A Comparative Study Analyzing Light Durations for the Growth of 'Rex Butterhead' Lettuce *Lactuca sativa* Utilizing GREENBOX Technology

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Abstract

Exponential population growth pressures the agriculture industry to provide fresh foods to both rural and urban areas. GREENBOX technology, based on Controlled Environment Agriculture (CEA) principles, addresses this challenge by optimizing conditions for leafy green crops. It integrates into urban infrastructure to mitigate the effects of urbanization on fresh food availability. GREENBOX controls environmental factors like temperature, humidity, light, and nutrient delivery to enhance crop growth. Given that artificial lighting is a major energy consumer in CEA, this study explored different photoperiods to improve energy efficiency. *Lactuca sativa* 'Rex Butterhead' lettuce was grown under three photoperiods: 16-hour light/8-hour dark (control), 14-hour light/10-hour dark, and 12-hour light/12-hour dark. Biomass parameters, including fresh and dry weight, leaf area, leaf count, and chlorophyll concentration, were measured. While all treatments produced viable crops above the expected harvest weight, the 16-hour photoperiod caused statistically significant ($p < 0.001$) differences in fresh weight, dry weight, leaf count, Specific Leaf Area (SLA), and Leaf Area Index (LAI) compared to the shorter photoperiods. No significant differences were found between the 12-hour and 14-hour treatments. These results suggest that photoperiod adjustments could enhance the efficiency and productivity of GREENBOX units for urban agriculture.

Species used in this study: Rex 'Butterhead' lettuce, *Latuca sativa* L.

Index words: Controlled Environment Agriculture, hydroponics, lettuce, photoperiod, urban agriculture.

Significance to the Horticulture Industry

With the rising pressures on food security, GREENBOX technology was developed as an avenue for fresh leafy vegetable crop production in urban settings. Urbanization continues to challenge the horticulture industry to provide innovative solutions to combat food insecurity and instances of food deserts. GREENBOX technology utilizes CEA coupled with hydroponics as an avenue for sustainable crop production. This provides many benefits to the horticultural industry, including higher quality, consistency, and yield of crops while also reducing the resources required compared to soil-based food production, resulting in a net increase in crop production rates. It also reduces the need for pesticides and increases the marketability for fresh, clean, nutritious produce. As light is a very significant factor in the production of nutritious leafy greens such as 'Rex Butterhead' lettuce (*Latuca sativa* L.), it can be altered in spectra, intensity, and duration to enhance crop productivity and nutritional value. Although artificial lighting may be the most effective resource in CEA, it is also the most unsustainable due to high energy usage and associated cost. Finding a suitable photoperiod that can reduce the energy consumption of GREENBOX technology without compromising the quality of crops can offer the horticulture industry a reliable, year-round opportunity for sustainable crop cultivation in urban areas.

Introduction

Light is an essential component of crop production as it is the primary driver of photosynthesis. The absorption of light by plants leads to the formation of many chemical substances, such as pigments, hormones, and carbohydrates, which are essential to plant growth and development (Burkholder 1936). Therefore, the viability of crops is dependent on light. Light intensity, light duration, and light spectrum may all be manipulated to improve crop growth and quality.

Plants utilize light spectra from red (740nm) to blue (435nm) to carry out various necessary processes and functions for growth. Visible radiation also influences crops' nutritional value as it is fundamental in synthesizing carbohydrates in leafy greens (Burkholder 1936). The light intensity can impact plants' morphological and physiological responses throughout their growth cycle, which may later affect their nutritional value (Zheng et al. 2020). Low light intensity can slow plant growth and productivity by impacting gas exchange, while an overabundance of light can negatively affect photosynthetic efficiency (Fan et al. 2013).

Duration of light is also an essential factor in crop productivity and nutritional value. Longer photoperiods can

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promote larger leaf size, chlorophyll content, and fresh weight in lettuce crops, which are associated with better nutritional quality (Iqbal et al. 2022). However, longer photoperiods may also result in tip burn and marginal leaf necrosis in leafy greens (Iqbal et al. 2022). Shorter photoperiods may cause leaves to stretch and become thinner and lighter in color due to reduced light exposure (Palmer and van Iersel 2020). Light intensity parameters are measured using the Daily Light Integral (DLI), which may be defined as the total amount of light received by a plant in a 24-hour period (Kozai 2018). The recommended DLI for leafy green crops such as lettuce is 17 mol·m^{-2}·day^{-1} (Paz et al. 2019). Most leafy greens require an average Photosynthetic Photon Flux Density (PPFD) of 100-300 umol·m^{-2}·s^{-1}, with a 10-18 hour photoperiod to avoid improper pigmentation (Cui et al. 2021).

