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Influence of Day and Night Temperature and Radiation Intensity on Growth, Quality, and Economics of Indoor Green Butterhead and Red Oakleaf Lettuce Production

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Abstract: Lettuce (*Lactuca sativa*) is among the most consumed vegetables worldwide and is primarily field-grown; however, indoor agriculture enables year-round, precise production. Through precise manipulation of the mean daily temperature (MDT) and photosynthetic photon flux density (*PPFD*), crop color, morphology, and yield can be altered. Therefore, we quantified how MDT and *PPFD* interact and developed models predicting yield and economic viability. Eleven days after sowing, green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï RZ' were transplanted into six deep-flow hydroponic tanks with day/night and MDTs of 22/15 °C (20 °C), 25/18 °C (23 °C), or 28/21 °C (26 °C), under light-emitting diodes providing a low or high *PPFD* of 150 or 300 μmol·m^{-2}·s^{-1} for 17-h·d^{-1}. As *PPFD* increased, shoot fresh mass (SFM) of 'Rex' increased by 29% (33.4 g). SFM of 'Rouxaï RZ' and shoot dry mass (SDM) of both cultivars was influenced by the interaction of MDT and *PPFD*. The greatest 'Rouxaï RZ' SFM (158.8 g) and SDM (6.42 g) were recorded at >20 °C MDT under the high *PPFD*; the lowest SFM (76.0 g) and SDM (3.17 g) occurred at 20 °C under the low *PPFD*. Similarly, 'Rex' SDM was greatest (7.36 g) and lowest (3.78 g) under the aforementioned MDTs and *PPFD*s. Increasing from the low to high *PPFD* increased tipburn incidence on 'Rouxaï RZ' from 0 to 25% and 'Rex' from 47 to 100%. 'Rouxaï RZ' had darker yellow-red foliage at lower MDTs under the high *PPFD*. A high MDT and low *PPFD* resulted in a lighter green. Finally, for the greatest SFM, while reducing energy costs as interpreted from the economic analysis, we recommend growing 'Rex' and 'Rouxaï RZ' under a *PPFD* of 150 and 300 μmol·m^{-2}·s^{-1}, respectively, at an MDT of 23 to 26 °C depending on the cost of temperature control.

Keywords: photosynthetic photon flux density; controlled environment agriculture; daily light integral; leafy greens; vertical farming

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1. Introduction

Vertical farms, warehouses, and shipping containers, collectively encompassing indoor agriculture (IA), provide the opportunity to produce year-round where growing seasons, land access, or food system infrastructure are limiting [1,2]. Set apart from greenhouses for its sole use of artificial lighting systems, IA facilities enable precise control of environmental conditions, improving produce quality and annual yields beyond that possible under field conditions [3,4]. In addition, these compact, closed systems offer more efficient use of resources such as land and water [5,6], contributing to a recent rising interest in producing leafy greens in IA [2]. Lettuce (*Lactuca sativa*) is particularly well-suited for IA production due to its compact growth, quick marketable biomass accumulation, and high market significance, enabling higher yield per area with efficient space utilization [1].

Parameters influential to plant growth, such as radiation duration, quantity, and quality; day and night temperatures; airflow; relative humidity (RH); vapor pressure deficit (VPD); and carbon dioxide (CO_2) concentration can all be precisely manipulated in IA [1–4].

Mean daily temperature (MDT) and the photosynthetic photon flux density (*PPFD*) strongly influence lettuce growth and development, as well as quality parameters such as flavor, color, nutrient content, and the occurrence of physiological disorders such as tipburn [4].

The MDT influences plant developmental rate, including the rate of germination, rooting, leaf unfolding, and flowering; phytochemical biosynthesis and accumulation; and overall quality, with different crops having specific temperature ranges conducive for development [7–9]. Overall plant growth, including shoot (branching, stem diameter, and leaf size) and root growth, foliage coloration, and flowering, is impacted by the daily light integral (DLI) and *PPFD* [10,11].

