (1988). Symbols correspond to the color for each treatment and shape represents the two PPFs (200 and 500 µmol m^{-2} s^{-1}), which are associated with DLIs of 11.5 and 29 mol m^{-2} d^{-1}. Symbol shape and color are consistent in all figures.

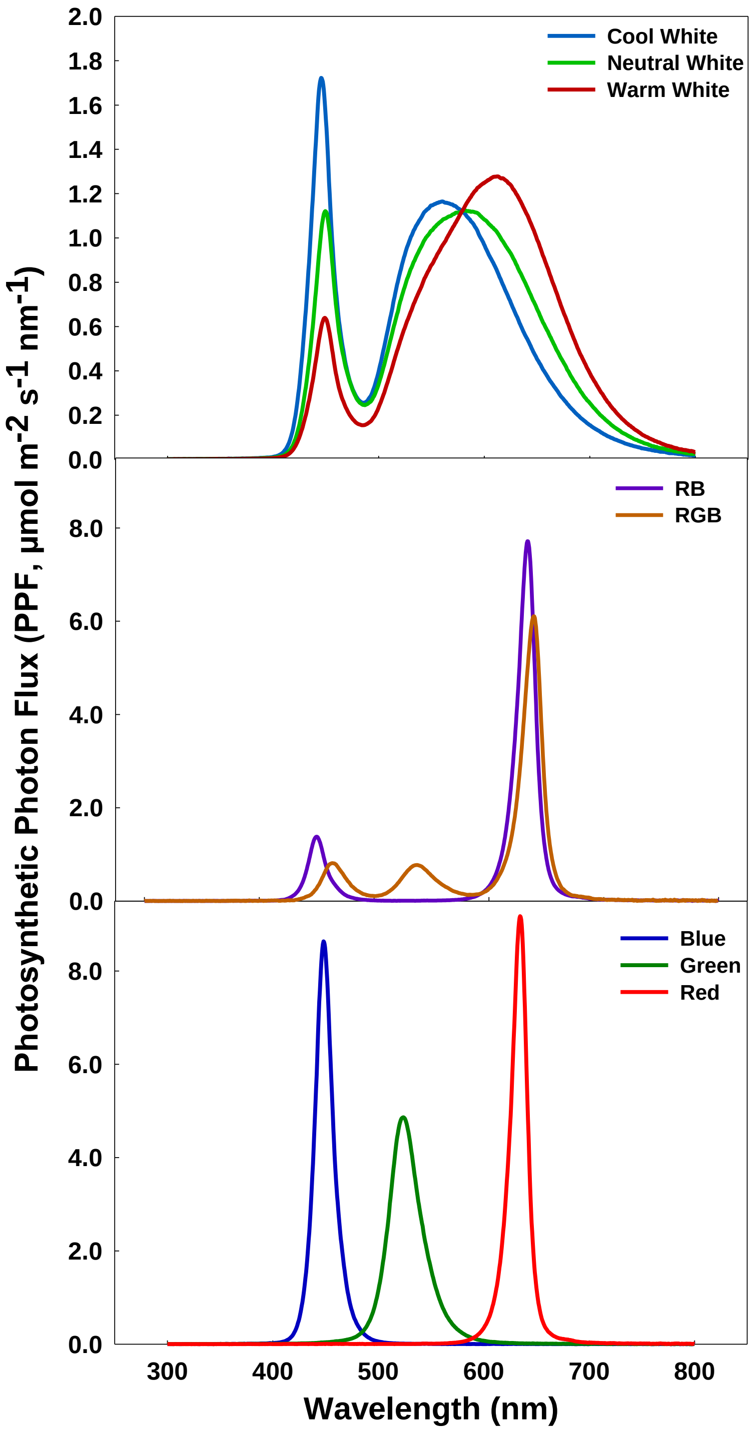

Fig. 2. Spectral distributions of all eight LED treatments, including: the three types of white LEDs, the red + blue (RB) and red + green + blue (RGB) LEDs, and the red, green, and blue monochromatic LEDs. Variation in spectral distribution between the 200 and 500 µmol m^{-2} s^{-1} treatments was negligible.

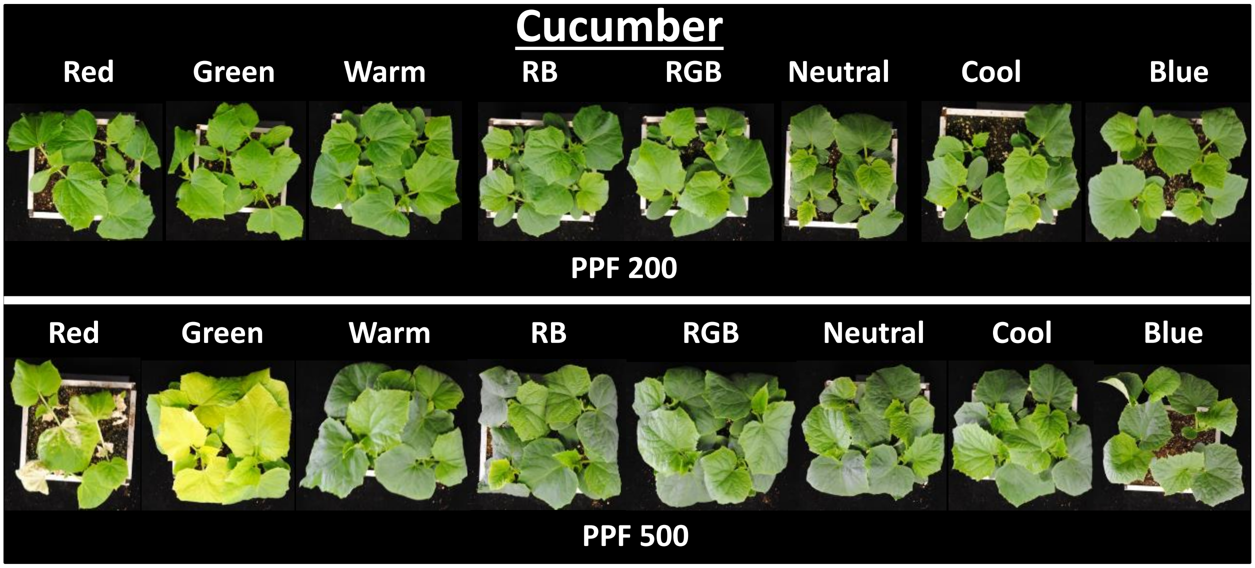

Fig. 3. Overhead view of cucumber plants at harvest for all eight LED treatments at both light intensities arranged from low to high BL fraction. There were four plants per treatment and three replicates per study. Note the in coloration with the green and red treatments at the 500 PPF level (DLI = 29 mol m^{-2} d^{-1}).

