Far-red Photons Increase Light Capture but Have Lower Photosynthetic Capacity Than Red Photons

Jun Liu
Department of Horticultural Sciences, Texas A&M AgriLife Research Center at Dallas, Texas A&M University, Dallas, TX 75252, USA

Marc W. van Iersel
Horticultural Physiology Laboratory, Department of Horticulture, University of Georgia, Athens, GA 30602, USA

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ABSTRACT. Far-red photons (700–750 nm) can accelerate crop growth during indoor production through both physiological and morphological processes. A previous study showed that far-red photons can drive photosynthesis with efficiency similar to that of traditionally defined photosynthetically active photons (400–700 nm) if they are provided together with shorter-wavelength photons. Far-red photons also promote leaf and canopy expansion, which can increase light interception and growth. This study aimed to distinguish the contribution of morphological and physiological changes to crop growth induced by substituting red photons with far-red photons. We studied the long-term effects of substituting red photons with far-red photons on canopy light interception and whole-plant photosynthesis. ‘Little Gem’ lettuce (Lactuca sativa) seedlings were grown under four light spectrums of the same total photon flux density (400–750 nm). In addition to a background of a mixture of white and blue photons of 150 μmol·m⁻²·s⁻¹, we provided 51 μmol·m⁻²·s⁻¹ red photons, far-red photons, or mixtures of red and far-red photons. In the first run, plants were harvested twice. The first harvest was at canopy closure, and the second harvest was when plants reached full size. In the second run, we harvested lettuce plants more frequently to minimize leaf overlap and interplant competition. We found that far-red photon substitution promoted leaf and canopy expansion and increased light interception. The effect of far-red photon substitution on leaf and canopy expansion was stronger in the second run than in the first run, likely because of lower plant density in the second run when plants were harvested more frequently. Far-red photon substitution of red photons decreased the amount of extended photosynthetically active radiation (ePAR) photons (400–750 nm) absorbed by leaves because of the lower leaf absorbance of far-red photons. The greater effect on canopy expansion in the second run of far-red photons substitution was able to exceed the reduction of ePAR photon absorption by leaves; therefore, we observed an increased crop gross photosynthetic rate (Pg) between the second and third harvests during the second run. However, during the first run, lower absorbance of ePAR completely offset the effect of the greater canopy size and light interception, and crop Pg was decreased in the first run before the first harvest. The changes in light interception and crop Pg resulting from far-red photon substitution did not affect dry weight. Far-red photons had photosynthetic activity when applied with a blue and white light mixture, but their efficiency was approximately half that of red photons, potentially because of the lower absorbance of far-red photons. In conclusion, far-red photon substitution of red photons increased canopy size but decreased ePAR photons absorbed by leaves and did not increase the final dry weight. Because far-red light-emitting diodes (LEDs) have higher efficacy for converting electricity into photons, including far-red LEDs in fixtures for sole-source lighting can reduce energy costs without decreasing lettuce yields.

Far-red photons (700–750 nm) are rarely used in sole-source lighting for controlled-environment agriculture. However, plants have evolved under sunlight for millions of years, and far-red photons comprise ~16% of photons in ePAR photons (400–750 nm) (Zhen and Bugbee 2020a). Far-red photons are important for efficient photochemistry and photosynthesis (Zhen and Bugbee 2020a; Zhen and van Iersel 2017) and can increase light capture and crop yield of numerous vegetable and ornamental crops under sole-source lighting by eliciting leaf elongation (Craver et al. 2018; Legendre and van Iersel 2021; Meng and Runkle 2019). With its benefits for both morphological and photosynthetic processes, there is increasing interest in far-red applications in controlled-environment agriculture. Far-red photons have photosynthetic activity when combined with photons with shorter wavelengths. However, far-red photons are traditionally considered to have very low photosynthetic efficiency because they have too little energy to drive photochemistry (Emerson and Rabinowitch 1960; Evans 1987; McCree 1971). Only photons with wavelengths of 400 to 700 nm are typically considered photosynthetically active and designated as photosynthetically active radiation (PAR) (McCree 1971, 1972). Higher plants have two photosystems, photosystem I (PSI) and photosystem II (PSII), that operate in series to drive linear photosynthetic electron transport. Far-red photons...
are unable to excite PSII, but they can excite PSI. Therefore, far-red photons have minimal photosynthetic activity when applied alone, but they can drive photosynthesis when applied along with PAR (Emerson and Lewis 1943; Evans 1987; Zhen and van Iersel 2017). Furthermore, PAR photons tend to over-excite PSII compared with PSI, except for light between 570 and 620 nm and >690 nm, resulting in a suboptimal quantum yield of photosynthesis (Evans 1987). Because PSII and PSI work linearly, these two photosystems must work at matching rates to achieve high linear electron transport rates. Far-red photons can balance the excitation energy between the two photosystems and increase $\Phi_{\text{PSII}}$ (Zhen and van Iersel 2017). Therefore, leaves exposed to both far-red and PAR photons often have higher photosynthetic rates than the sum of photosynthetic rates when far-red photons and PAR photons are applied separately. This synergistic effect of far-red photons on photosynthesis is known as the Emerson enhancement effect (Emerson 1957; Myers 1971). In recent years, the addition of far-red photons to PAR to improve the Emerson enhancement effect and increased leaf elongation and light capture on the growth and yield resulting from far-red photon inclusion. A previous study of long-term effects of far-red substitution reported a higher dry weight of lettuce grown under 300 $\mu$mol-m$^{-2}$-s$^{-1}$ PAR photons with 50 $\mu$mol-m$^{-2}$-s$^{-1}$ far-red photons compared with lettuce grown under 350 $\mu$mol-m$^{-2}$-s$^{-1}$ PAR photons, despite the lower chlorophyll concentration and leaf level absorption (Zhen and Bugbee 2020b). During that study, the photon flux density across the entire PAR range was decreased by substituting PAR with far-red photons. The effects of substituting photons in the PAR range with far-red on plant morphology are likely greater when only red photons are replaced with far-red photons because of the importance of the ratio of red to far-red on phytochrome activity (Sager et al. 1988). Also, the previous study only tested the effect of far-red photon substitution compared with no far-red photon substitution on carbon assimilation and growth (Zhen and Bugbee 2020b). We aimed to quantify the response of lettuce carbon assimilation and growth to different amounts of far-red photon substitution. Thus, we quantified the effects of substituting 0 to 51 $\mu$mol-m$^{-2}$-s$^{-1}$ red photons with far-red photons on light capture and canopy photosynthesis of lettuce.