Photosynthesis is driven predominately by the available *PPFD*. Leaf photosynthetic rate increases linearly with *PPFD*, followed by a quadratic slope until the light saturation point, at which a greater *PPFD* does not further increase photosynthesis [12,13]. The ratio of plant productivity per *PPFD* is the light-use efficiency—increasing the *PPFD* above the light saturation point can reduce light-use efficiency as energy inputs increase without proportional yield responses. However, maintaining the *PPFD* at or below the saturation point while extending the day length can allow for yield increases [11,14]. The light saturation point and DLI response are highly species-specific, can vary between cultivars, and depend on leaf area index and other environmental factors, such as temperature and CO_2 concentration [12,15].

The response of lettuce to *PPFD* has been recorded in many studies [11,15–19]. Sago compared the growth of lettuce 'Pansoma' grown at 20 °C, 1200 $\mu mol \cdot mol^{-1}$ CO_2, and under *PPFDs* of 150, 200, 250, and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLIs of 13.0, 17.3, 21.6, and 25.9 $mol \cdot m^{-2} \cdot d^{-1}$) [18]. Shoot fresh mass (SFM) and dry mass (SDM), relative growth rate, leaf number, and tipburn occurrence all increased with increasing *PPFD*. SDM 35 d after sowing increased by 1.12-, 1.32-, and 1.42-fold under 200, 250, and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, compared to lettuce grown under 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, there was no difference in SDM between plants under 250 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating light saturation from 250 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [18]. Fu et al. grew romaine lettuce 'Lvling' under *PPFD*s of 100, 200, 400, 600, and 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLIs of 5, 10, 20, 30, and 40 $mol \cdot m^{-2} \cdot d^{-1}$) and a day/night temperature (14 h/10 h) of 20/16 °C (18.3 °C MDT) [15]. Plants under *PPFD*s of 200 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had high light-use efficiency and yield, with 400 and 600 $mol \cdot m^{-2} \cdot s^{-1}$ producing the largest yields and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ having the greatest light-use efficiency. Conversely, the lettuce had the lowest light-use efficiency and yields under 100 or 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Signs of stress were present under 600 and 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with the latter showing the highest level of stress as indicated by maximum photosystem II quantum yields (F_v/F_m) below 0.8. Due to high yield and relatively low-stress indicators, Fu et al. recommended maintaining a *PPFD* of 400 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for lettuce [15].

Kelly et al. found that green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï RZ' increased in SFM and SDM, leaf width and number, and chlorophyll concentration when DLIs increased from 6.9 to 15.6 $mol \cdot m^{-2} \cdot d^{-1}$ at an MDT of 22 °C, 60% relative humidity (RH), and 380 $\mu mol \cdot mol^{-1}$ CO_2 [11]. Additionally, the SFM under a DLI of 15.6 $mol \cdot m^{-2} \cdot d^{-1}$ was greatest under a *PPFD* of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 24 $h \cdot d^{-1}$ compared to the same DLI composed of *PPFD*s of 216 and 270 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with shorter photoperiods of 20 and 16 $h \cdot d^{-1}$, respectively. The SFM impact may be due to light-use efficiency decreasing under high *PPFD*s, alongside light saturation points being reached, at which increasing photoperiod may increase yield while greater *PPFD*s would not.

The influence of MDT on lettuce development, morphology, growth, and metabolism have been previously investigated [4,20,21]. Ouyang et al. grew 'Grand Rapids Tbr' at 16, 18, and 20 °C under a continuous *PPFD* of 210 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 30 days after transplant [21]. Lettuce SFM and height was 38 and 18% (9.9 g and 1.9 cm) greater at 20 °C than at 16 °C, and SDM was 14% (0.5 g) greater at both 18 and 20 °C than at 16 °C.