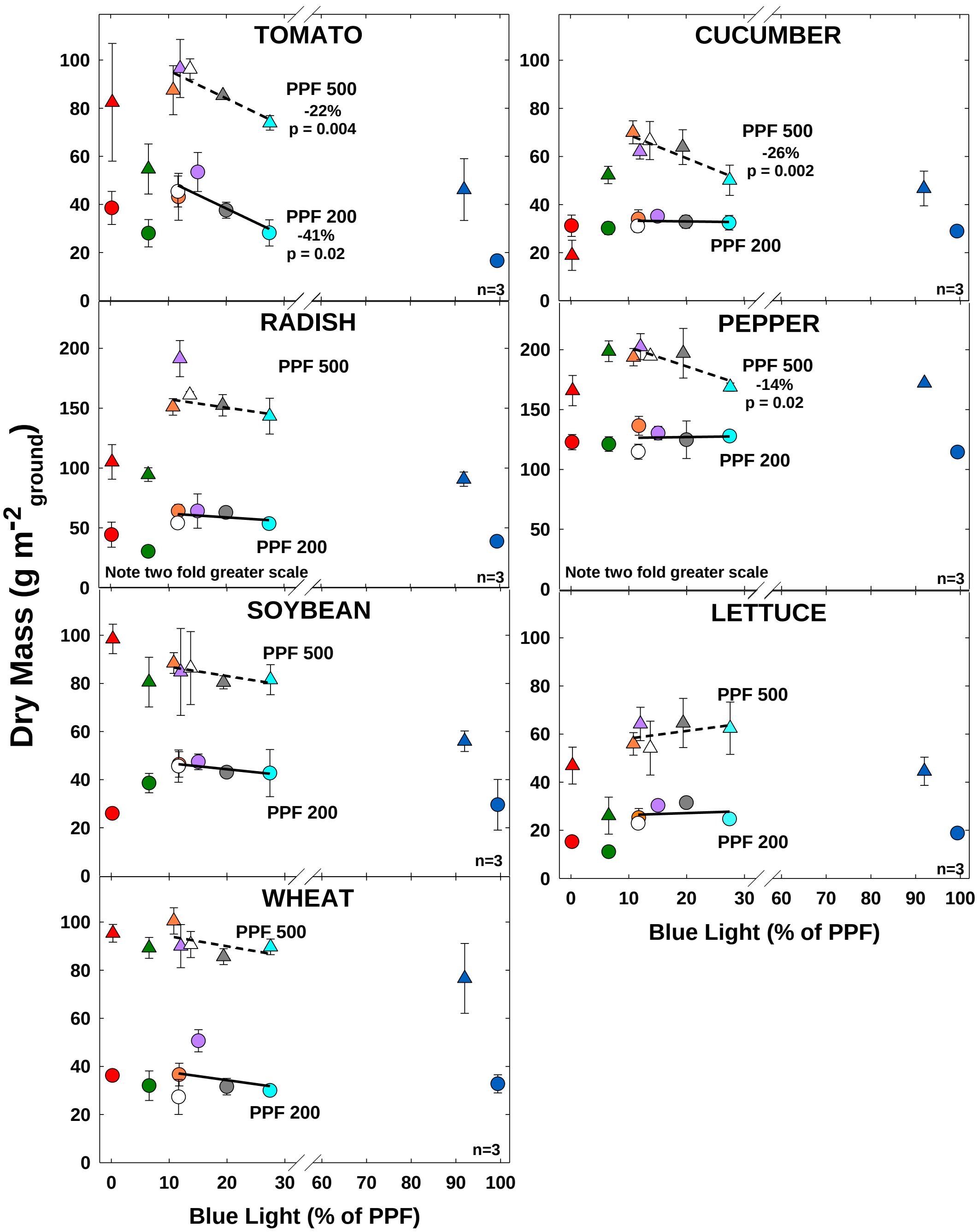

Fig. 4. The effect of percent blue light on dry mass (DM) gain for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Also note two-fold scale increase for DM in radish and pepper. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

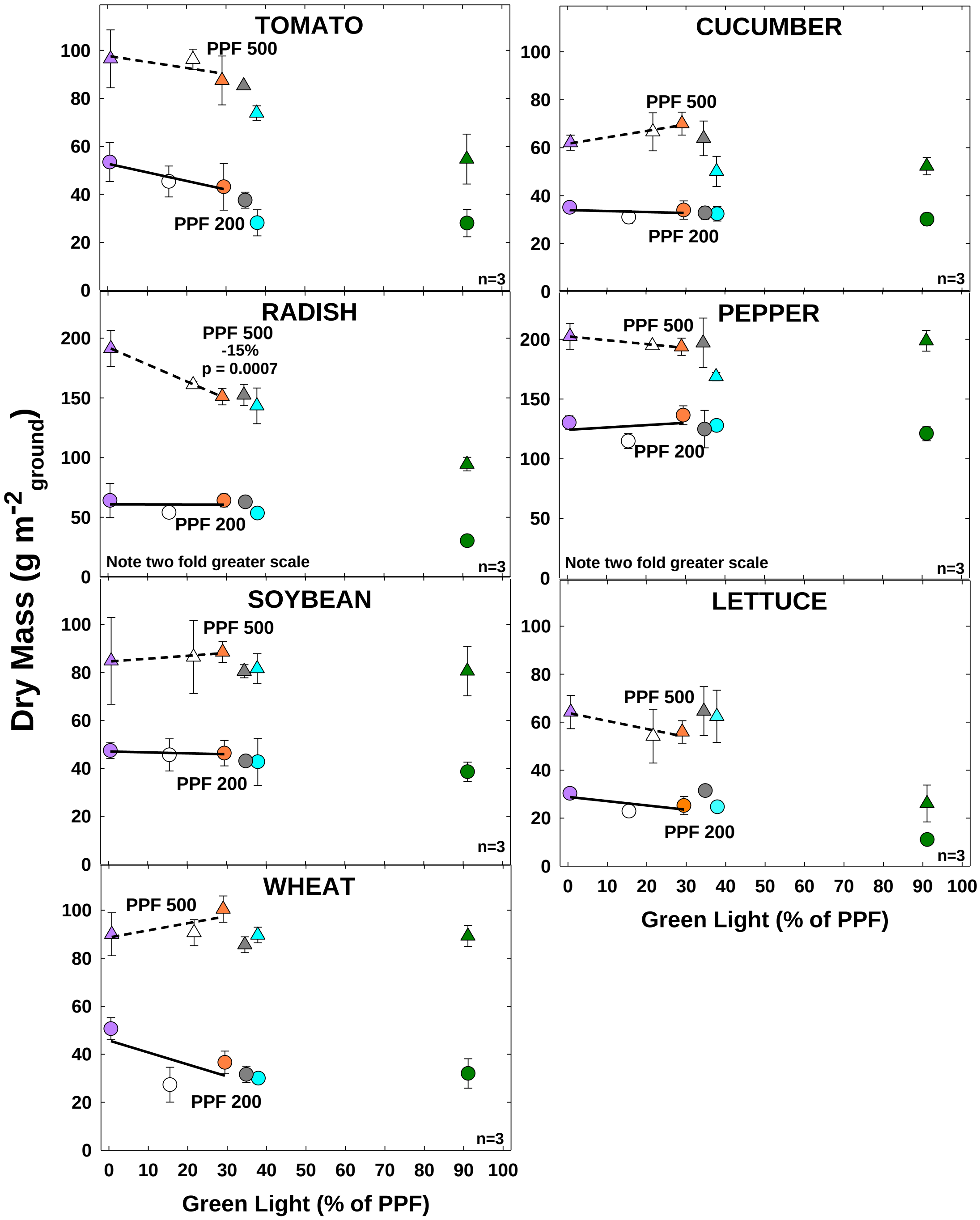

Fig. 5. The effect of percent green light on dry mass (DM) gain for seven species under two PPFs. Note two-fold scale increase for DM in radish and pepper. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown.