### Material and Methods

#### Plant material

Three lettuce (Lactuca sativa) seeds were sown in 10-cm square pots filled with a peat-based soilless substrate (Fafard 4P; SunGro Horticulture, Agawam, MA, USA). Eighteen pots of lettuce were placed in a 30.5-× 61-cm tray. Then, seeds were germinated in growth chambers (E15; Conviron, Winnipeg, MB, Canada) under a 16-h photoperiod with a photosynthetic photon flux density of 236 $\mu$mol-m$^{-2}$-s$^{-1}$ from warm-white fluorescent light. The temperature for germination was (mean ± SD) 23.9 ± 1.1°C, and the vapor pressure deficit was 0.56 ± 0.14 kPa. The CO$_2$ concentration was maintained at 816 ± 15 $\mu$mol-mol$^{-1}$. The pots were top-watered with tap water to keep them moist during germination. Seedlings were thinned to one per pot after emergence. Seedlings were selected for uniformity at 6 d after sowing for the first run and at 8 d after sowing for the second run. A total of 144 plants per run were selected and randomly distributed to eight trays with a capillary mat in the bottom. Then, these eight trays of 18 lettuce seedlings were transferred to a whole-plant gas exchange system, where they were exposed to different light treatments, and whole canopy gas exchange measurements were obtained.

The whole-plant gas exchange system consists of 10 acrylic gas exchange chambers, eight of which held one tray each (18 plants). Those eight gas exchange chambers were placed inside two growth
chambers (E15), with four gas exchange chambers inside each growth chamber. The other two chambers were left empty outside of the growth chambers as controls. The growth chambers were set at 10°C to remove the heat generated by the light-emitting diodes (LEDs) and condense water inside the gas exchange chambers to reduce the vapor pressure deficit. Temperature and relative humidity probes (HMP50; Campbell Scientific, Logan, UT, USA) measured temperature and relative humidity inside each gas exchange chamber. Temperature and relative humidity were recorded by a datalogger (CR10T, Campbell Scientific) every 15 min. Each gas exchange chamber was equipped with a heater that maintained the temperature of each chamber to a setpoint based on the temperature reading of a probe. A secondary temperature sensor (type T thermocouple) also measured the temperature of each chamber as a secondary temperature reading. The secondary temperature was recorded by a different datalogger (CR23X, Campbell Scientific) every 10 min. Air blowing into the gas exchange chambers was enriched with CO2 up to \(800 \mu \text{mol} \cdot \text{mol}^{-1}\). The temperature measured by the secondary temperature sensor and vapor pressure deficit was 23.0 ± 1.5°C and 1.2 ± 0.41 kPa, respectively, across this whole study. The average temperature in each chamber can be found in Supplemental Table 1. Plants were sub-irrigated when needed with a fertilizer solution of 100 mg L\(^{-1}\) N made with a water-soluble fertilizer (Peters Excel 15N–2.2P–12.4K Cal-Mag; Everris, Marysville, OH, USA). More details about the gas exchange system can be found in the work by van Iersel and Bugbee (2000).