The interaction of MDT and DLI on lettuce growth has also been investigated previously [19,21,22]. For instance, Lee et al. grew crisphead lettuce cultivars 'Adam', 'Manchu', and ‘Sensation’ at day/night temperatures (12 h/12 h) of 22/18 °C (20 °C MDT) or 18/16 °C (17 °C MDT) and under *PPFD*s of 150, 200, and 250 µmol·m^{-2}·s^{-1} for the first 30 d after transplant (DAT) [19]. From 30–60 DAT, the plants were grown at 18/16 °C (17 °C MDT) or 18/14 °C (16 °C MDT). For each cultivar, leaf number increased with temperature, while *PPFD* only impacted ‘Manchu’ leaf number at the lower temperature when increased from 150 to 250 µmol·m^{-2}·s^{-1}, increasing from 22 to 27 leaves. Leaf biomass was lowest at the high MDT and 150 µmol·m^{-2}·s^{-1} for all cultivars, with the greatest leaf biomass occurring at the high MDT and 250 µmol·m^{-2}·s^{-1} for ‘Sensation’, low MDT, and 250 µmol·m^{-2}·s^{-1} for ‘Adam’, and 250 µmol·m^{-2}·s^{-1} at either MDT or 200 µmol·m^{-2}·s^{-1} at the low MDT for ‘Manchu’. These findings exemplify that there are cultivar-specific responses to MDT and *PPFD*.

The sustainability of IA spans several broad categories, including its environmental, social, and economic impact [23]. In this study, the latter is estimated by identifying how revenues and key variable production costs are affected by changes in MDT and *PPFD* in lettuce production. Temperature and lighting contribute directly to one of the largest variable production costs in IA production: energy costs [24–26].

Given the strong influence of MDT and *PPFD* on lettuce growth, development, and quality, identifying conditions for improved resource efficiency and yield in IA is needed. Therefore, the objectives of this study were (1) to quantify if lettuce growth, development, quality, and yield are influenced by the interaction of MDT and *PPFD*; (2) to develop models that predict yield and economic viability under various *PPFD*s and MDTs. We postulated that (1) increasing *PPFD* will increase biomass production but increase the occurrence of tipburn; (2) higher temperatures will increase leaf number for both cultivars while reducing ‘Rouxaï RZ’ red pigmentation intensity and profitability.

2. Materials and Methods

Plant material and propagation conditions. On 28 April and 9 June 2020, seeds of red oakleaf lettuce ‘Rouxaï RZ’ and green butterhead lettuce ‘Rex’ (Rijk Zwaan; Salinas, CA, USA) were sown into 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Gordan, Milton, ON, Canada). The cultivars were selected due to their use in previous indoor production studies and commercial relevance. Plugs were presoaked in deionized water with a pH of 4.4 to 4.5 adjusted using diluted (1:31) 95 to 98% sulfuric acid (J.Y. Baker, Inc.; Phillipsburg, NJ, USA). The plug trays were covered with translucent plastic domes for 3 d to maintain high humidity during germination. Trays were placed in a walk-in growth chamber (Hotpack environmental room UWP 2614-3; SP Scientific, Warminster, PA, USA) with an MDT of 22 °C, CO_2 concentration of 500 µmol·mol^{-1}, and RH of 60%. Light-emitting diode (LED) fixtures (Ray66 Indoor PhysioSpec; Fluence Bioengineering, Austin, TX, USA) provided a total photon flux density (*TPFD*, 400–800 nm) of 180 µmol·m^{-2}·s^{-1} and a light ratio (%) of 19:39:39:3 blue (400–500 nm): green (500–600 nm): red (600–700 nm): far-red (700–800 nm) radiation for 24 h. After 3 d, the photoperiod was reduced to 20 h until transplant at 11 d. Seedlings were sub-irrigated with deionized water supplemented with water-soluble fertilizer providing (in mg·L^{-1}): 125 N, 18 P, 138 K, 73 Ca, 47 Mg, 1.56 Fe, 0.52 Mn, 0.36 Zn, 0.21 B, 0.21 Cu, 35 S, and 0.01 Mo (12N–1.8P–13.3K RO Hydro FeED; JR Peters, Inc., Allentown, PA, USA). The pH and electrical conductivity (EC) were adjusted to 5.6 and 1.6 dS·m^{-1}, respectively, as determined with a pH/EC probe (HI 991,301 pH/TDS/Temperature Monitor; Hanna Instruments, Smithfield, RI, USA). The pH was adjusted using potassium bicarbonate and sulfuric acid, while the EC was adjusted by adding deionized water and concentrated nutrient solution.