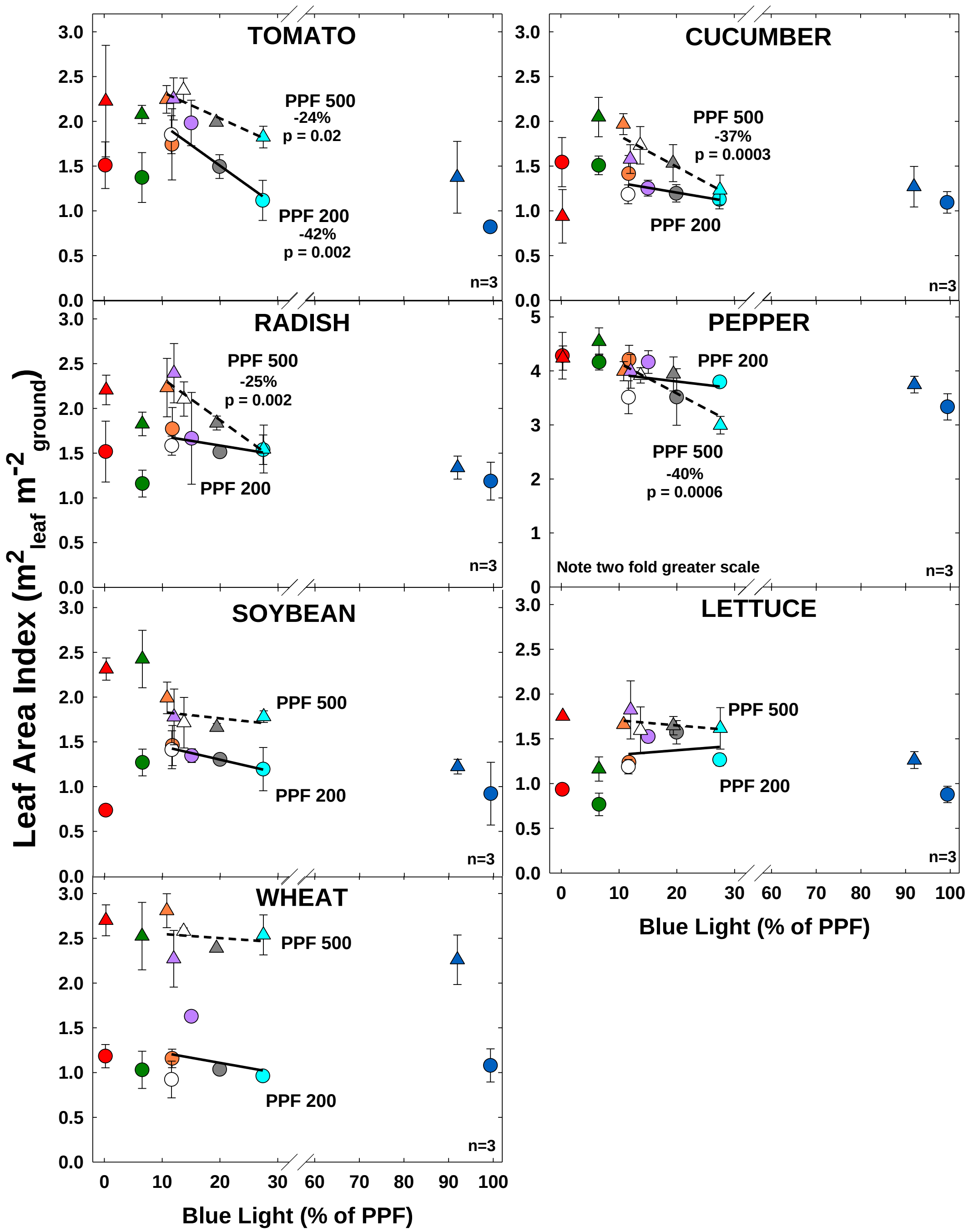

Fig. 6. The effect of percent blue light on leaf area index (LAI) for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Also note two fold scale increase for pepper. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

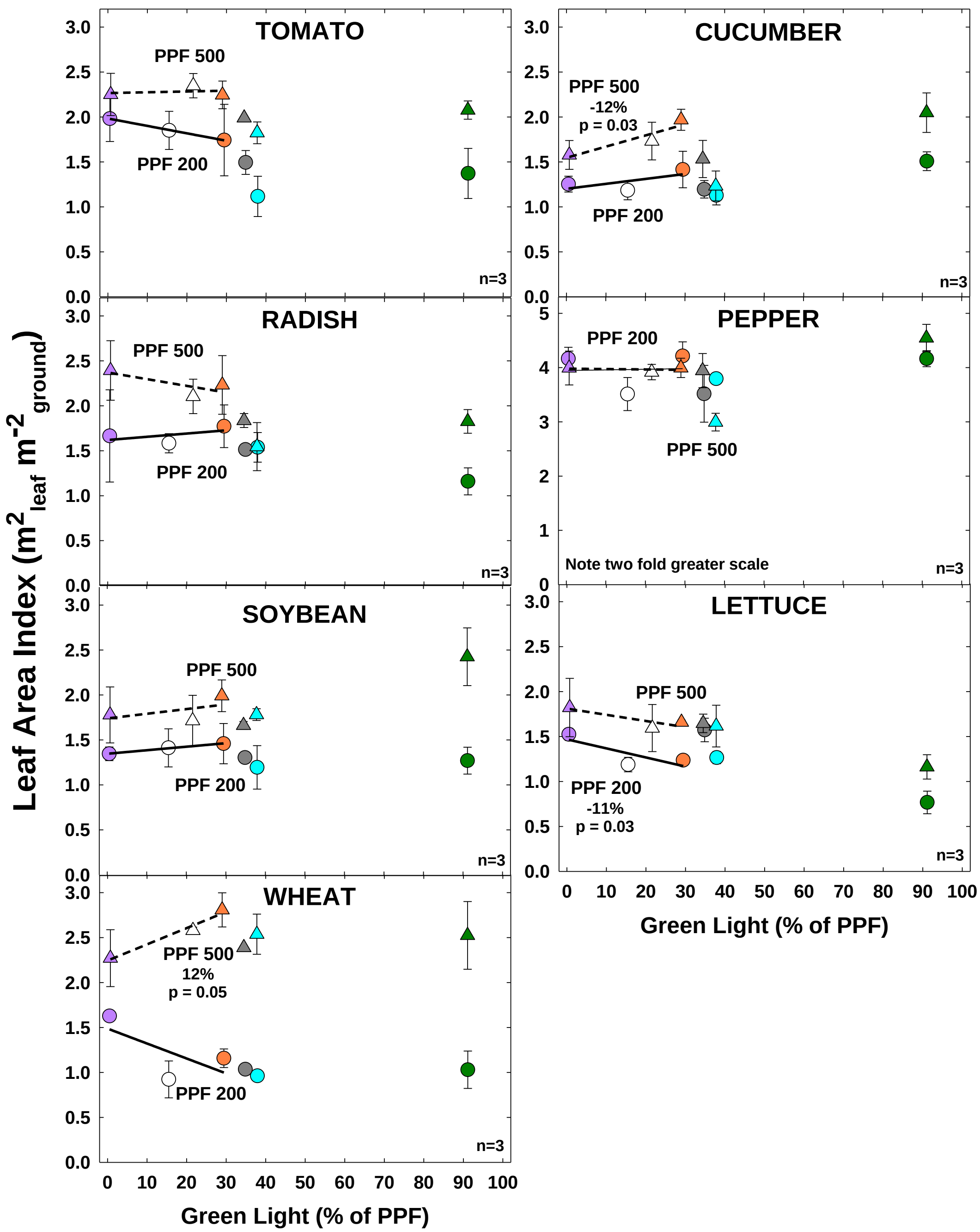

Fig. 7. The effect of percent green light on leaf area index (LAI) for seven species under two PPFs. Note two fold scale increase for pepper. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown.