Light treatments

We substituted different amounts of red photons with far-red photons to test the effects of red and far-red photons. The PAR was provided by commercial LED grow lights (Pro 650; LumiGrow, Emeryville, CA, USA). Far-red photons were provided by custom-made LED bars (peak at 738 nm; full width at half maximum = 20 nm). The LEDs were placed on top of each acrylic gas exchange chamber. Reflective walls were placed between gas exchange chambers to block light pollution between chambers. All plants were grown under a 16-h photoperiod with an ePAR of (mean ± SD) 205 ± 2 μmol m\(^{-2}\) s\(^{-1}\) for the first run and 208 ± 1 μmol m\(^{-2}\) s\(^{-1}\) for the second run. The background light was set as \(\sim 150 \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\) of white and blue photons from LEDs, to which 51 μmol m\(^{-2}\) s\(^{-1}\) of red and far-red photons were added (0/51, 17/34, 34/17, and 51/0 μmol m\(^{-2}\) s\(^{-1}\) of red/far-red photons) to create four light treatments with different ratios of red to far-red photons. Light spectral distribution was measured by a spectroradiometer (PS-100; Apogee Instruments, Logan, UT, USA) at the center of each gas exchange chamber. The detailed spectral distributions can be found in Supplemental Table 1. The four light treatments with different ratios of red to far-red photons are referred to by the far-red photon portion relative to ePAR (%FR) because it was shown to be a better metric than phytochrome photo-equilibrium or the ratio of red to far-red photons (Kusuma and Bugbee 2021). The %FR was calculated based on measured light spectrums. Hereafter, the four light treatments are referred to as 2.5%, 9.2%, 16.5%, and 23.6% FR in the first run and 3.2%, 11.0%, 19.0%, and 27.4% FR during the second run.

A randomized complete block design was used, with the four light treatments randomly assigned to the four gas exchange chambers within each growth chamber. The two growth chambers were treated as two replications in each run. All plants within each gas exchange chamber were exposed to the same light treatment and treated as one experimental unit.

Data collection and statistical analyses

**Photosynthetic measurements.** The whole-plant gas exchange system required 10 min in the first run and 20 min in the second run to complete one round of gas exchange measurements of all ten chambers. The time to flush air in tubing of the whole-plant gas exchange system was increased to further eliminate noise in data in the second run. Therefore, the measurement duration was longer in the second run. The chamber-level net photosynthetic rate (crop \(P_g\); micromoles per second) of lettuce plants in each gas exchange chamber was recorded by a datalogger. The average daily \(P_m\), dark respiration (crop \(R_d\); micromoles per second), and gross photosynthetic rate (crop \(P_g\); micromoles per second) were calculated for each chamber. Crop \(P_g\) was calculated as \(P_g = P_m + |R_d|\), assuming respiration is the same in the dark and in the light (van Iersel and Bugbee 2000). We also calculated the average daily plant level \(P_m\), \(P_m\), and \(R_d\) (micromoles per second per plant) by dividing the crop \(P_g\), \(P_m\), and \(R_d\) by the number of plants in the gas exchange chambers on each day.

The daily carbon gain per plant (micromoles per day per plant), which is the net amount of carbon gained and a measure of growth rate, was calculated as:

\[
\text{DCG} = (P_g \times 16 \text{ h} - |R_d| \times 8 \text{ h}) \times 3600 \text{ s}^{-1} \times 10^{-6} \text{ mol} \cdot \text{mol}^{-1}
\]

The cumulative carbon gain (CCG) per plant (moles per plant) was calculated as the integral of the daily carbon gain over time. To test the reliability of our whole-plant gas exchange system, the total CCG was calculated for each run by integrating the CCG of all plants in the system throughout the life cycle of plants. We also calculated the total dry weight of each run by adding together the shoot dry weight of plants during all harvests; thereafter, the CCG and shoot dry weight were regressed. To further quantify the photosynthetic activity of far-red photons, at 21 d and 29 d after light treatment of the first run and at 9 d and 32 d after light treatment of the second run, we briefly turned off the far-red photons and measured the change in photosynthesis in response to the reduction of far-red photons. At 32 d after light treatment of the second run, near the end of this study (the last harvest was at 33 d after light treatment), we also turned off the red lights and measured the changes in photosynthesis in response to red photons. Because the white and blue background lights have red and far-red photons, the decrease in chamber \(P_g\) occurred in response to the reduction of red and far-red photon flux density (PFD).

**Morphology and growth.** We obtained top view canopy images of the plants to quantify the projected canopy size (PCS) and estimate canopy light interception. In the first run, we photographed the whole tray of lettuce plants in situ with a cellphone camera. The images were obtained twice per week until the canopies were too large to be captured within the camera’s field of view, 20 d after the plants were moved into the whole-plant gas exchange system. The PCS was quantified with Image J (National Institutes of Health, Bethesda, MD, USA) by extracting the area of green pixels in the photos. In the second run, the whole tray was imaged by our custom-made chlorophyll fluorescent imaging (CFI) system, similar to that described by Kim and van Iersel (2022). The plants were imaged twice per week throughout the whole growing cycle. Photos from the chlorophyll fluorescent imaging system were analyzed using Python programming language following a similar principle as that reported by Kim and van Iersel (2022).
We grew two rounds of lettuce plants, which we denoted as the first run and the second run. During the first run, we harvested nine plants at 22 d after light treatment when the canopy was closed. The first harvest followed a checkered pattern, and every other plant on each tray was picked. The remaining nine plants were harvested 35 d after light treatment when the canopy was closed again. Plant densities were 97 plants/m² before and 48 plants/m² after the first harvest. In the second run, we harvested nine plants at 12 d after light treatment, five plants at 18 d after light treatment, and the remaining four plants at 33 d after light treatment. During the first harvest, we similarly followed the checkered pattern. During the second harvest, we randomly picked five plants and redistributed the remaining four plants on the tray to minimize leaf overlapping. We harvested more frequently in the second run to minimize leaf overlap and light competition among plants. Plant densities were 97 plants/m² before the first harvest, 48 plants/m² before the second harvest, and 22 plants/m² before the third harvest. The total leaf area, length of the longest leaf, and dry weight of the harvested plants were measured, and the SLW was calculated as the leaf dry weight divided by the leaf area. Leaf samples were also collected for a chlorophyll content analysis at the first harvest in the first run and at the third harvest in the second run.