Hydroponic systems. On 9 May and 20 June 2020, 14 seedlings of each cultivar were transplanted 20-cm-apart into six 250 L, 0.9-m-wide by 1.8-m-long deep-flow hydroponic systems (Active Aqua premium high-rise flood table; Hydrofarm, Petaluma, CA, USA) distributed within three walk-in growth chambers described previously. Each hydroponic system contained a 4-cm-thick extruded polystyrene foam sheet to float on the nutrient solution. Plastic net baskets were placed into 4-cm-diameter holes in the polystyrene foam, and seedlings were placed in the baskets, so the rockwool was in contact with the nutrient solution. Deionized water supplemented with water-soluble fertilizer providing (in mg·L^{-1}) 150 N, 22 P, 166 K, 87 Ca, 25 Mg, 1.9 Fe, 0.62 Mn, 0.44 Zn, 0.25 B, 0.25 Cu, and 0.01 Mo (12N–1.8P–13.3K RO Hydro FeED; JR Peters, Inc.), and 0.31 g·L^{-1} magnesium sulfate (Pennington Epsom salt; Madison, GA, USA). The EC and pH were adjusted daily to maintain an EC of 1.7 dS·m^{-1} and pH of 5.6, as described previously. Air pumps (Active Aqua 70 L·min^{-1} commercial air pump; Hydrofarm) connected to air stones (Active Aqua air stone round 10.2 cm × 2.5 cm; Hydrofarm) were used to increase the dissolved oxygen concentration.

Growth chamber environmental conditions. The air day/night (17 h/7 h) and MDT set points in each growth chamber were 22/15 (20 °C), 25/18 (23 °C), or 28/21 (26 °C), measured every 5 s by a resistance temperature detector (Platinum RTD RBBJL-GW05A-00-M 36B; SensorTec, Inc., Fort Wayne, IN, USA) and logged by a C6 controller (Environmental Growth Chambers, Chagrin Falls, OH, USA). *PPFD*s of 150 or 300 μmol·m $^{-2}$·s^{-1} were provided for 17 h·d^{-1} by LED fixtures (Ray66; Fluence Bioengineering), providing a DLI of 9.2 and 18.4 mol·m^{-2}·d^{-1}, respectively, averaged over several measurements (Table 1). The LEDs were mounted ~130 and 95 cm above the crop canopy for the 150 and 300 μmol·m^{-2}·s^{-1} treatments, respectively. Every 15 s, water temperature, leaf temperature, and *PPFD* were measured using a thermistor (ST-100; Apogee Instruments, Logan, UT, USA), infrared thermocouple (OS36-01-T-80F; Omega Engineering, INC. Norwalk, CT, USA), and quantum sensor (LI-190R; LI-COR Biosciences, Lincoln, NE, USA), respectively, with means logged every hour by a CR-1000 datalogger (Campbell Scientific, Logan, UT, USA). A CO_2 concentration of 500 μmol·mol^{-1} was maintained in each chamber with compressed CO_2 injection, measured with a CO_2 sensor (GM86P; Vaisala, Helsinki, Finland) and logged by a C6 Controller (Environmental Growth Chambers) every 5 s. Relative humidity was maintained at 58.5% (±4.6).

Table 1. Mean (± sd) day and night air temperature, canopy and water temperatures; photosynthetic photon flux density (*PPFD*); vapor pressure deficit (VPD); and carbon dioxide (CO_2) concentrations during 36 or 37 d of indoor deep flow hydroponic production for butterhead lettuce (*Lactuca sativa*) 'Rex' and red oakleaf lettuce 'Rouxaï RZ', respectively.