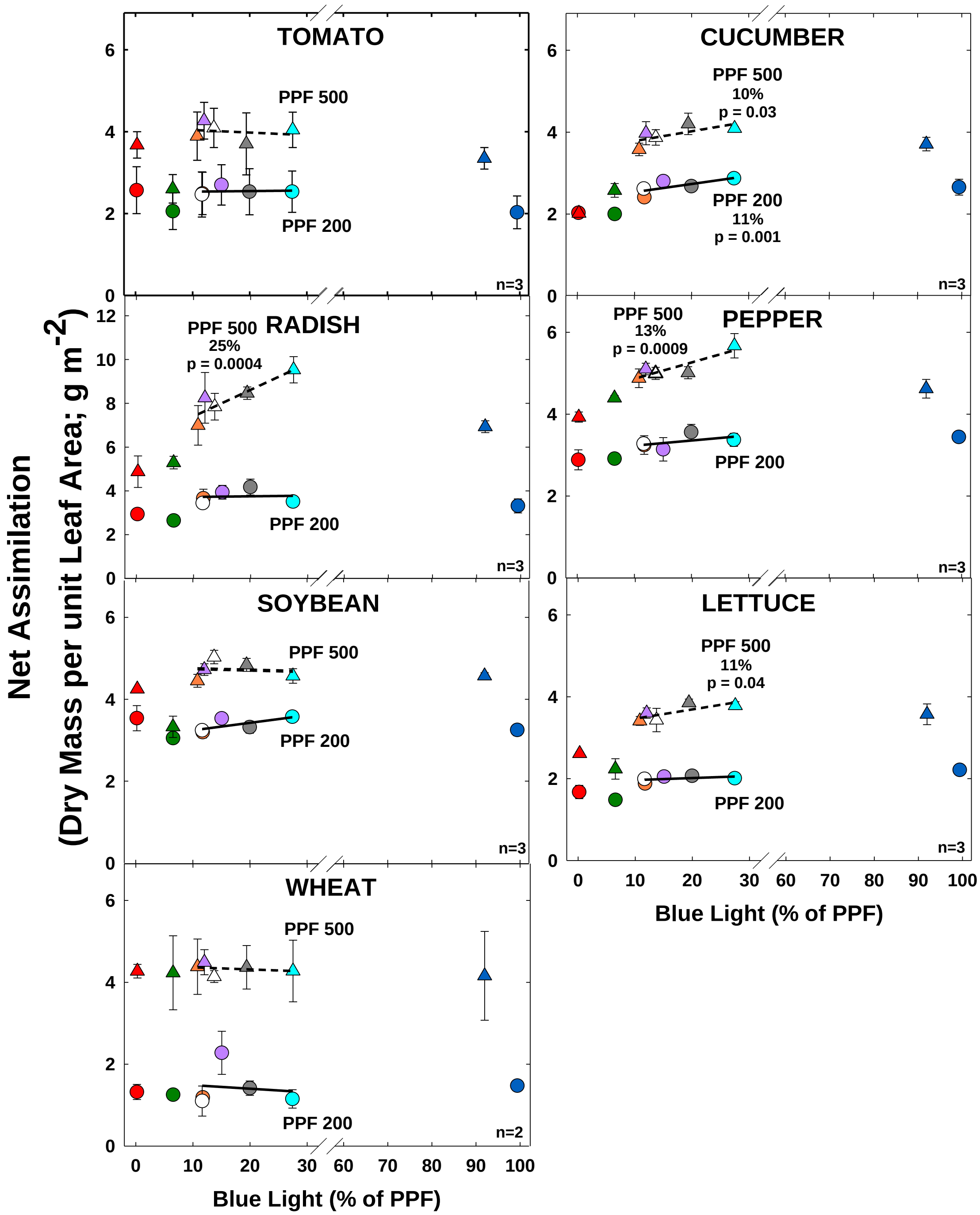

Fig. 8. The effect of percent blue light on net assimilation for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

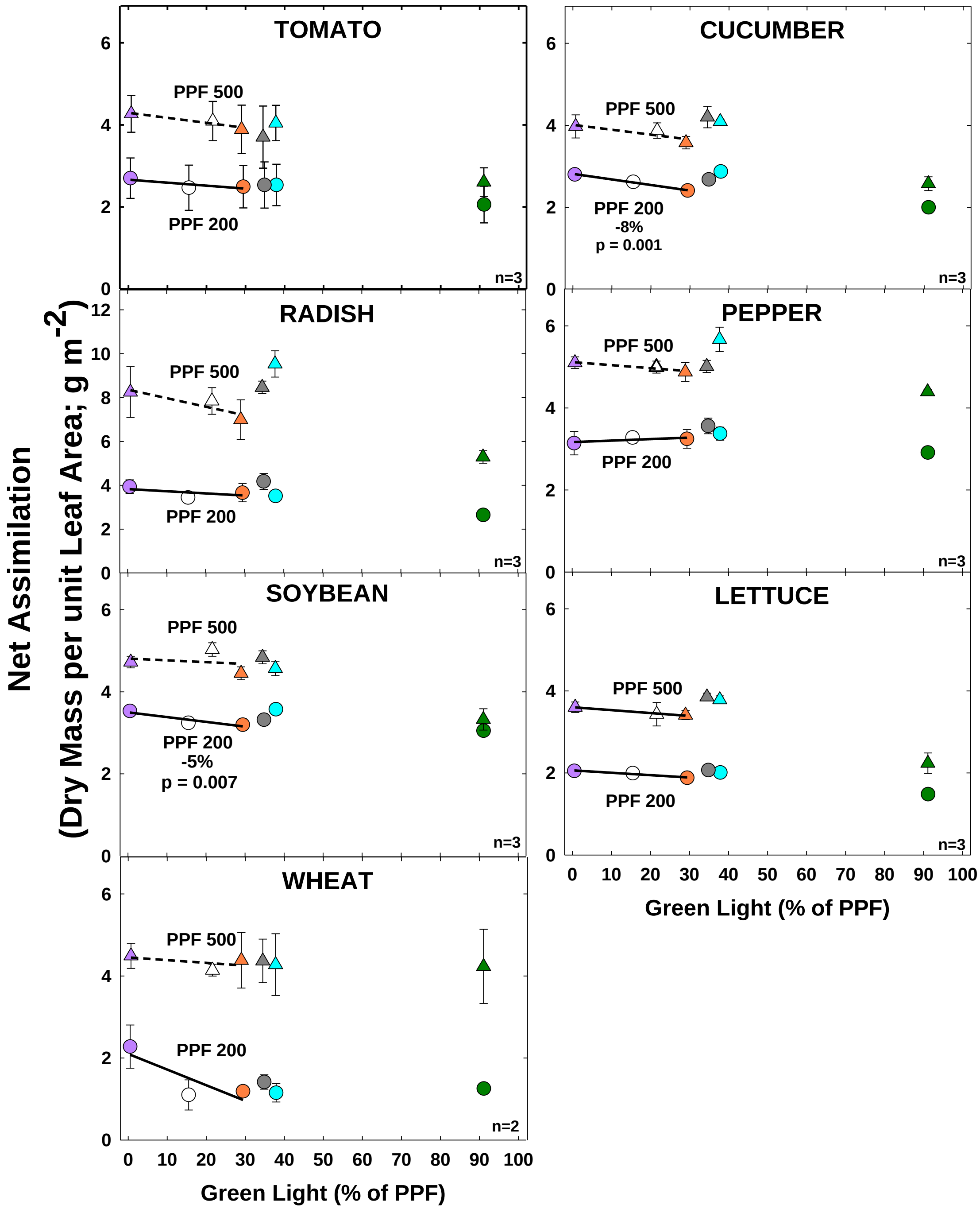

Fig. 9. The effect of percent green light on net assimilation for seven species under two PPFs. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown. Regression line includes the RB, RBG and warm white treatments for each PPF. When significant, p-values and percent change are shown.