We also measured leaf-level light absorptance at each harvest, except for the first harvest of the second run, when the leaves were too small. Light absorptance was measured using a method similar to that described by Zhen et al. (2019). Three newly expanded leaves were selected from each chamber for light absorbance measurement. Light spectrums were measured by a spectroradiometer (PS-100). An LED light with multiple LEDs with different spectrums (GN-DMX512-D LED System; EyeHortilux, Mentor, OH, USA) was used to provide light ranging from 400 to 770 nm. To measure transmittance, the light spectrum of the LED light was first measured inside a dark room with the spectroradiometer directly under the LED light. Then, a lettuce leaf was placed between the LED light and the spectroradiometer, with the adaxial side facing up, to measure the transmitted light spectrum under the leaves. Then, light transmittance of the leaves was calculated at 1 nm resolution as the transmitted spectrum divided by the LED light spectrum. Light reflectance of leaves was measured using a fiber-optic cable perpendicular to the adaxial surface of lettuce leaves under the LED light. Then, the reflectance spectrum was divided by the reflectance spectrum of a Halon white standard (AS-004, Apogee Instruments) to calculate the leaf reflectance. Leaf absorptance was calculated as 100% − reflectance − transmittance. The average absorptance spectrum of the three leaves from the same chamber was used for later analyses. The amount of ePAR photons absorbed by leaves was calculated by multiplying the light spectrum of the grow light with the absorptance spectrum of the respective leaf.

Fig. 1. The length of the longest leaf of ‘Little Gem’ lettuce plants increased linearly with the far-red photons portion relative to extended photosynthetically active radiation (ePAR) photons in the grow light spectrum at the first 22 d after light treatment started and the second (35 d after light treatment started) for the first run (A) and at the first 12 d after light treatment started and the second harvest 18 d after light treatment started) for the second run (B).
DATA ANALYSIS. The effect of the %FR of the growing spectrum on various parameters was tested using spreadsheet software (Excel; Microsoft Corp., Redmond, Seattle, WA, USA) with a regression analysis; chamber temperature was a covariate because temperature varied among different chambers. Linear regression was conducted for the total CCG of each run and total shoot dry weight in Excel as well. We also tested the correlation between the length of the longest leaf and projected canopy size, light absorptance and SLW, and the correlation between P_g and PCS, amount of ePAR photons absorbed by leaves, and %FR. The multiple regression was performed using statistical software (JMP Pro version 15; SAS Institute Inc., Cary, NC, USA). The total PCS and the amount of ePAR photons absorbed by leaves between the two runs were compared using a paired t test in Excel.

Results and Discussion

Far-red photon substitution of red photons induced leaf expansion and hyponasty and increased the projected canopy size. Morphological changes in lettuce plants induced by far-red photons were visible early. In the first run, at 10 d after light treatment, a greater %FR increased seedling height (Supplemental Fig. 1A). In the second run, the effect of %FR was already visible at 7 d after light treatment (Supplemental Fig. 1B). The length of the longest leaf increased linearly with %FR at all harvests (Fig. 1). In the first run, we observed 1.5-mm and 1.1-mm increases in the longest leaf length at the first and second harvests, respectively, with each 1% increase in %FR (Fig. 1A). The increase of the longest leaf length was more substantial in the second run. With every 1% increase in %FR, the longest leaf length increased 1.5 mm at the first harvest and 2.0 mm at the second harvest (Fig. 1B). The increase in plant height is likely a result of both an increase in leaf length and a change in leaf angle. Far-red photons stimulates upward growth of leaves to form a steeper leaf angle, known as hyponasty. Our observations of taller plants and longer leaves align with the typical symptoms of a shade avoidance response (Gommers et al. 2013; Ruberti et al. 2012).

The PCS in the first run was not increased by increasing the %FR until 20 d after light treatment (Fig. 2A), which was the last day that PCS was measured. With the %FR increasing from 2.5% to 23.6%, the PCS increased from 137 to 164 cm²/plant (20%) (Fig. 2A, Supplemental Fig. 2). For the second run, the PCS increased with increasing %FR starting from 18 d after light treatment until the end of the study at 32 d after light treatment (Supplemental Fig. 2). In the second run at 21 d after light treatment, the increase in PCS was much greater (from 147 to 238 cm²/plant or 61.6%) compared with that of the first run, despite similar %FR (Fig. 2B).
Previous studies showed that inclusion of far-red photons can induce leaf expansion and, thus, canopy enlargement. The canopy enlargement resulted in greater light interception by the crop and higher dry weight accumulation under sole-source lighting (Craver et al. 2018; Hernandez and Spalholz 2019; Jin et al. 2021; Kubota et al. 2012; Legendre and van Iersel 2021; Li and Kubota 2009; Meng and Runkle 2012; Park and Runkle 2017; Zhen and Bugbee 2020a, 2020b; Zou et al. 2019). During our study, we similarly noted increased leaf expansion in response to %FR, as is evident from the greater length of the longest leaf (Fig. 1) and increased plant height (Supplemental Fig. 1), although the effect on leaf expansion was greater in the second run than in the first run. The effect of the %FR on canopy enlargement was also more evident in the second run (Fig. 2). We found a strong correlation between the PCS and the longest leaf length for both runs, suggesting that the treatment effects on canopy enlargement were a consequence of effects on leaf expansion. In the first run, with an increase of 1 cm in the longest leaf length at 22 d after light treatment, PCS at 20 d after light treatment increased 3.7 cm²/plant ($R^2 = 0.81; P < 0.01$). In the second run, the PCS at 21 d after light treatment also strongly correlated with the longest leaf length at the nearest harvest (18 d after light treatment) ($R^2 = 0.78; P < 0.01$). Each 1-cm increase in longest leaf length at 18 d after light treatment was associated with an 18.8-cm²/plant increase in PCS at 21 d after light treatment, which was much higher than that in the first run. Our data showed that substitution of red photons with far-red photons resulted in increased leaf expansion and canopy size. The effect of far-red PFD on leaf length and canopy size was greater in the second run than in the first run.