In conventional soil-based agriculture, the light duration of crops varies with the seasons. During the approach of winter, the duration of daylight decreases, resulting in shorter days, while the opposite occurs during the approach of summer. These seasonal changes cause variations in DLI which can affect concentrations of nitrate, sugars, and other metabolites in various leafy green vegetables (Gent 2014). Supplemental lighting is needed for the efficient growth of plants year-round because the northern portions of the United States receive approximately three to five times more sunlight in June than in January (van Iersel and Gianino 2017). Controlled Environment Agriculture (CEA) commonly uses soilless cultivation techniques such as hydroponics (Khan et al. 2021, Paz et al. 2019), which consist of enclosed or semi-closed spaces that reduce the impacts of harmful insects and other pests and optimize environmental factors such as light and temperature, to provide consistency and predictability of food production (Kikuchi et al. 2018). Considering crop productivity strongly depends on the crop's photosynthetic rate, having the ability to control light parameters is a significant advantage in the crop production process (Ahmad et al. 2019). Therefore, the quality of lighting elements within CEA is crucial to successful crop production.

Light-emitting diode (LED) lights are commonly used for indoor crop production due to their high energy efficiency and spectral specificity (Wollaeger and Runkle 2014). Although the lighting element can be the most expensive component of CEA, it is also the most effective because it offers flexibility in light spectra, light intensity, and photoperiod (Morrow 2008). GREENBOX technology is a controlled environment system with ideal environmental conditions, enhanced through artificial lighting, environmental sensors, ventilation systems, and nutrient delivery systems (Singh and Yang 2021). GREENBOX technology was developed for urban crop production using principles of CEA at the University of Connecticut in response to increased pressures on food security (Liu et al. 2018, Singh et al. 2021a, 2021b, 2022, Yang et al. 2017). GREENBOX technology was designed for localized crop production in urban warehouse settings. The versatility of CEA (such as GREENBOX technology) and its ability to be used or installed near consumers can increase resource use efficiency (Xydis et al. 2020). By reducing the carbon emissions used in transport of produce, we can take the first steps to reducing the carbon footprint for CEA crop production (Xydis et al. 2020). Previous studies determined the technical and financial feasibility of crop production utilizing GREENBOX technology (Buss et al. 2023a, 2023b, Carroll et al. 2023, Griffith et al. 2023, Singh et al. 2021a, 2021b, Singh, et al. 2023a, 2023b, 2023c). In previously conducted studies, GREENBOX technology utilized LED lighting to implement a standard lighting photoperiod for crop production: a 16-hour light-on period and an 8-hour dark period. A knowledge gap exists on the viability of utilizing other photoperiod regimes for crop production using GREENBOX technology.

The main objective of this study was to assess the viability of fresh crop production using GREENBOX technology by implementing different lighting regimes. We tested whether the assembled GREENBOX units using commercially available materials provide optimal environmental conditions and biomass output to support crop production when utilizing varying photoperiods, which has implications on operation costs due to lighting elements being a large component of expenditures with Controlled Environment Agriculture.

Materials and Methods

Location. The experiments were conducted in the Aquarium Room (Academic Building 9, #114) at Florida Gulf Coast University (FGCU) in Fort Myers, Florida, United States of America. Fort Myers is in southwest Florida along the Gulf of Mexico, resulting in a generally subtropical climate consisting predominantly of hot, humid summers, and moderately cold winters lasting only a few weeks. The summer months are April through October, with an average temperature between 23.9-32.2 C (75-90 F) (NOAA 2023). The winter months are November through March, with average temperatures between 13.9-27.2 C (57-81 F) (NOAA 2023). The aquarium room had warehouse-like conditions, including tall ceilings, windows, and climate control. The temperature was maintained between 20.5 C and 22.2 C (69-72 F) year-round. The FGCU Aquarium room is rectangular, with an area of 85.25 m^2 (3356.3 in^2) and dimensions of 8.4 m (330.7 in) × 10.1 m (397.6 in). The floor is angled towards two large drains in the floor for drainage associated with experiments.