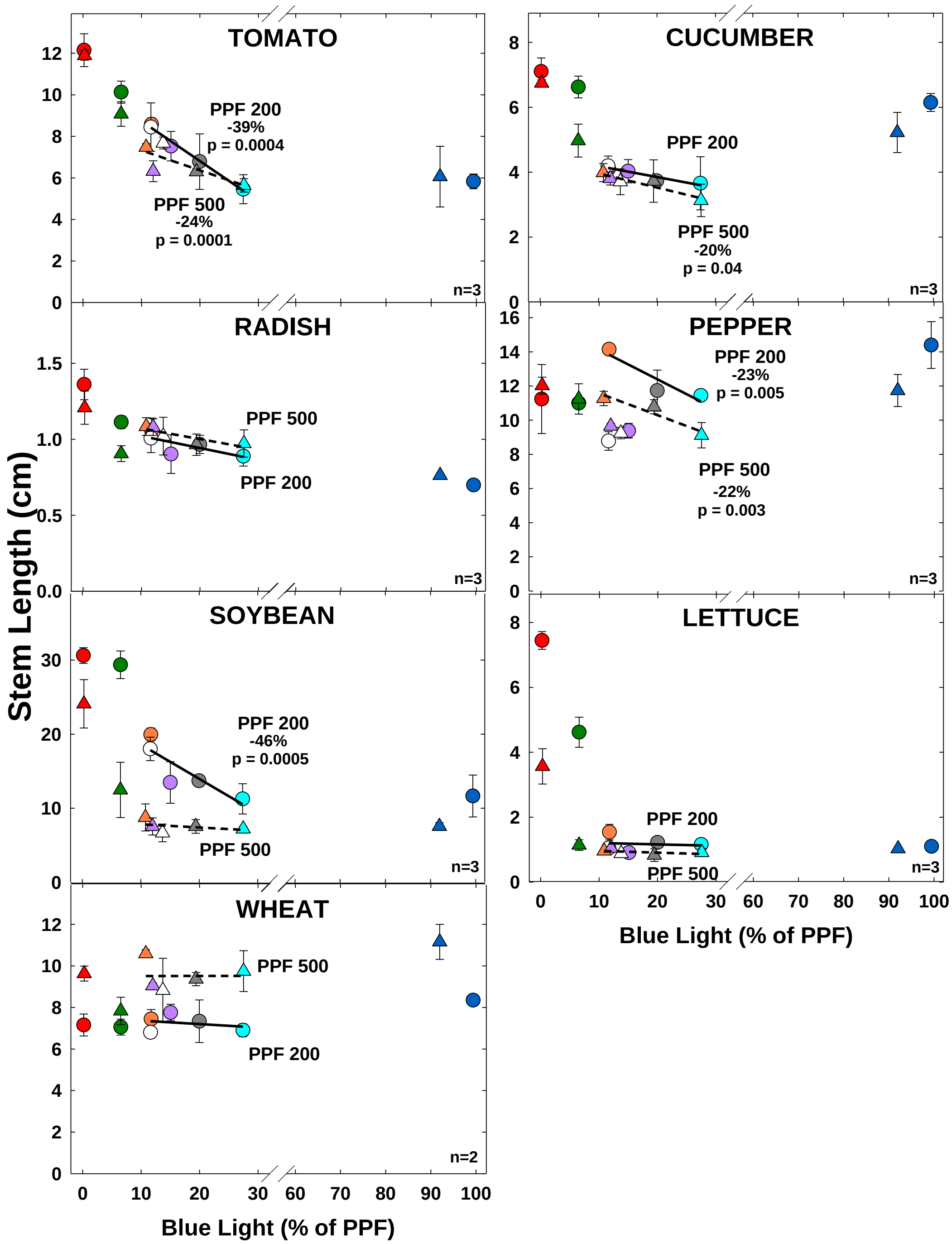

Fig. 10. The effect of percent blue light on stem length for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

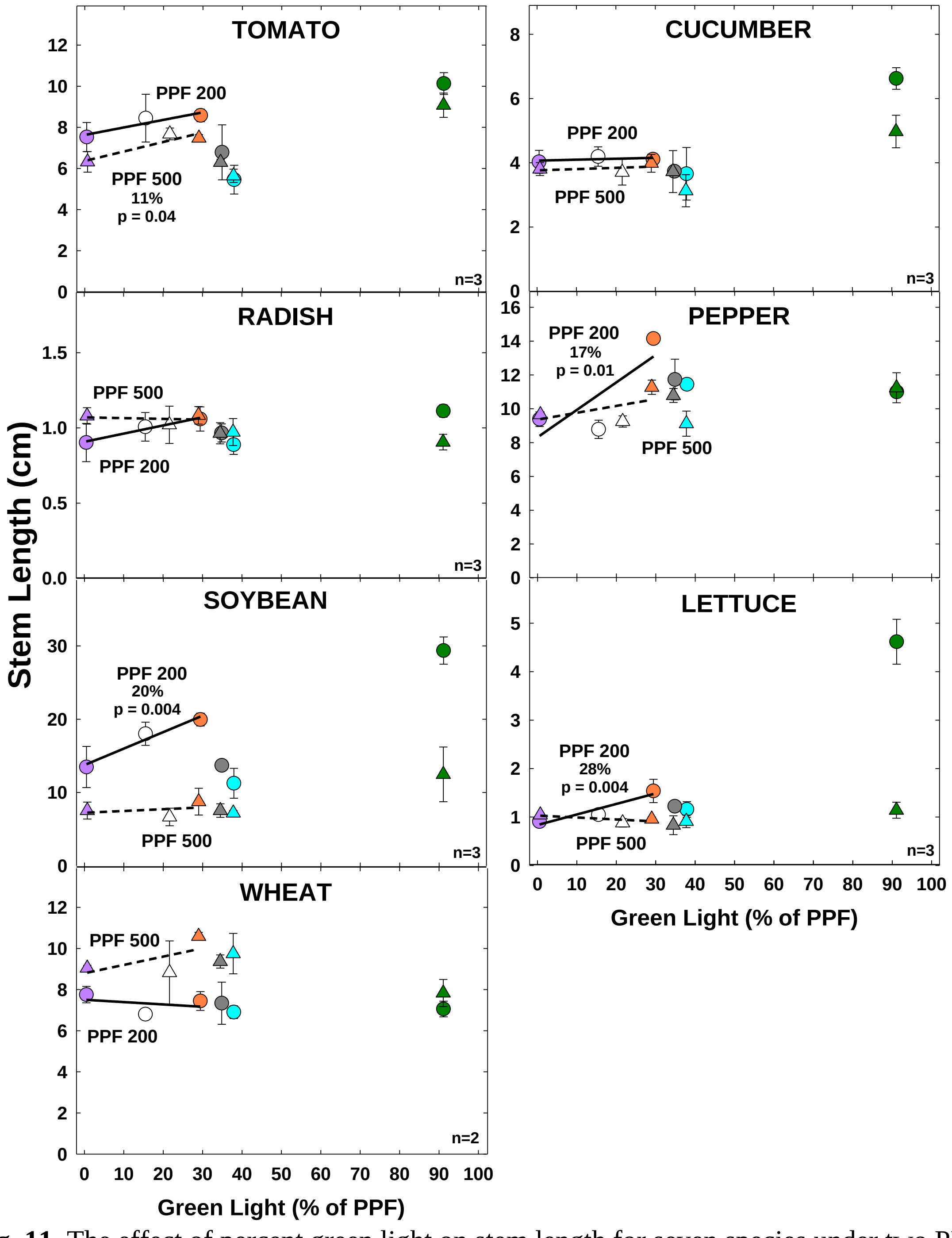

Fig. 11. The effect of percent green light on stem length for seven species under two PPFs. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown.

