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Photochemical Characterization of Greenhouse-grown Lettuce (*Lactuca sativa* L. 'Green Towers') with Applications for Supplemental Lighting Control

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Abstract. **Plant light use efficiency decreases as light intensity is increased, and a better understanding of crop-specific light responses can contribute to the development of more energy-efficient supplemental lighting control strategies for greenhouses. In this study, diurnal chlorophyll fluorescence monitoring was used to characterize the photochemical responses of 'Green Towers' lettuce (*Lactuca sativa* L.) to photosynthetic photon flux density (*PPFD*) and daily light integral (DLI) in a greenhouse during a production cycle. Plants were monitored continuously for 35 days, with chlorophyll fluorescence measurements collected once every 15 minutes. Quantum yield of photosystem II (ΦPSII) decreased exponentially with *PPFD*, whereas electron transport rate (ETR) increased asymptotically to 121 μmol·m^{-2}·s^{-1}. Daily photochemical integral (DPI) is defined as the integral of ETR over a 24-hour period; DPI increased asymptotically to 3.29 mol·m^{-2}·d^{-1} with increasing DLI. No effects of plant age or prior day's DLI and a negligible effect of *PPFD*s 15 or 30 minutes before measurements within days were observed. Simulations were conducted using the regression equation of ETR as a function of *PPFD* {ETR = 121 [1 – exp(–0.00277*PPFD*)]} to illustrate methods of increasing photochemical light use efficiency for improved supplemental lighting control strategies. For a given DLI, DPI can be increased by providing light at lower *PPFD*s for a longer period of time, and can be maximized by providing light with a uniform *PPFD* throughout the entire photoperiod. Similarly, the DLI required to achieve a given DPI is reduced using these same methods.**

Supplemental lighting can improve the profitability of greenhouse crop production, and a better quantitative understanding of plant responses to *PPFD* can facilitate the development of more efficient crop-specific control strategies for greenhouse supplemental lighting (van Iersel, 2017). Chlorophyll fluorescence measurements are a rapid and reliable means of probing the light reactions of photosynthesis directly (Baker, 2008). During the light reactions, some of the light energy absorbed by chlorophylls and accessory pigments migrates to photosystem II (PSII) reaction centers, resulting in the splitting of water molecules, liberating electrons and protons. The freed electrons are used to regenerate nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) via the electron transport chain, and a proton gradient across the thylakoid membrane drives adenosine triphosphate (ATP) synthase, regenerating ATP. These energy-rich molecules—NADPH and ATP—provide the reducing power and chemical energy for carbohydrate production in the Calvin-Benson-Bassham cycle. However, not all light absorbed by photosynthetic pigments is used to drive the light reactions; a significant amount is dissipated as heat, and a small fraction is reemitted as fluorescence. By measuring the fluorescence emitted by chlorophyll *a* molecules before and during short exposure to a beam of light with sufficient intensity to saturate the PSII reaction centers completely (a "saturating pulse"), Φ_{PSII} can be quantified directly. Φ_{PSII} is a unitless measure of the efficiency with which absorbed light is used to drive photochemistry in the light-adapted state of PSII. The dark-adapted value of the quantum efficiency of PSII (F_v/F_m) is an indicator of maximum potential photochemical efficiency. Combined with *PPFD*, Φ_{PSII} is used to calculate the rate of linear electron transport through PSII (the ETR), an estimate of the overall rate of the light reactions of photosynthesis (Baker and Rosenqvist, 2004; Genty et al., 1989; Maxwell and Johnson, 2000). To distinguish measurements based on chlorophyll fluorescence from other measures of photosynthesis such as gas exchange or oxygen evolution, data related to Φ_{PSII} and ETR are referred to as *photochemical* rather than *photosynthetic* herein.

Chlorophyll fluorescence is an ideal tool for understanding crop-specific photochemical responses to *PPFD*. Chlorophyll fluorometers are generally small and portable, with simple operation that requires no recalibration. Measurements can be collected quickly in situ, and are noninvasive and accurate (Baker and Rosenqvist, 2004; Maxwell and Johnson, 2000). An exact correlation between ETR and CO_2 fixation rates may be difficult to establish because the products of the light reactions can be used to drive processes other than the Calvin-Benson-Bassham cycle. Photorespiration is a major sink for NADPH and ATP in C3 plants (Krall and Edwards, 1992), and NADPH may be used as an electron donor for nitrate reduction (Tischner, 2000). Freed electrons may reduce O_2 at photosystem I (Mehler reaction, or water–water cycle) rather than be used to produce NAPDH (Polle, 1996), and ATP can be used for chloroplast functions such as protein repair and nucleotide metabolism (Murata and Nishiyama, 2018; Spetea et al., 2004). Thus, the relationship between ETR and CO_2 fixation depends on many factors, including temperature, relative humidity, CO_2 concentration, and water and nutrient availability. However, ETR can be taken as a relative indicator of overall photosynthetic rates, and hence plant growth. Furthermore, compared with gas exchange, ETR of C3 plants is relatively insensitive to changes in environmental variables other than light (Murchie and Lawson, 2013). Thus, chlorophyll fluorescence measurements provide a convenient, rapid, accurate, and robust means of evaluating photochemical responses to *PPFD*.