Temperature (°C)				PPFD (μmol·m^{-2}·s^{-1})	VPD (kPa)		CO_2 (μmol·mol^{-1})
Air Day	Air Night	Canopy	Water		Day	Night	
22.0 ± 0.1	15.3 ± 0.1	22.2 ± 3.3	20.6 ± 1.0	150.0 ± 1.6 307.8 ± 5.8	1.08 ± 0.05	0.67 ± 0.03	499.2 ± 44.9
24.9 ± 0.4	18.3 ± 0.2	25.7 ± 3.7	23.9 ± 1.0	150.9 ± 3.4 299.9 ± 9.3	1.22 ± 0.06	0.81 ± 0.04	503.1 ± 51.4
28.1 ± 0.5	21.3 ± 0.4	28.0 ± 3.1	26.4 ± 1.1	150.8 ± 5.0 299.4 ± 10.2	1.73 ± 0.20	1.17 ± 0.13	497.8 ± 58.4

Growth data collection and analysis. Parameters assessed for lettuce quality included the foliage coloration of 'Rouxaï RZ', relative chlorophyll concentration (RCC), the maximum photosystem II quantum yields (F_v/F_m), and the dry mass. The foliage coloration of ten 'Rouxaï RZ' plants in each treatment was measured 35 d after sowing with a tristimulus colorimeter (Chroma Meter CR-400; Konica Minolta Sensing, Inc., Chiyoda, Tokyo), reported as International Commission on Illumination (CIE) L*a*b* color space values, which were then converted to hue angle (h°) and chroma (C*) as suggested by McGuire [27]. The RCC of the most recent fully expanded leaf of ten plants of each cultivar in each treatment was then estimated with a SPAD meter (MC-100 Chlorophyll Meter; Apogee Instruments, Logan, UT, USA). One leaf of ten plants per treatment was then dark acclimated for >15 min using three of the manufacturer-supplied clips and then exposed to 3500 μmol·m^{-2}·s^{-1} of red radiation (peak wavelength 650 nm) to saturate photosystem II and the fluorescence was measured, averaged, and reported as F_v/F_m by a portable chlorophyll fluorescence meter (Handy Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, UK).

'Rouxaï RZ' and 'Rex' were harvested 36 and 37 d after sowing, respectively. SFM (g), length and width (cm) of the sixth fully expanded leaf, and leaf number (when >5 cm) were measured on ten plants of each cultivar per treatment. Plant height from the roots to the highest point of the foliage and the width at the widest point and perpendicular from the widest point was measured with a ruler and recorded. Incidence, but not severity, of tipburn was recorded. To provide an integrated measurement of plant size, the growth index (GI) was calculated (GI = {plant height + [(diameter 1 + diameter 2)/2]}/2) [28]. The plant material was placed in a forced-air drier maintained at 75 °C for at least 3 d, weighed, and SDM was recorded.

An economic analysis was conducted using a simplified economic model developed to estimate the economic viability under various PPFDs and MDTs. This economic analysis integrates revenues and the most significant variable production costs in an IA farm [25]. It is assumed that MDT and PPFD will affect plant growth and, therefore, yields and associated revenues, while electricity costs will be affected by changes in PPFD. Labor, consumables, and packaging costs are also tested for aggregated effects of variable production costs.