Experimental setup. The experimental setup was nearly identical to Buss et al. (2023b) and Griffith et al. (2023) and similar to (Singh et al, 2023a). We assembled two GREENBOX units for lettuce crop production using hydroponic Nutrient Film Technique (NFT) systems (Buss et al. 2023a, Singh et al. 2023a). The GREENBOX units were equipped with an artificial LED lighting element, environmental monitoring control modules, and soilless cultivation systems (Singh et al. 2021a). An illustration of the experimental setup is presented in Figure 1.

Environmental sensors (Extech SD800 Environmental Control System with SD Memory Card, Vernon Hills, Illinois) with the capability to monitor carbon dioxide, temperature, and relative humidity were used in the experimental setup. The lighting elements in this experimental setup were used for seedling and crop production. The GREENBOX unit containing the 14-hour treatment utilized one Phantom

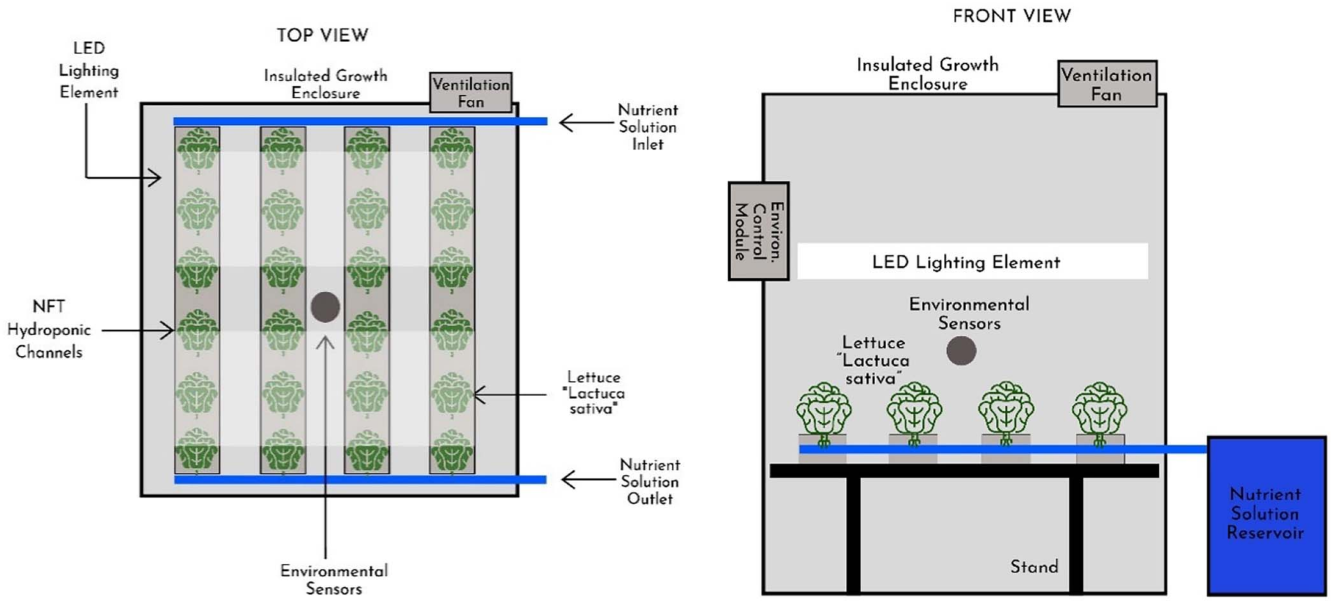


Fig. 1. Illustrations of the experimental setup utilizing an NFT system in the GREENBOX units. The GREENBOX units are shown in a horizontal top view and a front cross-sectional view.

PHENO 440 LED 100-277V 440W MP Spectrum light, while the GREENBOX unit containing the 12-hour treatment utilized one AgroLED Sun 48 LED 6500K - 120 Volt light in addition to eight FREELICHT (Amazon Inc., Seattle, Washington) LED lights to achieve an average DLI of 30 $mol{\cdot}m^{-2}{\cdot}day^{-1}$. The data collected from the control treatment utilizing the 16-hour light and 8-hour dark photoperiod was from a previously conducted experiment (Singh et al. 2023a). The lighting element used was LED light source (powerPAR PPLF44, Hydrofarm LLC, Petaluma, California) producing 160 watts with 131 lumens per watt rating efficiency, providing a light of 40,000 K color temperature for a rated diode life of 50,000 hours (Singh et al. 2023a).