Light response curves collected using chlorophyll fluorescence measurements are typically performed over a relatively brief period (often just a few minutes) with a highly focused light source and may not represent photochemical responses accurately under variable ambient light conditions (Rascher et al., 2000). Photoprotective processes affect photochemical light use efficiency by reducing the amount of absorbed light energy transferred to PSII reaction centers, and may operate over longer timescales. Because the accumulation of excess light energy in the light-harvesting complexes can lead to light-induced damage of PSII reaction centers (photoinhibition), plants have evolved a variety of interrelated photoprotective mechanisms by which excess absorbed light energy can be dissipated safely as heat, including molecular reorganization of PSII and the xanthophyll cycle (Demmig-Adams et al., 2012; Horton, 2012; Rochaix, 2014; Ruban, 2015). As *PPFD* increases, a larger fraction of absorbed light is dissipated as heat, resulting in a decrease in Φ_{PSII} (Baker, 2008; Maxwell and Johnson, 2000). Fluctuations in *PPFD* throughout the course of a day can lead to variations in Φ_{PSII} as a result of the up- or downregulation of the xanthophyll cycle.

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The xanthophyll cycle is the process by which the accumulation of protons leads to acidification of the thylakoid lumen, activating violaxanthin de-epoxidase, which catalyzes the de-epoxidation of violaxanthin to form antheraxanthin and zeaxanthin. This chemical conversion of the xanthophyll pigments facilitates the dissipation of excess light energy as heat. It reverses relatively slowly, over a scale of several minutes, through epoxidation catalyzed by zeaxanthin epoxidase. Because of this slow relaxation, transient exposure to high light levels may lead to decreases in photochemical efficiency (relative decreases in Φ_{PSII} and ETR) for several minutes even if *PPFD*s subsequently decrease to much lower levels (Demmig-Adams et al., 2012; Kaiser et al., 2018; Ruban, 2015). Photochemistry-induced acidification of the thylakoid lumen can further affect rates of electron transport by inhibiting plastohydroquinone oxidation by the cytochrome b_6f complex, thereby decreasing the rate of linear electron transport through PSII, in a process known as photosynthetic control (Foyer et al., 2012).

Light response curves collected over a short period of time may also be inadequate to describe photochemical responses for an entire growing period because photosynthetic rates can vary with leaf or plant age (Locke and Ort, 2014; Salmon et al., 2011) and can be affected by slow acclimation to light intensities. Acclimation to light intensities over the course of hours or days can lead to changes in the overall light response through mechanisms such as chlorophyll antennae rearrangement or changes in cellular metabolism and translation, and nuclear transcription, induced by chloroplast signaling (Dietz, 2015; Ruban, 2015). If factors such as ontogeny or acclimation impact the overall photochemical light response significantly, light response curves collected over only a few minutes may not describe realistic photochemical responses for a crop sufficiently, and longer term monitoring would be needed to characterize the photochemical response during a production cycle. Diurnal chlorophyll fluorescence monitoring can be used to gain a more detailed understanding of the photochemical light response under greenhouse lighting conditions (Weaver and van Iersel, 2016). This method consists of measuring chlorophyll fluorescence and *PPFD* over the course of several days, with measurements taken at regular intervals. In general, a 15-min interval between chlorophyll fluorescence measurements is sufficiently long to avoid measurement-induced photoinhibition resulting from the repeated application of saturating light pulses (van Iersel et al., 2016).

Although supplemental lighting can improve the growth, quality, and profitability of greenhouse-grown crops, the electricity requirement of supplemental lights can account for as much as 30% of the operating cost of a greenhouse (van Iersel and Gianino, 2017; Watson et al., 2018). The advent of light-emitting diode (LED) technology for horticultural lighting has facilitated the development of innovative approaches to providing and controlling greenhouse supplemental lighting (Morrow, 2008; Pinho et al., 2012; Singh et al., 2015). LED fixtures have several distinct advantages over the high-intensity discharge (HID) lamps traditionally used for greenhouse lighting, including their relatively high efficacy, low radiant heat load, and variable spectra. Another unique feature of LEDs is that the intensity of their light output can be controlled precisely and rapidly in a manner that is not possible with HID lamps. Lighting control systems that use this dimmability have the potential to reduce the electricity costs associated with providing supplemental light, and to increase the efficiency with which supplemental light is used for promoting plant growth. These adaptive, or dynamic, supplemental LED lighting control systems operate by keeping the LED lights off when ambient *PPFD* exceeds a predefined threshold *PPFD*. When ambient *PPFD* falls below this level, supplemental light is provided so that the combined *PPFD* of the LED lights and sunlight reaches, but does not exceed, the threshold. This ensures that supplemental light is provided only when the overall *PPFD* is relatively low, and the supplemental light can be used more efficiently by plants, because plant light use efficiency invariably decreases at greater *PPFD*s (Pinho et al., 2013; van Iersel and Gianino, 2017).