The smaller effect of far-red substitution on leaf length and canopy enlargement in the first run than in the second run may be related to plant density. Previous studies have found that high planting density can reduce the effect of far-red photons on crop growth and yield (Jin et al. 2021; Shibuya et al. 2020). At 20 d after light treatment in the first run, we had not harvested any plants; therefore, the plant density was 97 plants/m². For the second run, at 21 d after light treatment, the lettuce plants had already gone through two harvests (at 12 and 18 d after light treatment), and the plant density was reduced to 21.5 plants/m². At a high plant density, the canopy closes faster, and there is limited benefit to making larger leaves with far-red photon substitution (Jin et al. 2021; Shibuya et al. 2020) because increased canopy size increases interplant competition for light. The effectiveness of far-red photon substitution on leaf expansion and canopy enlargement may be higher when plants do not receive any additional signal, which also indicates shaded conditions (Jin et al. 2021; Shibuya et al. 2020). It is possible that, in our study, the difference in planting density caused by different
harvest times between the two runs resulted in a greater effect of far-red photon substitution on leaf length and PCS in the second run (Fig. 2).

**Photosynthetic rate was affected by far-red PFD through effects on canopy enlargement and leaf absorptance**

Crop \( P_g \) did not consistently respond to \( \%FR \) between the two runs (Figs. 3 and 4). In the first run, \( P_g \) decreased with increasing \( \%FR \) at 7 to 10, 13 to 16, and 19 to 21 d after light treatment. The negative effect of far-red photon substitution on \( P_g \) disappeared after the first harvest at 22 d after light treatment (Fig. 4A and 4C). At 20 d after light treatment, for example, when \( \%FR \) increased from 2.5% to 23.6%, crop \( P_g \) decreased by 26.2% (Fig. 3A). In the second run, \( P_g \) increased with \( \%FR \) in ePAR from 21 to 25 d after light treatment (Fig. 4B and 4D). At 21 d after light treatment, crop \( P_g \) increased linearly with \( \%FR \) by 31.5% when \( \%FR \) increased from 3.2% to 27.4% (Fig. 3B). It was worth noting that harvests and, therefore, reducing plant density seemed to interact with far-red photon substitution on crop \( P_g \). During the first run, the decrease in crop \( P_g \) diminished after the first harvest (Fig. 4A and 4C). In the second run, an increase in crop \( P_g \) induced by far-red photon substitution appeared only after the second harvest, when the plant density was reduced to 22 plants/m\(^2\) (Fig. 4B and 4D).

The leaf level light absorptance of lettuce only decreased with increasing \( \%FR \) at the first harvest of the first run (Supplemental Figs. 3 and 4). At this time, the decrease in leaf-level absorptance was not observed in the blue waveband (400–499 nm), whereas the light absorptance across the green (500–599 nm), red (600–699 nm), far-red (700–750 nm), and ePAR wavebands (400–750 nm) all decreased with increasing \( \%FR \) (Supplemental Fig. 4A). The difference in light absorptance of leaves in response to \( \%FR \) was not observed during the second harvest of the first run or during the two harvests of the second run (Supplemental Figs. 3B–D and 4B–D). Despite the inconsistent effect of far-red photon substitution on leaf-level light absorptance, lettuce leaves grown under higher \( \%FR \) absorbed less ePAR photons because red photons were substituted with more poorly absorbed far-red photons (Fig. 5, Supplemental Fig. 4).

We did not find any effect of far-red photon substitution on the chlorophyll a and b concentrations, total chlorophyll concentration, and chlorophyll a-to-b ratio in either runs (Supplemental Fig. 5). The response of SLW to increasing \( \%FR \) was strongest in younger plants and decreased with increasing \( \%FR \) at the first harvest of the first run and at the first two harvests of the second run (Fig. 6). For the last harvest of both runs, \( \%FR \) no longer affected SLW (Fig. 6). A comparison of the first two harvests of the second run showed that the decrease in SLW was more significant at the first (0.36 g·m\(^{-2}\) decrease in SLW per 1% increase in \( \%FR \)) than at the second harvest (0.25 g·m\(^{-2}\) decrease in SLW per 1% increase in \( \%FR \)) (Fig. 6). The leaf absorptance across the ePAR waveband at the first harvest of the first run was positively correlated with SLW (\( R^2 = 0.71; P = 0.01 \)). At the second harvest of the second run, SLW was similarly correlated with leaf absorptance across the ePAR region (\( R^2 = 0.50; P = 0.05 \)). However, for the last harvest of both runs, when
Far-red PFD no longer affected absorptance, SLW and absorptance were not correlated ($P > 0.50$).