Space optimization: A base production model is firstly defined by setting aside a propagation area where lettuce grows up to transplant (SpcBT) and a production area where lettuce grows after transplant (SpcAT) according to a space ratio (SpcR), which is estimated as the ratio of the density before transplant (DenBT) to the density after transplant (DenAT). Given the relatively lower density after transplant, SpcAT is a multiple of a base area (B) and this space ratio. To estimate the minimum space required to grow these crops, this simulation establishes a minimum base area of 1 m^2 for propagation. The minimum size production module, or total farm size (TSpc), becomes the sum of a base area SpcBT and SpcAT. In this base production module, harvest occurs at the end of each cycle, which comprises a number of days before transplant (DBT) and days after transplant (DAT). However, a commercial farm requires continuous production for daily harvest. The criterion is met by using the length of cycle before and after transplant as multipliers to define the respective space required. Length of cycles, therefore, determines the number of growing modules operating sequentially. Total farm size producing daily harvest is thus estimated as:

$$\text{TSpc} = (\text{SpcBT} * \text{DBT}) + (\text{SpcAT} * \text{DAT}), \text{ where SpcBT} = \text{B and SpcAT} = \text{B} * \text{SpcR}.$$

Costs and revenues are applied to relevant areas. For broader applications, results are reported per area ($\$\cdot m^{-2}$). Economic results are described from a cost minimization approach where the sum of variable production costs per area is represented as a proportion of revenues per area.

Electricity costs: The increased cost of electricity with greater *PPFD* were accounted for, but changes in heating or cooling costs to maintain each MDT were not accounted for, instead being kept constant. The model considers a 30% load in electricity costs in HVAC use; this may cause inaccuracies in situations where *PPFD* output influences temperature and required HVAC costs.

Specifically, electricity costs were estimated based on photoperiod (PhPer) and PPFD, applying an efficacy rate (ηPAR) of 2.5 and an electricity price rate of $0.10 per kWh in all scenarios. DLI is estimated as a function of the photoperiod and PPFD used in each stage (*i*), either before transplant (BT) or after transplant (AT). Daily energy cost (DEC) per m^2 takes the ratio of DLI to light efficacy (ηPAR) and multiplied by the electricity rate in kWh (CE):

$$\text{DLI}_i = \text{PhPer} * 3600 * \text{PPFD}$$

$$\text{DEC}_i = (\text{DLI}_i/\eta\text{PAR})/3{,}600{,}000 * \text{CE}$$

Annual electricity costs (AnnEC) associated with lighting system and HVAC is assumed to be continuous throughout the year (y = 360 days on a financial year) and applied to the growing area respectively to the entire propagation and production areas, respectively to PPFD settings:

$$\text{AnnEC} = [(\text{DEC}_{\text{BT}} * \text{SpcBT} * \text{DBT}) + (\text{DEC}_{\text{AT}} * \text{SpcAT} * \text{DAT})] * (1 + \text{HVAC}) * \text{y}$$

Labor costs: Labor costs were estimated as the number of hours spent per day per square meter in five general labor activities. The number of labor hours per square meter before transplant (L_{BT}) includes labor hours spent on seeding, while labor after transplant (L_{AT}) includes activities deemed to take place at the SpcAT area, including transplanting, harvesting, and packaging. It is assumed that cleaning (L_C) occurs daily in the entire farm area (TSpc). The estimation of the number of hours per activity takes two steps. It applies the ratio of one hour of labor dedicated to these five activities estimated by Kozai to the average labor hour per m^2 on a day [25] on a small farm (equal to or smaller than 10,000 sq.ft. or 930 m^2). Labor hour per m^2 was estimated to be 0.038 of an hour, based on Agrilyst reported average total labor hours and average farm area [29]. These estimates show seeding taking 11% of the daily labor time per m^2, transplanting and cleaning taking approximately 16% each, packaging 25%, and harvesting 32%. Total labor hours per day (L_D) for the entire growing area becomes:

$$L_D = (\text{Lc} * \text{TSpc}) + (L_{BT} * \text{SpcBT}) + (L_{AT} * \text{SpcAT})$$