Providing supplemental light in a manner that allows it to be used most efficiently by a crop has the potential to decrease the amount of supplemental light, and thus the total amount of electricity required, for crop growth. For example, using simulations based on historical weather data and cultivar-specific light responses, Weaver and van Iersel (2018) estimated that the amount of supplemental light required for early season production can be reduced by 24% for *Petunia ×hybrida* 'Daddy Blue' and 37% for *Impatiens walleriana* 'Super Elfin XP Violet' using an adaptive lighting control approach that accounts for crop light use efficiency. Thus, understanding species- or cultivar-specific photosynthetic or photochemical responses to *PPFD* can facilitate the implementation of lighting control strategies that use the dimmability of LEDs fully and reduce electricity costs by providing supplemental light according to a specific crop's ability to use that light efficiently.

Lettuce is an important greenhouse crop because there is a continuous demand for a supply of fresh leafy greens, production cycles are relatively short, and lettuce can be produced year-round in greenhouses if appropriate environmental conditions (e.g., light, temperature) are provided. Supplemental lighting for hydroponic greenhouse lettuce production has been the subject of a great deal of research, and some of the most advanced supplemental lighting strategies developed to date have focused on lettuce production (Albright et al., 2000; Bumgarner and Buck, 2016; Seginer et al., 2006). In our study, in situ diurnal chlorophyll fluorescence monitoring was used to evaluate the photochemical performance of a greenhouse-grown crop of a romaine-type lettuce cultivar (*Lactuca sativa* L. 'Green Towers') under growing conditions comparable to a commercial production environment. Specific hypotheses tested were whether the current ETR is affected by previous *PPFD*s during a day, and whether photochemical efficiency is affected by plant age or previous day's DLI. In addition to quantifying instantaneous photochemical responses to *PPFD*, the integral of ETR over individual measurement days was calculated and defined as the DPI ($mol \cdot m^{-2} \cdot d^{-1}$), the integral of ETR over a 24-h period. Last, we conducted simulations to demonstrate how these data can be used to develop energy-efficient supplemental lighting strategies, and outline general methods for using adaptive lighting control to improve crop light use efficiency by decreasing the DLI required to achieve a given DPI, or increasing the resulting DPI for a fixed DLI.

Materials and Methods

The study was conducted in a glass-covered greenhouse in Athens, GA, during Mar. and Apr. 2015. The mean relative humidity ($\pm\sigma$) was 66.3 ± 16.3%, the mean temperature was 21.4 ± 1.7 °C, and the mean DLI was 13.9 ± 6.8 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 1). Seeds of 'Green Towers' lettuce were sown in 10-cm square pots filled with a peat–perlite substrate (Fafard 2P; Sun Gro Horticulture, Agawam, MA). Fifteen plants were grown on ebb-and-flow benches and fertigated daily with a 100 $mg \cdot L^{-1}$ N liquid fertilizer (15N–2.2P–12.45K; 15–5–15 Cal-Mag; Everris, Marysville, OH). The plants were grown without shading to ensure that measurements could be taken under the widest range of DLIs and *PPFD*s possible.

Chlorophyll fluorescence monitoring was initiated 2 weeks after germination and was performed using a chlorophyll fluorometer and attached leaf clip with quantum sensor (JUNIOR-PAM; Heinz Walz, Effeltrich, Germany). The most recently fully expanded leaf was measured until the onset of head formation, after which the youngest fully expanded leaf exterior to the head was measured. Leaves were placed in the leaf clip and positioned such that the quantum

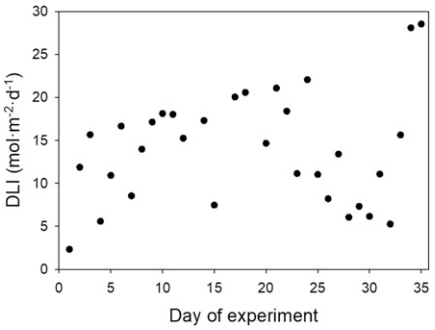

Fig. 1. Daily light integral (DLI) over the course of the study.

sensor was exposed fully to the south side of the greenhouse and not shaded by other leaves. Chlorophyll fluorescence measurements were taken once every 15 min to determine Φ_{PSII}, and *PPFD* was measured using the built-in quantum sensor on the leaf clip. ETR, an estimate of the rate of the light reactions of photosynthesis, was calculated from Φ_{PSII} and *PPFD* as ETR = $\Phi_{PSII} \times PPFD \times 0.84 \times 0.5$. This equation assumes that excitation energy is distributed evenly between PSII and photosystem I, and that 84% of incident light is absorbed by a leaf (Björkman and Demmig, 1987; Genty et al., 1989). After 48 h, a different plant was selected randomly for measurement, and measurements using the new plant commenced at least 1 h after sunset to verify that the F_v/F_m of the new leaf section was within an acceptable range: at least 0.78, with a theoretical maximum of around 0.85. Observations of F_v/F_m less than 0.78 indicate that the leaf is experiencing some type of stress and may be senescing. Values exceeding 0.85 are usually the result of measurement error, especially improper positioning of the fluorometer sensor head. This initial value was recorded and used as the value of F_v/F_m for subsequent analysis. Chlorophyll fluorescence monitoring continued in this fashion for 35 d and ended when the plants had formed a head and reached a salable size. Only one plant was measured at any given time because no treatments were applied or compared, and replications were not needed for a statistical analysis of the data.