Experimental procedure. Lettuce crop production lasted 44 days in total over August and September 2023, including a 14-day plug preparation period. On day 14, the lettuce plugs were transplanted into the NFT hydroponic systems. Following transplant, a 30-day growth cycle was carried out to expected harvest. We chose lettuce as our experimental subject for crop production because it is an agriculturally crucial leafy vegetable due to worldwide demand (Zheng et al. 2020).

The pelleted *Latuca sativa* 'Rex Butterhead' lettuce (Johnny's selected seeds, Fairfield, Maine) seeds were distributed into each hole of the OASIS® Horticubes (104 counts, OASIS® Grower Solutions, Kent, Ohio). The Horticubes were placed in a black tray 51 cm by 25 cm (20 in by10 in) Perfect Garden Seed Starter Grow Trays, Amazon Inc., Seattle, Washington), saturated with Reverse Osmosis (RO) water, and covered with newspaper. The covered seeds were placed in complete darkness for 48 hours to facilitate germination. Following the germination period, the seeds were placed under artificial LED lighting. The lights were programmed to provide 16 continuous hours of light per day between 06:00 and 22:00 for plug preparation.

The seedlings were fertilized with a starter nutrient solution daily, to ensure total saturation of the horticubes throughout the seedling stage and promote growth. The starter solution was composed of 3.6 grams of "Jack's hydroponic 15.5-0-0" (calcium nitrate) (Jacks Nutrients, JR Peters, Inc., Allentown, Pennsylvania) and 3.8 grams of "Jack's hydroponic 5-12-26" (Jacks Nutrients, JR Peters, Inc., Allentown, Pennsylvania) for every 10 L of water.

When two true leaves had developed after the cotyledon, the plugs were transplanted into the NFT systems on the fourteenth day. A nutrient solution was prepared for fertigation in the NFT systems using 9.0 grams of "Jack's hydroponic 15.5-0-0" (calcium nitrate) (Jacks Nutrients, JR Peters, Inc., Allentown, Pennsylvania) and 9.4 grams of "Jack's hydroponic 5-12-26" (Jacks Nutrients, JR Peters, Inc., Allentown, Pennsylvania) for every 10 L of water.

Due to the nutrient uptake by plants during growth, the elemental composition of the nutrient solution may be altered in a closed-loop system. Therefore, monitoring the composition of the nutrient solution regularly was essential. The optimal temperatures to grow lettuce are 17-28 C (63-82 F), the optimum pH is 5.8, and the electrical conductivity (EC) is 1.5 mS (Holmes et al. 2019). The nutrient solution was maintained with a target pH of 5.8 Standard Units (SU) and a targeted EC of 1.5-2.0 mS. The pH and EC of the nutrient solution were measured every three days and adjusted accordingly. If the pH was below the target pH of 5.8 standard units, we increased the pH by adding an alkali (0.05 M NaOH), and if the pH was above the target pH of 5.8 SU, we added an acid (0.05 M HCl). If the EC was below the target EC of 1.5 mS, we increased it by adding fertilizer based on initial calculations. If the EC exceeded the target of 2.0 mS, we decreased the EC by increasing the water content through dilution with RO water.

Following the transplant, the lettuce crops were subjected to different photoperiods. In treatment one, the lights were set to give the crops fourteen hours of continuous light between 6:00 and 20:00. In treatment two, the lights were set to provide the crops with twelve hours of continuous light between 6:00 and 18:00.

Data acquisition. We collected different types of data in this experiment, including environmental, biomass, and algal growth. We collected environmental data such as temperature, relative humidity (%), and CO_2 (ppm). Data was collected every 10 seconds of the growth cycle. We used 4G SD cards to store data, later exported as a Comma Separated Values (CSV) file. The amount of water vapor in the air can be described by relative humidity (RH) or vapor pressure deficit (VPD). Environmental sensors (Extech SD800 Environmental Control System with SD Memory Card, Vernon Hills, Illinois) were calibrated regularly following manufacturers' recommendations. Light intensity was monitored using a PPFD light sensor (FH-100 Light Meter PAR Meter, Amazon Inc., Seattle, Washington) The measuring range is up to 400,000 lx and 6000 (umol·m^{-2}·s^{-1}). PPFD measurements are automatically converted into DLI using the TUYA app, which was connected to a mobile device using Bluetooth. Three PPFD (umol·m^{-2}·s^{-1}) and DLI (mol·m^{-2}·day^{-1}) light measurements were taken daily from different points within the GREENBOX units. All values were averaged to represent the environmental conditions accurately throughout the growth cycle. The 16-hour control treatment data was obtained from a previous study with the same environmental conditions (Singh et al. 2023a).