Annual wages paid (AnnWg) is the product of daily labor in number of hours in one year by hourly wages (Wh), estimated to be on average $12.46 per hour, and a 20% benefit loading (Wb):

$$\text{AnnWg} = L_D * (\text{Wh} * (1 + \text{Wb})) * \text{y}$$

Consumables costs: Seeds and growing media are the only two input costs considered in this analysis as these are one of the highest variable costs in production. These costs contribute to a better understanding of the distribution of these variable production costs, although not expected to affect results on a per m^2 basis. The cost of seeds (CSeed) is the average wholesale price for lettuce seeds, at $0.03/seed. As for growing media (CMed), 1-inch rockwool hydroponic grow cubes starters were considered, at the cost of $0.035 per unit, following average market prices. These are assumed to be single-seeded in the plant propagation stage and then transferred with the plant upon transplant into the production area. As such, along with seeds, these costs occur daily as a product of plant density before transplant (DenBT) per m^2 in each module of the propagation area (SpcBT). On an annual basis, the cost of inputs is estimated as follows:

$$\text{AnnCInp} = (\text{CSeed} + \text{CMed}) * \text{DenBT} * \text{SpcBT} * \text{y}$$

Packaging costs are considered as well to evaluate whether more packaging material is required for increased yields. A unit price (CPck) of $0.04 is considered, following average market prices, and applied directly to daily harvest (DH) weight which is converted into oz and divided by size of package (Sz) of 4.5 oz. Annual cost of packaging becomes:

$$\text{AnnCPck} = ((\text{DH} * \text{y}) * \text{lb} * 16)/\text{Sz} * \text{CPck}$$

Revenues: Daily harvest (DH) is estimated as a product of density per m^2, individual plant mass, and the size of each module of production area (SpcAT) being harvested daily. Annual revenue (AnnRev) becomes the product of daily harvest by the number of days in a financial year (y), converted from harvest in grams to pounds (lb.), and the retailer price (P) per pound adjusted by retailers' margin (RetM). The latter adjustment provides the model with the flexibility to estimate the impact of market price changes. Changes in MDT and

PPFD are not expected to affect crop loss and shrinkage, so these are set as 100% harvest sales and zero shrinkage in all scenarios.

$$\text{AnnRev} = \text{DH} * \text{y} * \text{lb} * \text{P} * \text{RetM}$$

Given the lack of formally reported market prices for premium lettuce at the time of this work, retail prices were obtained through an online search of groceries stores' websites based in the U.S. Midwest (e.g., Wholefoods, Meijer, Kroger, Aldi), in Fall 2020. Market prices for selected leafy greens include products that were advertised as differentiated produce, ready for consumption, and sold in hard plastic packages. An average package size of 4.5 oz was adopted for the model simulation. Model simulations adopted the average price for single varieties at $13.41/lb. Wholesale prices were estimated using a standard industry gross margin of 50%, applied over the cost of goods sold (COGS).

The experiment was arranged in a split-block design with three temperature (three growth chambers) treatments as the main factor with two *PPFD* sub-factors, with 10 plants of each cultivar per treatment combination. The experiment was completed twice in time, and the growth chamber temperature treatments were randomized. Data were analyzed separately by cultivar with SAS (version 9.4; SAS Institute, Cary, NC, USA) mixed model procedure (PROC MIXED) for analysis of variance (ANOVA), tests of normality and homogeneity of variances were performed, and pairwise comparisons were performed with Tukey-Kramer difference test ($p \leq 0.05$). SigmaPlot (version 14.5, Systat Software, Inc., San Jose, CA, USA) was used for regression analysis.

3. Results

3.1. Shoot Fresh and Dry Mass

The SFM of 'Rouxaï RZ' was influenced by the interaction of MDT and *PPFD* (Table 2; Figure 1A). Increasing the MDT from 20 to 23 °C under a *PPFD* of 150 and 300 μmol·m^{-2}·s^{-1} increased SFM by 30 and 42% (by 22.9 and 44.6 g), respectively, while SFM did not further increase at an MDT of 26 °C. At MDTs of 20, 23, and 26 °C, increasing the *PPFD* from 150 to 300 μmol·m^{-2}·s^{-1} increased SFM by 41, 53, and 56% (by 30.8, 52.5, and 57.1 g), respectively. For 'Rex', raising the *PPFD* from 150 to 300 μmol·m^{-2}·s^{-1} increased SFM by 29% (33.4 g) among all MDT treatments.