DLI was calculated by integrating *PPFD* over each 24-h period, with *PPFD* assumed to be constant for each 15-min increment of the 24-h period. DPI was calculated by integrating ETR over each 24-h period, with ETR assumed to be constant for each 15-min increment of the 24-h period. The 24-h period was defined as beginning and ending at midnight. The apparent saturating *PPFD* for ETR was calculated as the *PPFD* at which 90% of the asymptote of ETR was reached. The apparent saturating DLI for DPI was calculated as the DLI at which 90% of the asymptote of DPI was reached.

Regression analyses were performed using SigmaPlot (version 13; Systat Software, Inc., San Jose, CA). Regression analysis was used to evaluate ETR and Φ_{PSII} as functions of *PPFD* for all days pooled and for individual days, and to evaluate F_v/F_m as a function of measurement day and preceding day's DLI. ETR was fit as a function of *PPFD* using the equation $ETR = a[1 - e^{-b(PPFD)}]$, Φ_{PSII} was fit as a function of *PPFD* using the equation $\Phi_{PSII} = c + a[e^{-b(PPFD)}]$, and DPI was fit as a function of DLI using the equation $DPI = a[1 - e^{-b(DLI)}]$, where a, b, and c are regression coefficients. To test the hypothesis that plant age affected photochemical capacity, daily asymptotes of ETR were analyzed as a function of plant age for all measurement days with at least two observations of *PPFD* greater than 831 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the apparent saturating *PPFD* for the pooled ETR response. The analysis was restricted to days on which saturating *PPFD*s were observed to ensure that an accurate approximation of the asymptote could be obtained. These asymptotes were also analyzed as a function of the previous day's DLI to test whether acclimation to the previous day's DLI affected the current day's photochemical capacity. To test the hypothesis that previous *PPFD*s affected current photochemistry within days, Φ_{PSII} was analyzed as a quadratic function of current *PPFD* and the observed *PPFD*s 15 and 30 min prior ($PPFD_{15}$ and $PPFD_{30}$, respectively) for all days, using polynomial regression with a general linear model (Proc GLM, SAS version 9.2; SAS Institute, Cary, NC) according to the model: $\Phi_{PSII} = a_0 + a_1 \times PPFD + a_2 \times PPFD^2 + a_3 \times PPFD_{15} + a_4 \times PPFD_{30}$, where $a_0, \ldots, a_4$ are regression coefficients. Significance was tested at $P = 0.05$. To test further the effect of within-day variations in *PPFD* on ETR and Φ_{PSII}, observations of ETR and Φ_{PSII} occurring before and after solar noon for nonzero *PPFD*s were compared and tested for significant differences at $P = 0.05$ using a mixed-model analysis of covariance, where day of experiment was treated as a random effect, time of day (before/after solar noon) was a fixed effect, and *PPFD* was a covariate. Analysis was performed using the general linear model in SAS (Proc GLM). The covariate effect was approximated using a ninth-order polynomial for ETR and a sixth-order polynomial for Φ_{PSII}, according to the model $y = a_0 + a_1 \times PPFD + \ldots + a_n \times PPFD^n$, where y is the dependent variable, n is the highest order of the polynomial, and $a_0, \ldots, a_n$ are regression coefficients. Polynomial order for each dependent variable was selected by using Taylor's theorem to determine the lowest order polynomial needed to replicate accurately the function values of the exponential equations fitted via regression analysis over at least 90% of the range of the *PPFD* data. Polynomial fit was verified using regression analysis in SAS, with model significance tested at $P = 0.001$.

Data from five measurement days were excluded from the analyses and graphs because observations of F_v/F_m recorded more than 1 h after sunset following the first photoperiod of diurnal measurement fell outside the acceptable range (0.78–0.85)—the same criteria used for the initial measurement of F_v/F_m at the onset of diurnal monitoring. In addition, observations were missing from 3 measurement days, and thus DPI and DLI were not calculated for these days.