A random number generator was used to choose lettuce heads for sampling. The biomass data of each lettuce head was monitored by destructive and nondestructive sampling every five days. The growth medium and roots were removed before taking the shoot fresh weight. The fresh shoot weight (g) was taken immediately after harvest to retain maximum moisture content; allowing evapotranspiration to occur may lead to inaccurate measurements. The dry weight (g) was recorded for each sample after all the leaves were collected in a brown paper bag and each bag was left in the drying oven for six days at 65 C (149 F).

Using a mobile device (iPhone 12 mini, Apple Inc., Cupertino, California), the leaf area was measured using the Leafscan app (Anderson and Anderson 2017). Measurements were completed using a reference measurement, four black dots equidistant from each other in the shape of a square, on a white sheet of paper. Then, each leaf was placed on the area of the paper without covering the black reference dots and photographed in the Leafscan app. The leafscan app compared the white area to the green area of the leaf and measured the leaf area in square centimeters (cm^2) with an accuracy of 0.01 cm^2. The data was collected and exported as a CSV file.

Four chlorophyll and nitrate concentration measurements per sample were taken using an AMTAST Chlorophyll Meter for estimating plant chlorophyll (Amazon Inc., Seattle, Washington). The measurement range for chlorophyll is 0.0-99.9 SPAD with an accuracy within 1 SPAD unit and 0.0-99.9 mg·g^{-1} for nitrogen content. The measurement area was 2 mm by 2 mm. The meter was powered by a 4.2 V rechargeable lithium battery with a capacity of 800MAH. To measure the chlorophyll content of the lettuce, light from two LED sources was emitted by the meter, one was red light (650 nm), and the other was infrared light (940 nm). Both light sources penetrate the leaf and hit the receiver. The light signal is then converted into an analog signal. The analog signal is amplified and converted into a digital signal by an analog/digital converter. The digital signal is processed by the microprocessor and the SPAD value is calculated and displayed on the LCD screen. The SPAD value was then converted to total chlorophyll content in (mg·cm^{-2}).

Using a NIX Pro 2 Color Sensor (Pro Color Sensor™, Nix Sensor Ltd., Ontario, Canada), one measurement from each plant was taken on the day of harvest. The NIX Color Sensor connects to a smart phone via Bluetooth. NIX color values were determined for each head of lettuce and displayed on the smart device. The measurements are taken in CIE2000, CIE76, CIE94G, CIE94T, CMC1:1, CMC2:1 values. The sensor is able to read and store up to 1,000 measurements. It uses four High-CRI LEDs designed specifically for color reproduction.

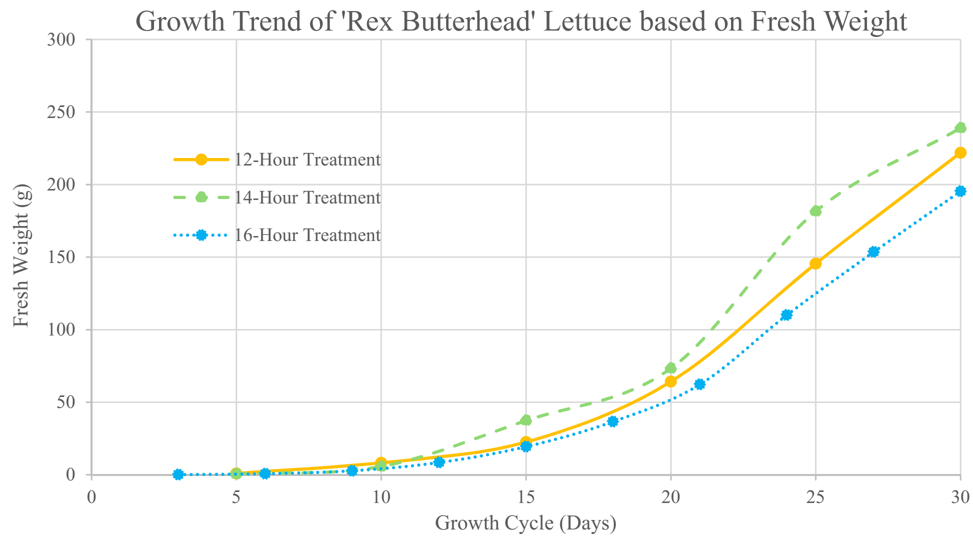
Algae sampling occurred every five days, starting at day zero. A 50 mL sample of nutrient solution was collected in a sterile centrifuge tube. The sample was incubated for two weeks and examined microscopically for the growth of algae, including cyanobacteria. The findings are described qualitatively.