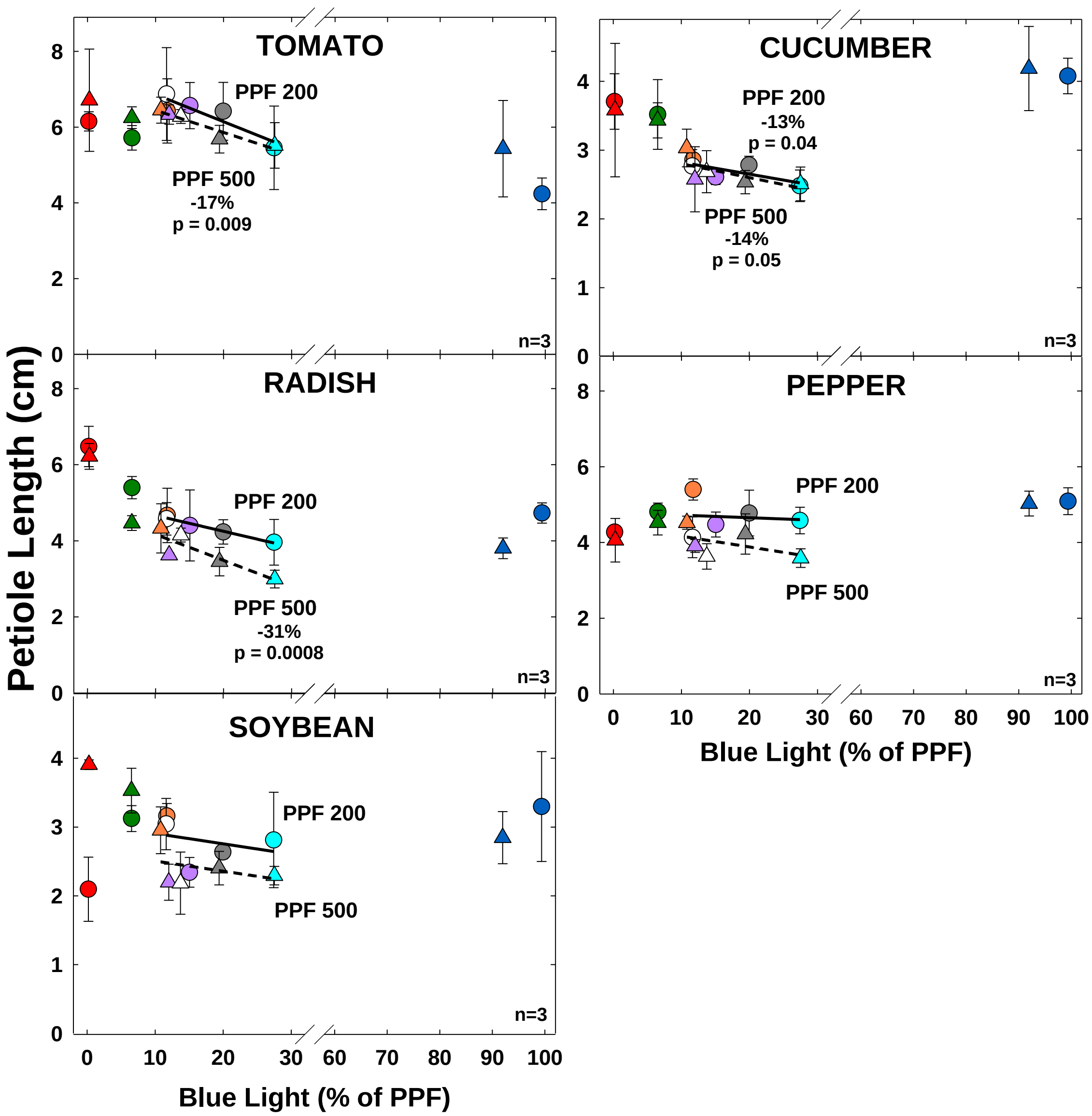

Fig. 12.The effect of percent blue light on petiole length for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

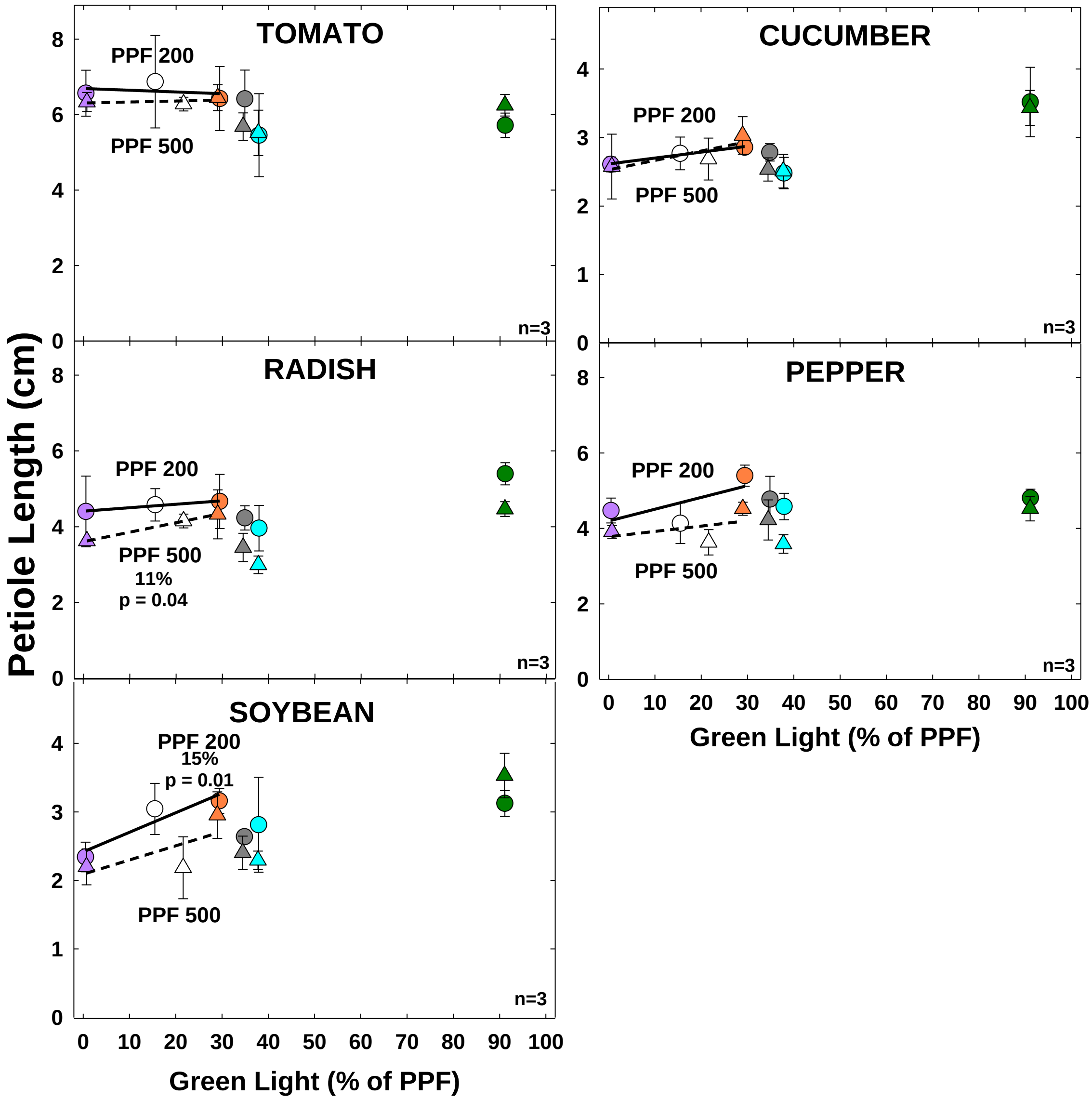

Fig. 13. The effect of percent green light on petiole length for seven species under two PPFs. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown.