Simulations were conducted based on the relationship between ETR and *PPFD*. A set of simulations was conducted in which the objective was to reach a DLI of 17 $mol \cdot m^{-2} \cdot d^{-1}$ with nine photoperiods (8–24 h, 2-h intervals) with a constant *PPFD*. The required constant *PPFD* for each photoperiod was determined by dividing 17 $mol \cdot m^{-2}$ by the photoperiod. ETRs corresponding to these *PPFD*s were calculated using the regression equation of ETR as a function of *PPFD*. Calculated ETRs were integrated over the photoperiod to obtain the DPI. Further simulations were conducted in which the objective was to reach a DLI of 17 $mol \cdot m^{-2} \cdot d^{-1}$ with a 12-h photoperiod using two *PPFD*s, each for half of the photoperiod, with a range of differences (0–700 $\mu mol \cdot m^{-2} \cdot s^{-1}$) between the two *PPFD*s ($\Delta PPFD$). The constant *PPFD* for the 0-$\mu mol \cdot m^{-2} \cdot s^{-1}$ difference scenario was calculated as described earlier to be 394 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For the remaining scenarios, the required *PPFD* for each half of the photoperiod was calculated by increasing or decreasing 394 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by one-half the required difference in *PPFD*. For each half of the photoperiod, ETR was calculated using the regression equation of ETR vs. *PPFD*, and DPI was obtained by integrating these values over the whole photoperiod. A third set of simulations was conducted in which the objective was to reach a DPI of 2.89 $mol \cdot m^{-2} \cdot d^{-1}$ with nine photoperiods (8–24 h, 2-h intervals) with a constant ETR (which corresponds to a constant *PPFD*). The required constant ETR was calculated for each photoperiod by dividing 2.89 $mol \cdot m^{-2}$ by the photoperiod. The corresponding *PPFD* was calculated using the inverse function of the regression equation of ETR as a function of *PPFD*: $PPFD = \ln(a/a - ETR)/b$, where a and b are regression coefficients. DLI was obtained by integrating this *PPFD* over the photoperiod.

Results and Discussion

Quantum yield of PSII decreased exponentially ($R^2 = 0.89$, $P < 0.0001$) as *PPFD* increased from 0 to ≈1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the greatest *PPFD* observed during this study (Fig. 2, top). This decrease in Φ_{PSII} was observed because, as *PPFD* increases, a greater proportion of absorbed light energy is dissipated as heat as a result of the operation of the xanthophyll cycle and other photoprotective processes, leaving a smaller fraction of the light to drive photochemistry (Demmig-Adams et al., 2012; Horton, 2012; Rochaix, 2014; Ruban, 2015). The response of ETR to *PPFD* was an exponential rise to a maximum (Fig. 2, bottom) with an asymptote of 121 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and an initial slope of 0.335 mol of electrons per mole of incident photons ($R^2 = 0.95$, $P < 0.0001$). The apparent saturating *PPFD* (reached at 90% of the asymptote of ETR) was 831 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

There was no significant change in the daily asymptotes of ETR throughout the course of the study (data not shown). This suggests that, for this cultivar, plant age has little impact on maximum photochemical capacity. Some of the variability in these data may have been the result of leaf (rather than plant) age, which was not documented. Similarly, F_v/F_m did not change significantly with plant age (data not shown), which could be the result of the short duration of the study or the relative insensitivity of F_v/F_m to leaf ontogeny. Although some chlorophyll fluorescence parameters may change with plant age, F_v/F_m is known to vary little with leaf age, except during senescence (Mauromicale et al., 2006; Šesták, 1999). Because plant age

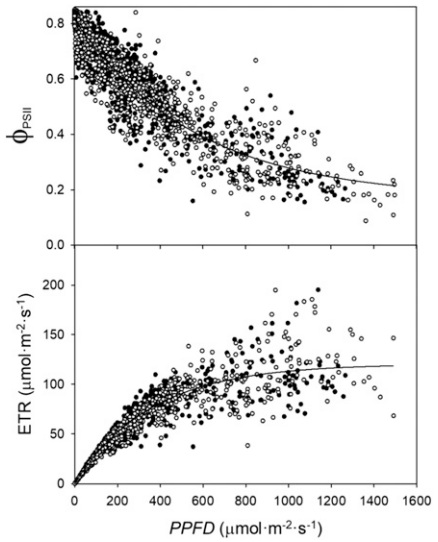

Fig. 2. Quantum yield of photosystem II (Φ_{PSII}) of 'Green Towers' lettuce as a function of photosynthetic photon flux density (*PPFD*) based on 35 d of constant diurnal monitoring. Closed symbols represent measurements taken before solar noon; open symbols represent measurements taken after solar noon. The regression line represents the equation $\Phi_{PSII} = 0.171 + 0.643e^{-0.00178PPFD}$, with $R^2 = 0.89$ and $P < 0.0001$ (top). Electron transport rate (ETR) of 'Green Towers' lettuce as a function of *PPFD* based on 35 d of constant diurnal monitoring. Closed symbols represent measurements taken before solar noon; open symbols represent measurements taken after solar noon. The regression line represents the equation $ETR = 121(1 - e^{-0.00277PPFD})$, with $R^2 = 0.95$ and $P < 0.0001$ (bottom).

did not affect photochemical characteristics, it is likely that diurnal chlorophyll fluorescence monitoring conducted over a much shorter period of time than the 35 d used in our study would be adequate to describe the photochemical light response of this cultivar over a production cycle. However, because only a small part of one leaf was measured at any given time, these results may not be indicative of entire canopies or the effect of aging on whole-canopy photochemistry.