Data processing and descriptive statistics. Using collected variables described in the previous section, we derived SLA (cm^2·g^{-1}), which is the ratio of total leaf area to dry weight of the crop, and LAI (cm^2·cm^{-2}), which is the ratio of lettuce head area over one square cm, total chlorophyll, and productivity (kg·m^{-2}).

Descriptive statistics were used to represent biomass data. The fresh and dry weights were displayed graphically to represent the growth trend (Fig. 2). Over the growth cycle, fresh weight (g), dry weight (g), total leaf area (cm^2), SLA (cm^2·g^{-1}), LAI (cm^2·cm^{-2}), and productivity (kg·m^{-2}) of lettuce crops in each hydroponic setting are presented in the following section. A permutation multivariate analysis of variance (PERMANOVA) was performed via the *vegan* package (Oksanen et al. 2022), followed by pairwise post-hoc analyses using PERMANOVAs and Wilcoxon Rank Sum tests, to understand crop performance differences at varying photoperiods. Statistical analysis was conducted using R statistical software (R Core Team 2022) at the 5% significance level.

Results and Discussion

All three treatments of light duration including the 12-hour treatment, 14-hour treatment, and 16-hour control treatment were able to produce lettuce. Lettuce exposed to 12 hours of light and 14 hours of light per 24-hour period for an entire growth cycle produced results that indicate GREENBOX technology may provide optimal environmental conditions necessary to reach the expected harvest weight. Table 1 summarizes environmental conditions such as PPFD (umol·m^{-2}·s^{-1}), DLI (mol·m^{-2}·day^{-1}), temperature (C), relative humidity (%), and carbon dioxide (ppm) inside GREENBOX units throughout the growth cycle.



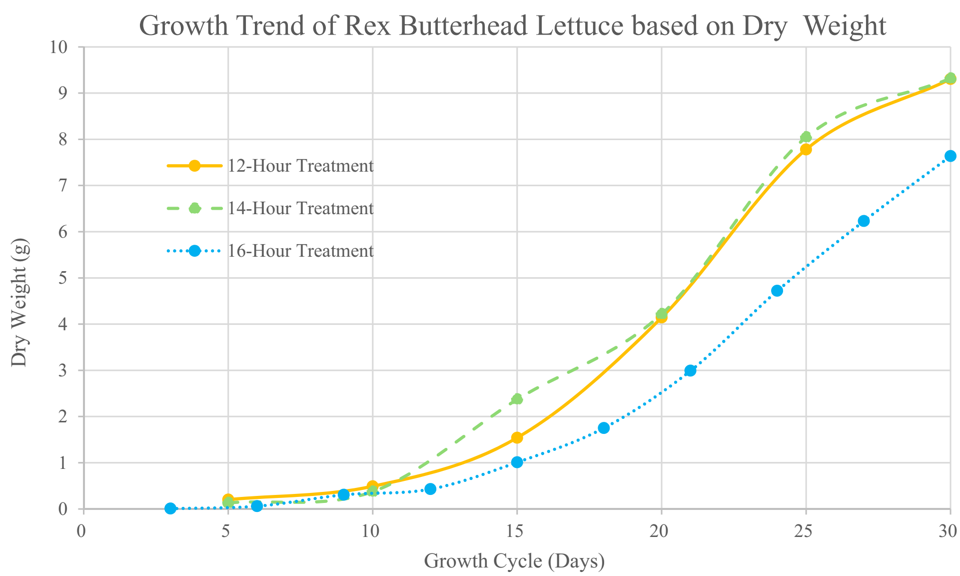


Fig. 2. Presents the growth trend following the fresh shoot and dry shoot weights over the growing cycle. The growth curve demonstrates similar growth rates for all treatments. As a result, we observe a slightly higher fresh and dry shoot weight for the 14-hour photoperiod than the 12-hour photoperiod on the day of harvest, however both are higher than the control treatment, the 16-hour photoperiod.