Supplemental Data

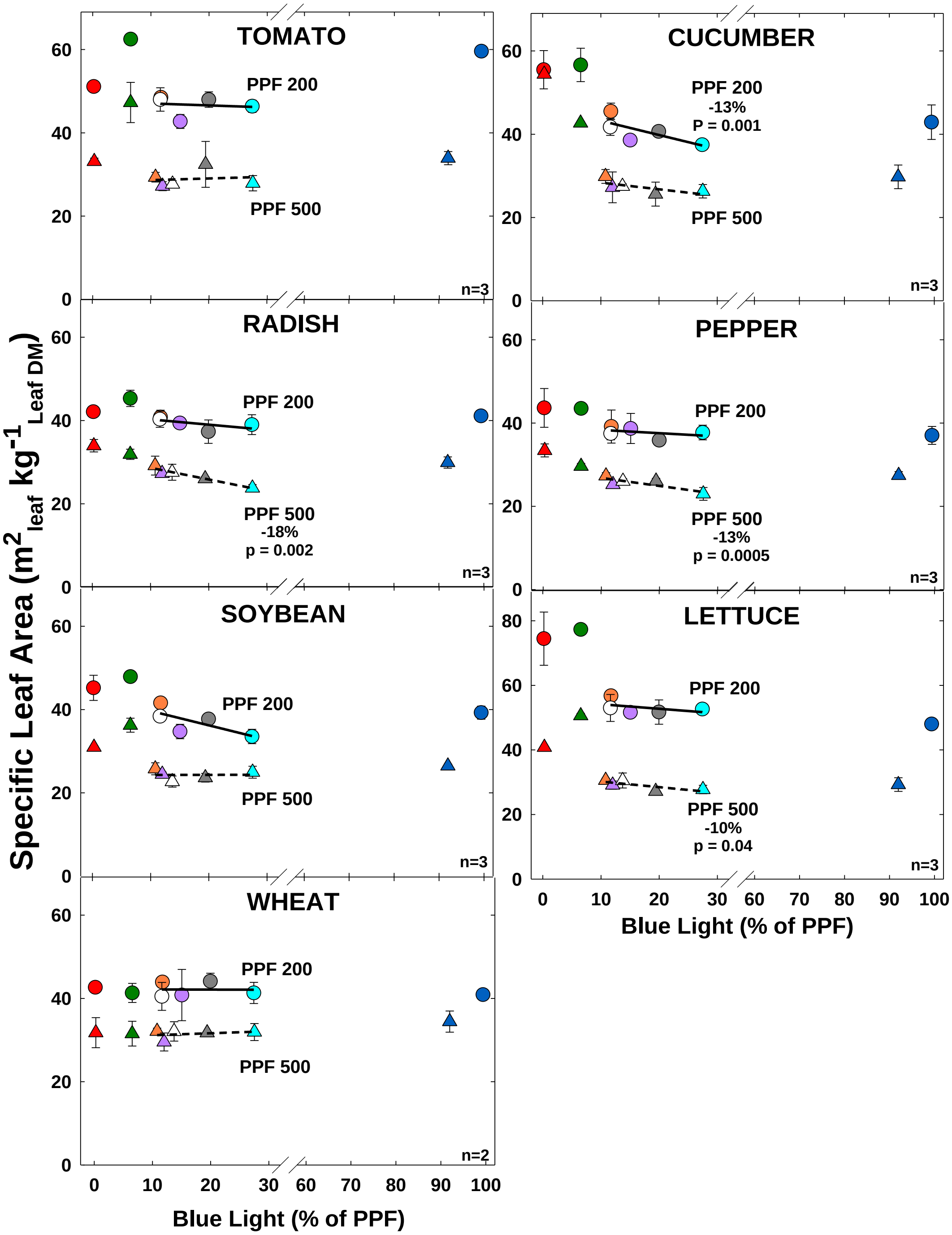

Figure S1. The effect of percent blue light on specific leaf area for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

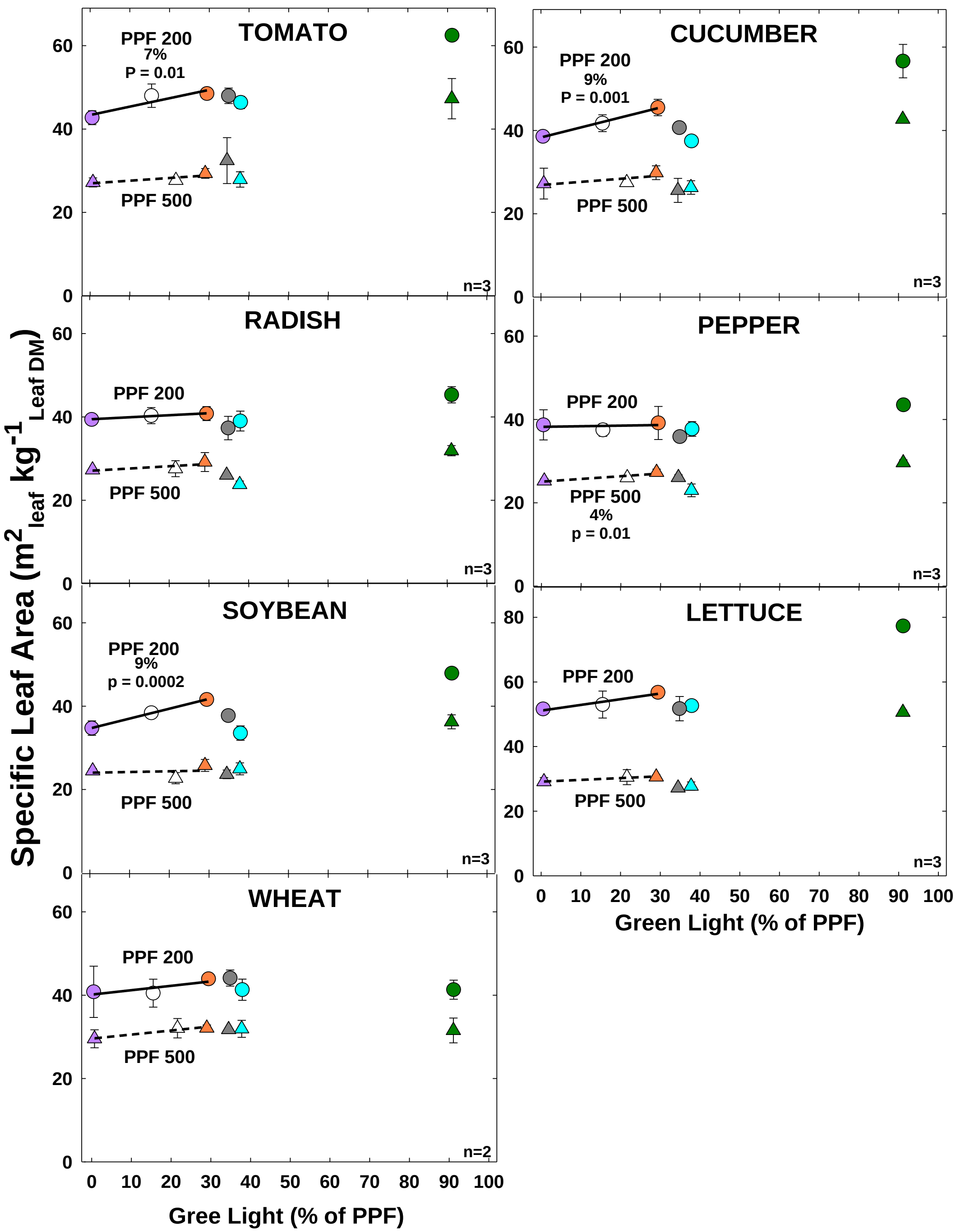

Figure S2. The effect of percent green light on specific leaf area for seven species under two PPFs. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown.