Fluctuating light levels can affect overall daily rates of photochemistry because photoprotective processes such as the xanthophyll cycle (Demmig-Adams et al., 2012), as well as photosynthetic control (Foyer et al., 2012), can inhibit photochemical light use for several minutes after transient exposure to high light intensities (Kaiser et al., 2018; Slattery et al., 2018). To test the hypothesis that previous light levels affect current photochemistry, Φ_{PSII} was analyzed as a quadratic function of current *PPFD* and linear effects of the *PPFD*s observed 15 and 30 min prior. Overall, the model fit well ($R^2 = 0.86$, $P < 0.0001$) and both $PPFD_{15}$ and $PPFD_{30}$ were highly significant ($P < 0.0001$), but contributed little to the overall model R^2 (partial $R^2 = 0.008$ and 0.005, respectively). Thus, *PPFD*s from the previous 15 and 30 min had a negligible effect on Φ_{PSII} (and hence ETR). Furthermore, there was no significant difference in observations of either Φ_{PSII} or ETR taken before vs. after solar noon (Fig. 2). These results are likely the result of the time resolution of our measurements; the 15-min interval needed to avoid measurement-induced photoinhibition is likely a sufficient span of time for xanthophyll cycle activity to relax almost completely after transient high light exposure. Zeaxanthin is converted back to the nonphotoprotective violaxanthin by zeaxanthin epoxidase on a scale of several minutes (Demmig-Adams et al., 2012; Kaiser et al., 2018). DLIs of individual measurement days also had no significant effect on F_v/F_m measured during the subsequent dark period or on the following day's asymptote of ETR (data not shown), and the study was conducted under a wide range of DLIs (Fig. 1). Thus, photochemical acclimation over a timescale of days was not observed in this study.

DPI, the integral of ETR over a 24-h period, was evaluated as a function of DLI. Like the response of ETR to *PPFD*, DPI increased exponentially to a maximum with DLI (Fig. 3; $R^2 = 0.82$, $P < 0.0001$), with an asymptote of 3.30 mol·m^{-2}·d^{-1}; 90% of this asymptote was reached at a DLI of 18.9 mol·m^{-2}·d^{-1} (apparent saturating DLI). Previous research showed that the ideal DLI for hydroponic greenhouse production of the bibb lettuce cultivar 'Ostinata' is 17 mol·m^{-2}·d^{-1}. At this DLI, growth rates were sufficiently high to guarantee rapid production without causing excessive leaf tip burn (Albright et al., 2000; Both et al., 1997). Interestingly, although a different cultivar was used, the saturating DLI found in our study deviates by only 11% from the recommended DLI based on growth trials (Both et al., 1997). This points to the potential utility of chlorophyll fluorescence monitoring for developing crop-specific DPI or DLI recommendations. However, it is important to recognize that DPI is not a direct function of DLI, but rather of the integral of ETR over a day. ETR in turn is a nonlinear function of *PPFD*, and hence DPI not only depends on DLI, but also on how observations of *PPFD* are distributed throughout the course of a day. Because of this, seasonal variation in daily distributions of *PPFD* would be expected to influence the observed response of DPI to DLI.

Lighting recommendations for greenhouse crops are currently made based on estimates of the range of DLIs required for ideal production of specific crops (e.g., Torres and Lopez, n.d.). However, with the same DLI, different DPIs can result from providing the same quantity of light with different distributions of *PPFD*, resulting from the nonlinearity of the ETR response. Although a clear correlation between DPI and crop growth has not yet been established, quantifying DPI provides a means of assessing the effectiveness of greenhouse supplemental lighting control strategies, assuming that an increase in DPI will result in greater growth rates. One means of increasing DPI for a given DLI is to extend the photoperiod, allowing supplemental light to be provided at lower *PPFD*s, thereby increasing the efficiency of photochemical light use and leading to greater DPIs. Figure 4 shows the *PPFD* required to reach a DLI of 17 mol·m^{-2}·d^{-1} using a constant *PPFD* at a range of photoperiods (8–24 h), with the corresponding calculated ETR and resulting DPI based on the regression equation of ETR as a function of *PPFD*. As the photoperiod is increased and the constant *PPFD* decreased, DPI increases from 2.81 mol·m^{-2}·d^{-1} with an 8-h photoperiod to 4.39 mol·m^{-2}·d^{-1} with a 24-h photoperiod (Fig. 4). This occurs because the rate of increase in ETR decreases exponentially as *PPFD* increases, because ETR as a function of *PPFD* is an exponential rise to a maximum. Evidence from previous research indicates that these simulated increases in DPI do indeed correspond to improved plant growth. Koontz and Prince (1986) showed that providing the same DLI with a 24-h photoperiod increased lettuce weight by 30% to 50% compared with a 16-h photoperiod. Soffe et al. (1977) demonstrated that extending the photoperiod from 12 to 16 h, while holding DLI constant at 5 MJ·m^{-2}, increased growth rates of seven vegetables:

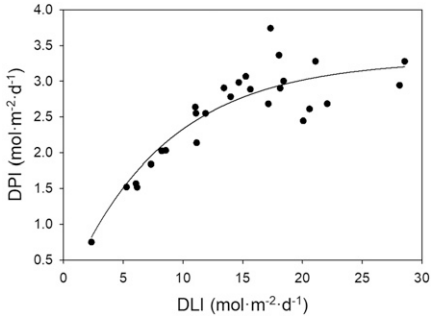

Fig. 3. Daily photochemical integral (DPI) of 'Green Towers' lettuce as a function of daily light integral (DLI) based on 35 d of diurnal chlorophyll fluorescence monitoring. The regression line represents the equation $DPI = 3.30(1 - e^{-0.122DLI})$, with $R^2 = 0.82$ and $P < 0.0001$.