The mean PPFD ranged from 593.7 to 688.3 umol·m^{-2}·s^{-1} for the 12-hour photoperiod and 513.7 to 695.0 umol·m^{-2}·s^{-1} for the 14-hour photoperiod. The DLI ranged from 24.8 to 29.8 mol·m^{-1}·day^{-1} for the 12-hour photoperiod and 25.9 to 35.0 mol·m^{-1}·day^{-1} for the 14-hour photoperiod. The PPFD and DLI measurements for the 12 and 14-hour treatments were similar to the averages for the 16-hour treatment. A linear relationship exists between plant growth and PPFD/DLI in controlled environments (Kubota 2016). Results from both the 12 and 14 hour treatments exceeded the recommended minimum DLI of 6.5 – 9.7 mol·m^{-2}·day^{-1} (Paz et al. 2019).

The mean temperature ranged from 20.2 to 32.5 C (68-90 F) for the 12-hour photoperiod and 20.0 to 29.8 C (68-86 F) for the 14-hour photoperiod. The temperatures in the 12 and 14-hour treatments are similar to the average temperatures in the 16-hour treatment. Due to the grow tent being indoors and thermal insulation inside both GREENBOX units, the temperature variation remained minimal. The temperature regime was within the optimal range of 17-28 C (63-82 F) (Holmes et al. 2019) for the majority of the growth cycle but increased for short periods of time.

The mean relative humidity ranged from 37.7 to 96.8% for the 12-hour photoperiod and 42.7 to 89.6% for the 14-hour photoperiod. The relative humidity stayed within the recommended 40-60% for most of the study but sometimes exceeded the recommended range for short time periods (Ryu et al. 2014). Maintaining relative humidity at an optimum level is essential in crop production because high relative humidity can lead to fungal disease and low relative humidity can lead to stunted growth (Ryu et al. 2014).

The mean CO_2 values ranged from 444 and 692 ppm for the 12-hour treatment and 443 to 833 ppm for the 14-hour treatment. The average values in the 12 and 14-hour treatment were higher than the average CO_2 values from the 16-hour control treatment.

The environmental conditions presented throughout this growth cycle are suitable for the year-round production of

Table 1. **Average light intensity in Photosynthetic Photon Flux Density plus one standard deviation (umol·m^{-2}·s^{-1}), Average Daily Light Integral (mol·m^{-1}·day^{-1}) plus one standards deviation, Average temperature plus one standard deviation, Average relative humidity (%) plus one standard deviation, and average CO_2 (ppm) plus one standard deviation in 12-hour treatment and 14-hour treatment systems using GREENBOX technology over the growth cycle. The 16-hour treatment data shows averages and one standard deviation for previously collected data (Singh et al., 2021a).**

Light treatment duration	PPFD (umol·m^{-2}·s^{-1})	DLI (mol·m^{-1}·day^{-1})	Temperature (°C)	Relative humidity (%)	CO_2 (ppm)
12 Hr.	633.5±24.4	27.3±1.2	26.3±3.1	59.6±11.3	499.8±29.6
14 Hr.	608.9±48.3	30.7±2.5	25.0±1.9	59.7±8.8	501.4± 29.4
16 Hr.	606	34.93	25.9±1.0	47.14±6.6	306.5±57.9

'Rex Butterhead' lettuce. The conditions in the 12- and 14-hour treatments were similar to the 16-hour treatment environmental conditions.

Table 2 presents the collected biomass data from crop harvest on Day 30. Both 12-hour and 14-hour treatments were compatible with GREENBOX technology and could carry out crop production to full harvest. The fresh weights for each photoperiod exceeded the average harvest weight of 181 g per lettuce head (Tokunaga et al. 2015). The 12-hour and 14-hour photoperiod had comparable fresh and dry weights, but the 14-hour treatment exhibited a greater statistically significant difference in the fresh weight ($p = 0.012$) and dry weight ($p = 0.001$) than the 16-hour photoperiod and data collected from previous GREENBOX growth cycles (Singh et al. 2021b, 2022, Singh and Yang 2021). Fresh and dry weights represent the aggregate gas exchange in photosynthesis and evapotranspiration throughout the growth cycle (Kubota 2016).