Far-red photon substitution resulted in inconsistent effects on crop $P_g$ in the two runs because of combined morphological and physiological changes induced by far-red photon substitution. In the first run, $P_g$ was negatively correlated with %FR before the first harvest (Figs. 3 and 4), despite higher PCS at 20 d after light treatment (Fig. 2). Multiple regression detected no correlation between crop $P_g$, PCS at 20 d after light treatment, and the amount of ePAR photons absorbed by leaves. The increase in PCS and decrease in the amount of ePAR photons absorbed by leaves in response to %FR may have offset each other. PCS increased 19.7%, whereas ePAR photons absorbed by leaves decreased 20.9% when %FR increased from 2.5% to 23.6%, possibly resulting in a similar amount of canopy light capture under different far-red PFD treatments. Therefore, the lower $P_g$ in response to higher %FR observed at 20 d after light treatment in the first run may have been caused factors other than light capture, for example, lower leaf-level quantum yield of photosynthesis, which was previously observed in lettuce in response to far-red supplementation (Zou et al. 2019). Zou et al. (2019) found that lettuce leaves grown under supplemental far-red photons had a lower leaf level quantum yield of photosynthesis based on absorbed photons. Therefore, they had a lower net photosynthetic rate than leaves grown without supplemental far-red photons when absorbing the same amount of PAR (Zou et al. 2019).

In the second run, %FR increased crop $P_g$ from 21 to 25 d after light treatment, contrary to the results from the first run (Figs. 3 and 4). Multiple regression indicated that crop $P_g$ at 21 d after light treatment was positively correlated with both PCS at 21 d after light treatment ($P < 0.01$) and ePAR photons absorbed by leaves ($P < 0.01$). The positive correlation of PCS to crop $P_g$ is consistent with the hypothesis that higher %FR promotes canopy enlargement and increases photon capture and, thus, crop $P_g$. The positive correlation between crop $P_g$ at 21 d after light treatment (Fig. 3B) and ePAR photons absorbed by leaves (Fig. 5) indicated that a lower amount of ePAR photons absorbed by leaves grown under higher %FR decreased light capture and photosynthesis. However, the lower amount of ePAR photons absorbed by leaves grown under higher %FR did not offset the higher light interception elicited by higher PCS because $P_g$ still increased with increasing %FR on this date (Figs. 3B and 4B): the PCS at 21 d after light treatment increased 61.6% when %FR increased from 3.2% to 27.4% (Fig. 2), whereas the amount of ePAR photons absorbed by leaves decreased by only 16.6% (Fig. 5).

At 32 d after light treatment in the second run, when crop $P_g$ no longer increased with increasing %FR, multiple regression showed that crop $P_g$ was negatively correlated with %FR ($P = 0.01$) but...
positively correlated with PCS ($P < 0.01$). At this time, crop $P_g$ no longer correlated with ePAR photons absorbed by leaves. The negative correlation between crop $P_g$ and %FR suggested a lower quantum yield of photosynthesis to use absorbed photons of leaves grown under high %FR, similar to what was observed by Zou et al. (2019). This may explain why far-red photon substitution no longer increased crop $P_g$ despite higher PCS. At this stage, individual leaves grown under high %FR still absorbed fewer ePAR photons (Fig. 5). However, the plants had multiple leaf layers, which may have diminished the difference in light capture induced by far-red photon substitution at the canopy level (Zhen and Bugbee 2020b). Therefore, crop $P_g$ was no longer correlated with the amount of ePAR photons absorbed by individual leaves.

In both runs, far-red photon substitution induced leaf expansion (Fig. 1), which increased PCS (Fig. 2, Supplemental Fig. 2). Far-red photon substitution also decreased ePAR photon absorbed by leaves in both runs (Fig. 5). Far-red photon substitution negatively affected crop $P_g$ in the first run, but it positively affected crop $P_g$ in the second run (Figs. 3 and 4). The contradictory effects of far-red photon substitution on crop $P_g$ between the two runs may be associated with differences in plant density. Between the two runs, plant density in the second run after the first harvest (12 d after light treatment) was lower than plant density at 12 d after light treatment in the first run. In the first run, because plant density (as well as light interception) was higher at 21 d after light treatment than that at 20 d after light treatment in the second run, far-red photon substitution may have limited benefits for light interception through leaf expansion for the first run (19.7% increase in PCS when %FR increased from 2.5% to 23.6% in the first run compared to 61.6% increase in PCS when %FR increased from 3.2% to 27.4% in the second run). The PCS of individual lettuce plants in the first run was consistently lower than the PCS of plants in the second run at similar times after light treatment (Fig. 2, Supplemental Fig. 2; note the difference in the scales of the y-axes). Crop $P_g$ in the first run stopped increasing before the first harvest but increased rapidly after the harvest reduced interplant competition (Fig. 4A). This validates the idea that, at high plant density, the effect of far-red photon substitution on light interception was limited. Furthermore, the higher plant density during part of the first run created more intracanopy shading than that in the second run (the total PCS was 1354 cm$^2$ at 20 d after light treatment in the first run, much larger than the 767 cm$^2$ measured at 21 d after light treatment in the second run; $P < 0.01$), which induced a more dramatic reduction in ePAR photons absorbed by individual leaves because of competition and possibly lower quantum yield of photosynthesis of leaves to use absorbed photons. Therefore, the higher plant density in the first run reduced the increase in PCS and magnified the
reduction in light absorptance by higher %FR compared with the second run. Consequently, in the first run, the smaller increase in PCS failed to compensate for the more dramatic decrease in leaf-level light absorptance and possibly lower quantum yield of photosynthesis of leaves at 20 d after light treatment, which resulted in lower crop \( P_g \) with increased far-red photon substitution.