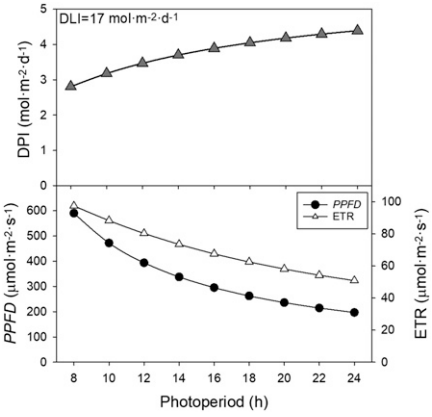

Fig. 4. Daily photochemical integral (DPI) resulting from reaching a daily light integral (DLI) of 17 mol·m^{-2}·d^{-1} with a constant photosynthetic photon flux density (*PPFD*) over a range of photoperiods required (top); required *PPFD*, and corresponding electron transport rate (ETR; calculated from equation in Fig. 2, bottom).

lettuce, celery (*Apium graveolens*), beetroot (*Beta vulgaris*), spinach beet (*Beta vulgaris*), radish (*Raphanus raphanistrum* ssp. *sativus*), cabbage (*Brassica oleracea*), and oilseed rape (*Brassica napus*). Because altering the photoperiod may have unintended consequences for flowering of many daylength-sensitive crops, extending the photoperiod may not always be an option. Another means of increasing DPI for a fixed DLI is to provide supplemental light with a more uniform (less variable) distribution throughout the photoperiod. Figure 5 illustrates this principle. If light is provided to reach a DLI of 17 mol·m^{-2}·d^{-1} with a 12-h photoperiod, a constant *PPFD* of 394 µmol·m^{-2}·s^{-1} would be required, resulting in a DPI of 3.47 mol·m^{-2}·d^{-1}. If the distribution of *PPFD* is altered such that light is provided to reach a DLI of 17 mol·m^{-2}·d^{-1} in 12 h, with a greater *PPFD* for half the photoperiod and a lesser *PPFD* for the other half (with the difference between these denoted as Δ*PPFD*), DPI will decrease with increasing Δ*PPFD*; and, at a Δ*PPFD* of 700 µmol·m^{-2}·s^{-1}, DPI is reduced to 2.58 mol·m^{-2}·d^{-1} (Fig. 5). Uniform distributions of *PPFD* are associated with greater DPIs than more variable distributions as a result of the nonlinearity of the ETR response; as *PPFD* is decreased or increased by the same amount from some initial value, the decrease in ETR at the lower *PPFD* will be greater than the increase in ETR at the higher *PPFD*, and the magnitude of this difference increases as the change in *PPFD* increases. The hypothesis that an increase in DPI resulting from improved uniformity of *PPFD* will improve crop growth is supported by past research. Aikman (1989) demonstrated the effect of lighting uniformity on tomato growth. Tomatoes were grown in growth chambers at a constant DLI with a consistent light level of 58 W·m^{-2} and with two variable light distributions, where the light was provided at 103 W·m^{-2} for the first half of the day and 13 W·m^{-2} for the second, or vice versa. Dry weight of plants grown under the uniform light intensity was, on average, 33% greater than in the other treatments. Although the simulations presented herein do not account for the interactions of supplemental LED lights and sunlight, adaptive lighting control can be used to improve the uniformity of *PPFD*s from LED lights and sunlight combined, and to minimize the *PPFD* provided by LED lights, thereby achieving equivalent increases in DPI (van Iersel and Gianino, 2017).

In a manner analogous to increasing DPI for a given DLI, the DLI required to reach a particular DPI can be reduced by providing supplemental light at lower *PPFD*s and/or with a more uniform *PPFD* distribution. Reducing the required DLI will decrease the total amount of supplemental light provided, which results in electricity savings. According to the regression equation of DPI vs. DLI (Fig. 3), a DPI of 2.89 mol·m^{-2}·d^{-1} corresponds to the recommended DLI of 17 mol·m^{-2}·d^{-1} for lettuce (Both et al., 1997). If light is provided to reach a DPI of 2.89 mol·m^{-2}·d^{-1} with a continuous *PPFD* over a range of photoperiods (8–24 h), the required DLI decreases as the photoperiod is extended (Fig. 6). The greatest DLI requirement, 18.4 mol·m^{-2}·d^{-1}, occurs with an 8-h photoperiod, whereas the DLI required for a 24-h photoperiod is only 10.1 mol·m^{-2}·d^{-1}, a 45% decrease. Similarly, for a fixed photoperiod and DPI, DLI will be reduced if supplemental light is provided with a more uniform distribution of *PPFD* (Weaver and van Iersel, 2018).