On the contrary, we observed a higher statistically significant difference ($p = 0.0001$) in SLA in the 16-hour treatment compared to the 12-hour and 14-hour treatments, which had comparable values. However, both 12-hour and 14-hour treatments resulted in a statistically greater number of leaves than the 16-hour treatment ($p = 0.02$ and $p = 0.009$ respectively). This may be because we observed a high quantity of leaves affected by tipburn in the 12 and 14-hour treatments. Tipburn is generally associated with high growth rates of lettuce (Frantz et al. 2004). High DLI may have caused localized calcium deficiency as the required calcium levels are not being distributed to the leaves fast enough (Frantz et al. 2004).The statistical analysis of SLA suggests that although the 12 and 14-hour treatments produced more biomass, the growth rate is fast enough to cause significant tipburn in 'Rex Butterhead' lettuce, thereby reducing the leaf area and quality of the lettuce produced. The occurrence of tipburn may result in lettuce produce being unsellable or with reduced marketability (Fang et al. 2020, Kaufmann 2023).

Chlorophyll and nitrate content was measured throughout the growth cycle for the 12 and 14-hour treatments to further inform us on the quality of lettuce produced. No statistically significant differences were observed between treatments ($p = 0.7254$, $p = 0.7564$, $p = 0.5113$, $p = 0.5343$ for chlorophyll and for nitrate for the 12-hour and 14-hour treatments respectively).

The PERMANOVA confirmed that the duration of light exposure is important ($F[2,23] = 16.44$, $p = 0.001$). Pairwise-PERMANOVAs revealed a significant difference between 14- and 16-hour exposure ($F[1,15] = 43.86$, $p = 0.001$), 12- and 16-hour exposure ($F[1,15] = 18.39$, $p = 0.001$), but not between 12- and 14-hour exposure ($F[1,16] = 0.17$, $p = 0.798$). Specifically, pairwise Wilcoxon rank sum tests found that the 16-hour treatment resulted in significantly greater SLA than the 12- ($p = 0.0001$) and 14-hour ($p = 0.0001$) treatments, significantly less fresh weight ($p = 0.012$) and dry weight ($p = 0.001$) than the 14-hour treatment, with fewer leaves than both the 12- ($p = 0.02$) and 14-hour ($p = 0.009$) treatments.

Using data collected from the NIX Color Sensor, a statistical analysis shows no significant differences ($p > 0.05$) in color data. Although, we observed slight visible differences in the color of the lettuce crops, no correlation was found between the different treatments and chlorophyll content.

The green algal genera *Chlorella, Desmodesmus, Monoraphidium,* and the cyanobacteria *Pseudanabaena* and *Schizothrix,* were all found to be present in the nutrient solution throughout the growth cycle (Fig. 3). Although algae can cause competition for water and nutrients with lettuce crops, there was not enough detected to cause a hindrance to crop growth.

In conclusion, we found that all three systems' environmental conditions, such as temperature, relative humidity, and CO_2, were similar and that all photoperiods could produce lettuce for consumption at the expected harvest weight following a 30-day growth cycle. These results are comparable to previous growing cycles using GREENBOX technology and other peer-reviewed literature. Statistical analysis suggests the 12-hour and 14-hour treatments can produce more lettuce in terms of biomass parameters such as fresh weight and dry weight than the 16-hour treatment. The main aim of this study was to compare the biomass parameters of 'Rex Butterhead'

Table 2. **Average shoot fresh weight (g), shoot dry weight (g), leaf area index (LAI), specific leaf area (SLA), leaf count (n), chlorophyll (SPAD), total chlorophyll (mg·cm^{-2}), nitrate (mg·g^{-1}), and productivity (kg·g^{-2}) for each treatment were recorded at harvest on day 30 of the growth cycle.**

	Fresh weight (g·$head^{-1}$)	Dry weight (g·$head^{-1}$)	LAI (cm^{-2}·cm^{-2})	SLA (cm^2·g)	Leaf count (n)	Chlorophyll (SPAD)	Total chlorophyll (mg·cm^{-2})	Nitrate (mg·g^{-1})	Productivity (kg·m^{-2})
12-Hour Treatment	221.95	9.30	6.16	241.44	55	32.64	0.028	13.0	5.33
14-Hour Treatment	239.03	9.32	6.32	226.73	59	32.16	0.027	12.8	5.74
16-Hour Control Treatment	200.41	7.33	5.85	455.53	46	NA	NA	NA	6.78

Fig. 3. These images show the different species of algae and cyanobacteria found in the nutrient solution. through the 30-day growth cycle. A) *Chlorella* and *Pseudodanabena* B) *Desmodesmus* C) *Schizothrix and* D) *Monophidium.*

lettuce using different photoperiods using GREENBOX Technology. The results from this work would inform future design iterations of GREENBOX technology and how to reduce the energy output produced from artificial lighting without compromising biomass productivity and the nutritional quality of 'Rex butterhead' lettuce.

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