Fig. 7. The relative decrease in gross photosynthetic rates (\( P_g \)) of ‘Little Gem’ lettuce plants in response to far-red lights being turned off in the first run (A) and to far-red (B) and red lights (C) being turned off at the end of the second run. ePAR = extended photosynthetically active radiation photons.
Far-red photons have lower photosynthetic activity than red photons

To quantify the photosynthetic activity of far-red photons at 21 d after light treatment (Supplemental Fig. 6) and 29 d after light treatment of the first run and 32 d after light treatment of the second run, the far-red LEDs were turned off temporarily. At 32 d after light treatment of the second run, we also turned off the red LEDs to quantify the change in the photosynthetic rate in response to reduction in red photons. $P_g$ decreased when far-red LEDs were turned off and increased when they were turned back on, indicating that far-red photons had photosynthetic activity (see an example in Supplemental Fig. 6). The change in $P_g$ correlated with the change in %FR (Fig. 7), with similar effects at 21 and 29 d after light treatment in the first run. Similarly, the responses of $P_g$ to far-red LEDs being turned off were similar at 9 d after light treatment and at 32 d after light treatment in the second run; therefore, the data for different days within each run were pooled and analyzed together (Fig. 7). The slopes of the regression lines were 0.43 and 0.56 for the first run and second run, respectively. A slope of 1 would indicate that a 1% change in %FR decreases $P_g$ by 1%, and that the quantum yield of photosynthesis (moles of carbon assimilated per mole incident photons) of the light being turned off equaled the average quantum yield of all photons in the grow light spectrum. Thus, these data suggest that the photosynthetic efficacy of the far-red photons was approximately half that of the average of all photons. At the end of the second run, we also turned off the red LEDs, and $P_g$ decreased by 0.98% for each 1% reduction of red photons in ePAR (Fig. 7C), suggesting that red photons had a quantum yield of photosynthesis similar to the average of the entire spectrum. These results indicate that far-red photons had significant photosynthetic activity, but it was substantially lower than that of red photons.

The decrease in $P_g$ in response to both red and far-red photon removal in our study was linear throughout the range of PFDs (0–51 μmol·m$^{-2}·s^{-1}$) (Fig. 7); therefore, it is likely that they were driving photosynthesis at their maximum quantum yield. The quantum yield of photosynthesis of red photons was 1.75-times (0.98/0.56) that of far-red photons. The lower light absorbance of far-red photons may be responsible for the lower quantum yield of photosynthesis of far-red photons than that of the red photons. The leaf level absorbance of red photons was 88.3% ± 3.3%, 1.97-times that of far-red photons (46.9% ± 3.0%) at the third harvest of the second run (Supplemental Fig. 4D); however, at the whole canopy level, the difference in absorbance between red and far-red photons would be smaller than at the leaf level. Another reason for the lower quantum yield of far-red photons is that only far-red photons absorbed by the light-harvesting complex surrounding PSI can be used for electron transport. Far-red photons absorbed by the PSII light-harvesting complex cannot be used for photosynthesis because they do not have enough energy to excite photosystem II.
The final dry weight of lettuce was unaffected by %FR (Fig. 8). The CCG was closely correlated with the total shoot dry weight summed over all harvests for both runs (Fig. 9) and unaffected by %FR ($P = 0.17$ for the first run and $P = 0.09$ for the second run). Apparently, the change in canopy carbon assimilation in response to far-red PFD was too small to have a significant effect on the CCG and shoot dry weight.