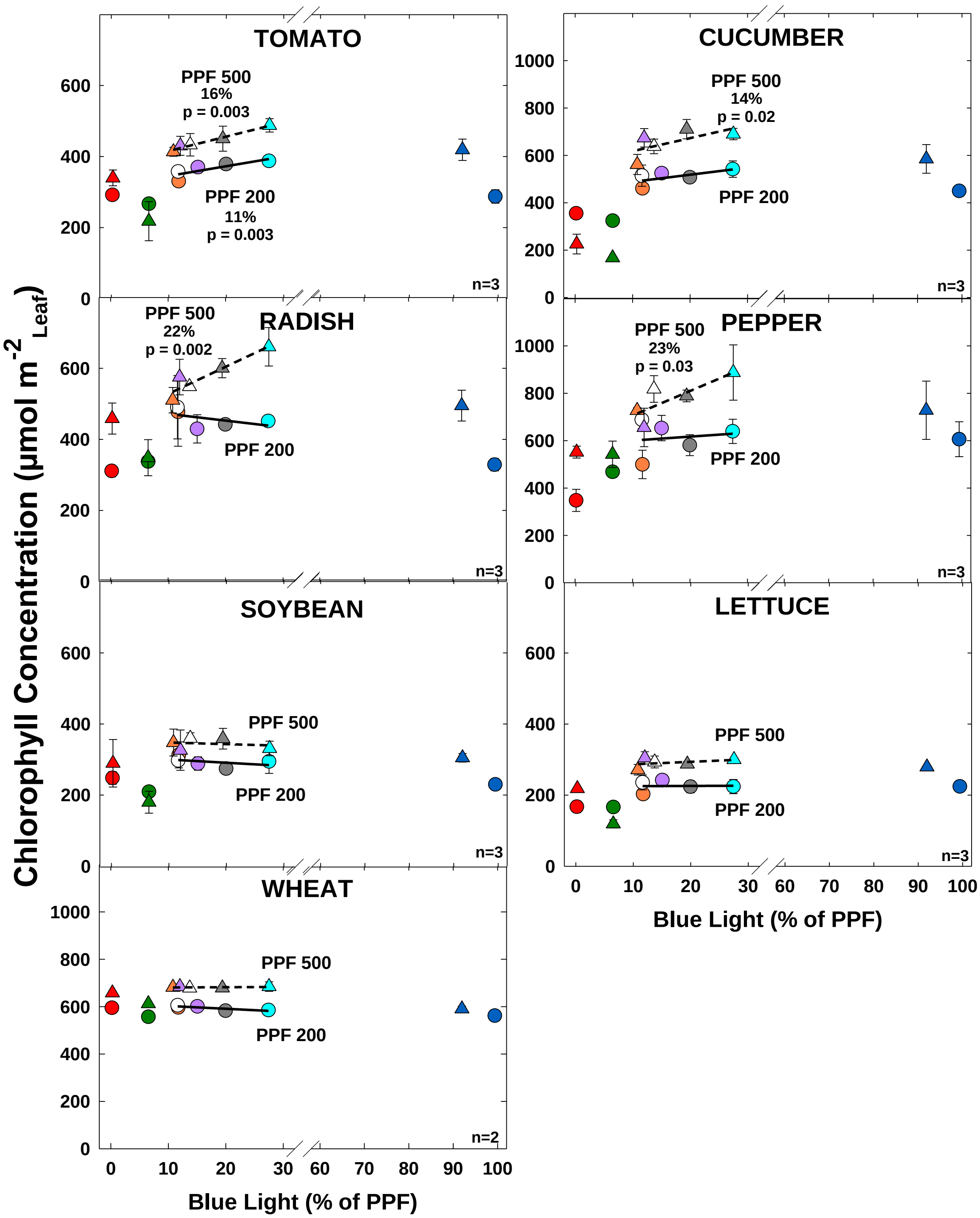

Figure S3. The effect of percent blue light on chlorophyll concentration for seven species under two PPFs. Note scale break for percent BL between 30 and 60. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the four treatments with comparable green and red wavelengths for each PPF. When significant, p-values and percent change are shown.

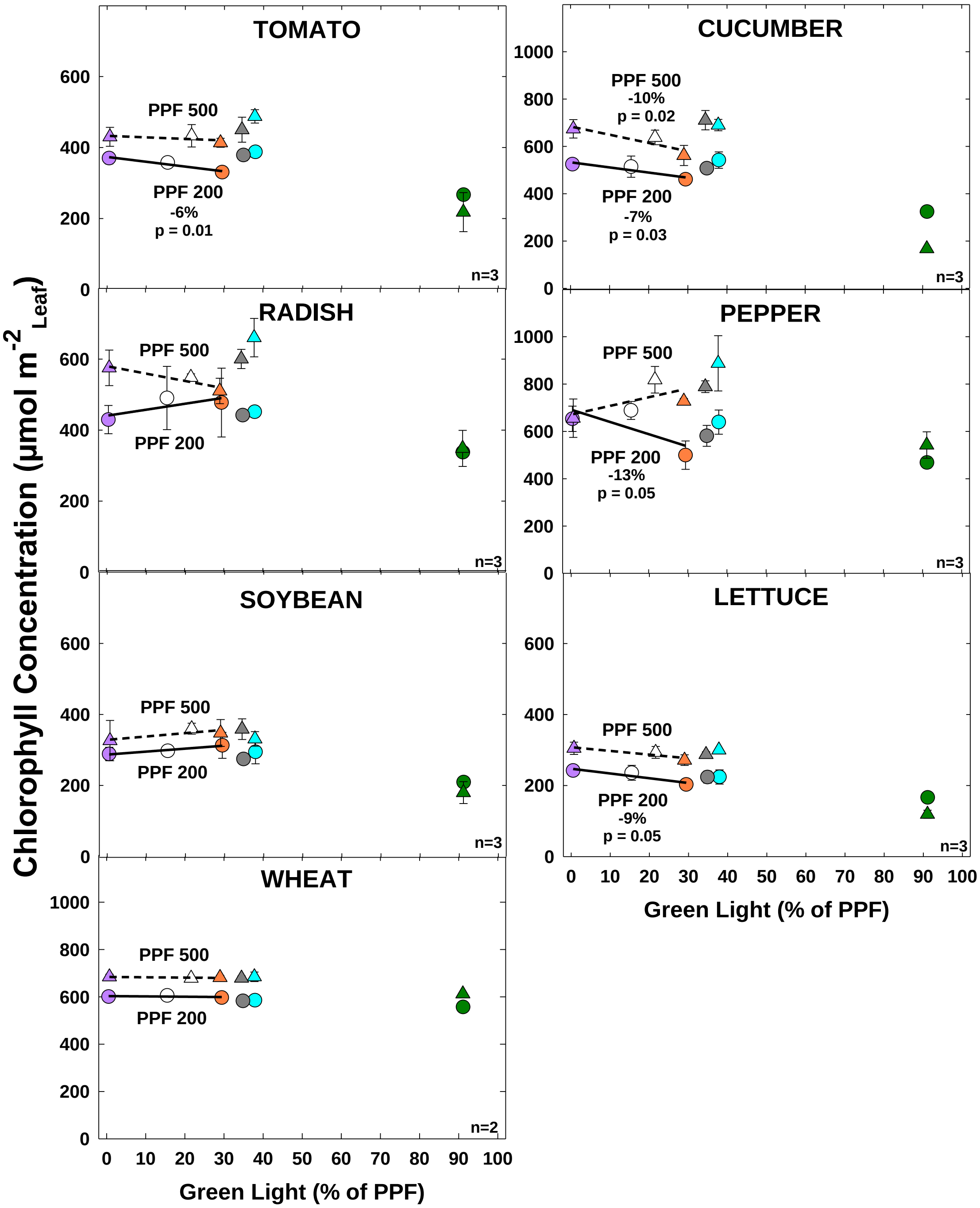

Figure S4. The effect of percent green light on chlorophyll concentration for seven species under two PPFs. Each data point shows the mean and standard deviation of three replicate studies for each species (n=3). Some error bars are smaller than the symbol size. See Fig. 1 for symbol color and shape legend. To minimize confounding spectral effects the regression line includes only the three treatments with comparable blue and red wavelengths for each PPF. When significant, p-values and percent change are shown.