Control strategies for greenhouse supplemental lighting that account for daily requirements of photosynthesis or photochemistry have been developed with the goal of reducing electricity costs by decreasing the amount of supplemental lighting required, or providing supplemental lighting when electricity is less expensive (Clausen et al., 2015; Wang et al., 2018; Watson et al., 2018; Weaver and van Iersel, 2018). Kjaer et al. (2011) demonstrated that the electricity cost associated with supplemental lighting can be reduced by 25% without affecting the overall quality of two ornamental *Campanula* species when supplemental lights are controlled by the DynaLight system. This system accounts for electricity prices and photosynthetic rates to achieve a specified DPI with the lowest possible electricity cost, using a canopy photosynthesis model based on *PPFD*, temperature, and CO_2 concentration (Aaslyng et al., 2003; Clausen et al., 2015; Kjaer et al., 2012). Implementing such strategies requires evaluating crop-specific light response and establishing recommendations for daily photosynthesis or photochemistry for individual crops. The results of our study demonstrate that the response of ETR to *PPFD*, as determined using diurnal chlorophyll fluorescence monitoring, is robust to plant age, within-day fluctuations in *PPFD*, and previous day's DLI for the lettuce cultivar studied. Additional research, including greenhouse growth trials, is needed to evaluate the relationship between DPI and crop growth, and to establish methods for determining crop-specific DPI requirements.

Conclusions

The photochemical responses of 'Green Towers' lettuce were found to be consistent throughout the course of our study, and were unaffected by plant age, or previous *PPFD*s or DLIs within or across days. This suggests that, although diurnal chlorophyll fluorescence monitoring throughout a production cycle provides valuable insight, photochemical light response curves collected for a shorter period of time should be adequate for characterizing crop-specific photochemical responses to develop supplemental lighting control strategies. ETR is an asymptotically increasing function of *PPFD*, and therefore daily photochemical light use efficiency can be improved by providing supplemental light at relatively low *PPFD*s over an extended period of time, or by providing supplemental light in a uniform manner. For a given DLI, DPI can be increased by applying these principles. Similarly, the DLI required to achieve a given DPI can be reduced. Further research is needed to assess the effectiveness of supplemental lighting control strategies that account for these dynamics, and to determine whether greenhouse crop production can be improved by providing supplemental light in a photochemically efficient manner.

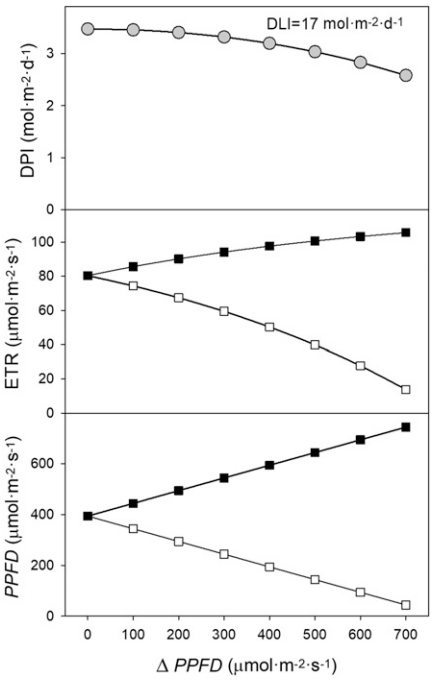

Fig. 5. Daily photochemical integral (DPI) resulting from reaching a daily light integral (DLI) of 17 mol·m^{-2}·d^{-1} with a 12-h photoperiod using two photosynthetic photon flux densities (*PPFD*s), each for half of the photoperiod, with a range of differences between the two *PPFD*s (Δ*PPFD*) (top). Required *PPFD*s (bottom), and corresponding electron transport rates (ETRs) (middle) are shown. For Δ*PPFD* = 0, only one *PPFD* is used.

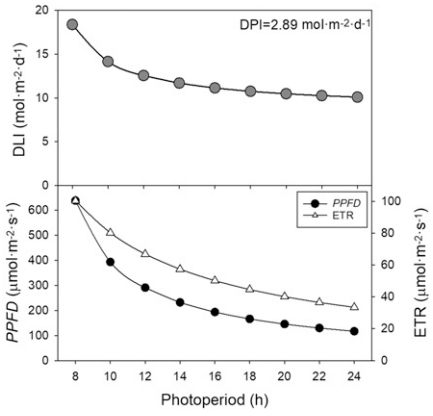

Fig. 6. Daily light integral (DLI) needed to reach a calculated daily photochemical integral (DPI) of 2.89 mol·m^{-2}·d^{-1} with a constant photosynthetic photon flux density (*PPFD*) over a range of photoperiods (top); required electron transport rate (ETR) and corresponding *PPFD* (bottom) based on the regression equation in Fig. 2 (bottom).

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