The CCG calculated from canopy photosynthesis measured by the whole-plant gas exchange system correlated strongly with the final dry weight of lettuce plants (Fig. 9), indicating that the whole-plant gas exchange system provided a reliable measurement of carbon assimilation of lettuce plants. The slopes of the regression equations were 24.4 g mol$^{-1}$ for the first run and 29.4 g mol$^{-1}$ for the second run (Fig. 9). Assuming that root tissue comprised 10% of the total dry weight, we can estimate that, per mole of assimilated carbon, the total dry weight of the lettuce plant increased by 24.4 g mol$^{-1}$/0.9 = 27.1 g mol$^{-1}$ for the first run and 29.4 g mol$^{-1}$/0.9 = 32.7 g mol$^{-1}$ for the second run. Then, the carbon content of plants in the two runs can be estimated as 12 g mol$^{-1}$/27.1 g mol$^{-1}$ = 0.443 g g$^{-1}$ for the first run and 12 g mol$^{-1}$/32.7 g mol$^{-1}$ = 0.367 g g$^{-1}$ for the second run. Previous studies reported a carbon content of green lettuce of 0.36 to 0.40 g g$^{-1}$, depending on nitrogen availability (Becker et al. 2015). Zhen and Bugbee (2020b) assumed that the carbon content of green lettuce plants was 0.4 g g$^{-1}$ in their study, which resulted in close estimation of lettuce total dry weight and cumulative daily carbon gain. During our study, the estimated carbon content values of 0.443 and 0.367 g g$^{-1}$ were close to the previously reported carbon contents of lettuce, which indicated that our whole-plant gas exchange system generated reliable data.

In conclusion, during our study, far-red photon substitution induced leaf expansion and canopy enlargement, which increased canopy light interception. Far-red photon substitution also reduced the amount of ePAR photons absorbed, simply because we replaced strongly absorbed red photons with less absorbed far-red photons. This reduction in absorbed ePAR photons by leaves decreased the light energy available for photosynthesis and, thus, negatively impacted photosynthetic rates. In the first run, the reduction in the amount of ePAR absorbed by leaves possibly offset the benefit of larger PCS on canopy level photosynthesis. However, in the second run, larger canopy size increased canopy level $P_C$, despite lower leaf level ePAR photon absorbance with far-red photon substitution. The different effect of far-red photon substitution on crop $P_C$ between the two runs is likely a result of different plant densities; high plant density during much of the first run limited the increase in canopy size in response to far-red photon substitution, which failed to compensate the decrease in canopy level $P_C$ caused by the reduction in amount of ePAR absorbed. Furthermore, far-red photons had photosynthetic activity when applied with blue and white background light but had substantially lower photosynthetic activity than red photons. The canopy-level quantum yield of photosynthesis of red photons was 1.75-times that of far-red photons. The final dry weight and cumulative carbon gain of lettuce plants were unaffected by the far-red photon portion relative to ePAR in either run. It is worth noting that these observations may be limited to ‘Little Gem’ lettuce because the response to far-red photon inclusion is cultivar-specific, as observed by Liu and van Iersel (2022). Our results suggest a potential cost-saving opportunity for growers because far-red LEDs have higher efficacy ($\mu$mol photon output per joule of energy consumed) than blue, red, and white LEDs (Kusuma et al. 2020). Thus, partially replacing LEDs that provide PAR with far-red LEDs can reduce power consumption without a negative impact on crop growth.

References Cited
Craver JK, Boldt JK, Lopez RG. 2018. Radiation intensity and quality from sole-source light-emitting diodes affect seeding quality and
Supplemental Fig. 1. Lettuce height increased linearly with the far-red photons portion, relative to extended photosynthetically active radiation (ePAR) photons, at 10 d after light treatment started for the first run (A) and at 7 d after light treatment started for the second run (B). Note the different y-axis scales.

Supplemental Fig. 2. Projected canopy size (PCS) per plant from 1 to 20 d after light treatment started in the first run (A) and throughout the experiment in the second run (B). *PCS increased with the increasing far-red photons portion relative to extended photosynthetically active radiation (ePAR) photons (shown in the legend) (α = 0.05). Note the different y-axis scales.
Supplemental Fig. 3. Leaf level light absorptance of lettuce plants grown with different far-red photons portion, relative to extended photosynthetically active radiation (ePAR) photons, in the first harvest of the first run (A), second harvest of the first run (B), second harvest of the second run (C), and third harvest of the second run (D). The far-red photons portion relative to ePAR values are shown in the graphs and with their corresponding line patterns.
Supplemental Fig. 4. The response of lettuce leaf absorptance of blue (400–499 nm, solid blue circles), green (500–599 nm, green diamonds), red (600–699 nm, red triangles), far-red (700–750 nm, dark red squares), and extended photosynthetically active radiation photons (ePAR) photons (400–750 nm, black dots) in response to far-red photons portion relative to extended photosynthetically active radiation (ePAR) at the first harvest of the first run (A), second harvest of the first run (B), second harvest of the second run (C), and third harvest of the second run (D). A higher far-red photon portion relative to ePAR linearly decreased absorptance of green, red, and far-red photons only in the first harvest of the first run (A), as indicated by the regression lines.
Supplemental Fig. 5. The chlorophyll a and b concentrations, total chlorophyll concentration (A and C), and chlorophyll a:b ratio (B and D) were not affected by the increasing far-red photons portion relative to extended photosynthetically active radiation (ePAR) photons in the first run (A and B) or the second run (C and D).

Supplemental Fig. 6. Example of raw net photosynthetic rates ($P_n$) data at 21 d after light treatment started in the first run. Far-red lights were turned off at 1002 HR and turned back on at 1302 HR. The far-red photons portion relative to extended photosynthetically active radiation (ePAR) photons of each treatment is listed with the corresponding line color.
Supplemental Table 1. Light spectrum and average temperature in each gas exchange chamber under different far-red photon portions relative to extended photosynthetically active radiation (ePAR) photons in the first run and second run. Photon flux density of each waveband is shown.

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