Table 2. Influence of mean daily temperature (MDT; 20, 23, and 26 °C) and photosynthetic photon flux density (*PPFD*; 150 and 300 μmol·m^{-2}·s^{-1}) on growth index; leaf length and width (cm) and number (no.); shoot fresh and dry mass (g); relative chlorophyll concentration (RCC); tipburn incidence (TB); hue angle (h°); chroma (C*); and CIE L* color value of red oakleaf lettuce 'Rouxaï RZ' and green butterhead lettuce 'Rex'. Data represent the mean of two replications per cultivar with 10 samples. Analyses of variance for the effects of MDT and *PPFD* and their interaction are included below each cultivar mean. Within-column means with different letters were significantly different according to Tukey–Kramer difference test ($p < 0.05$).

MDT	PPFD	Growth Index	Leaf Length	Leaf Width	Leaf (No.)	Fresh Mass	Dry Mass	RCC	TB (%)	h°	C*	L*
						'Rouxaï RZ'						
20	150	17.2 b	13.1	17.2 b	17.1 c	76.0 c	3.17 d	18.9 b	0 b	107.8 a	17.7 c	37.3 b
	300	17.4 b	12.3	18.9 a	20.2 b	106.8 b	4.80 b	23.2 a	20 a	80.2 b	7.0 d	28.8 a
23	150	19.6 a	14.2	18.7 b	22.4 b	98.9 b	3.93 c	18.2 b	0 b	110.9 a	21.8 b	39.0 b
	300	20.1 a	14.2	20.4 a	28.0 a	151.4 a	6.05 a	21.8 a	15 a	84.8 b	8.2 d	30.5 a
26	150	20.9 a	14.9	19.5 b	22.6 b	101.7 b	4.12 c	18.5 b	0 b	113.6 a	27.3 a	39.8 b
	300	21.3 a	14.5	20.6 a	28.5 a	158.8 a	6.42 a	22.4 a	40 a	88.2 b	7.7 d	30.1 a
PPFD		NS [z]	NS	***	***	***	***	***	***	***	***	***
MDT		*	NS	NS	**	*	*	NS	NS	NS	**	NS

Table 2. *Cont.*

MDT	PPFD	Growth Index	Leaf Length	Leaf Width	Leaf (No.)	Fresh Mass	Dry Mass	RCC	TB (%)	h°	C*	L*
PPFD × MDT		NS	NS	NS	*	***	*	NS	NS	NS	***	NS
						'Rex'						
20	150	16.9 a	13.2 a	12.9 b	22.7 b	99.6 b	3.78 d	24.4 b	45 b	–[y]	–	–
	300	15.4 b	11.5 b	14.6 a	21.5 b	133.1 a	5.68 b	31.1 a	100 a	–	–	–
23	150	18.3 a	14.3 a	13.1 b	27.5 a	121.1 b	4.47 c	23.1 b	55 b	–	–	–
	300	17.3 b	12.6 b	14.3 a	28.0 a	149.9 a	6.94 a	30.5 a	100 a	–	–	–
26	150	19.5 a	14.4 a	13.7 b	30.9 a	129.2 b	4.45 c	23.6 b	40 b	–	–	–
	300	17.7 b	12.9 b	14.5 a	32.3 a	167.1 a	7.36 a	31.9 a	100 a	–	–	–
PPFD		***	***	***	NS	***	***	***	***	–	–	–
MDT		NS	NS	NS	*	NS	*	NS	NS	–	–	–
PPFD × MDT		NS	NS	NS	NS	NS	**	NS	NS	–	–	–

[z] NS—non-significant, *, **, *** represent non-significant or significant difference at $p \leq 0.05$, 0.01, and 0.001, respectively. [y] Data not collected.

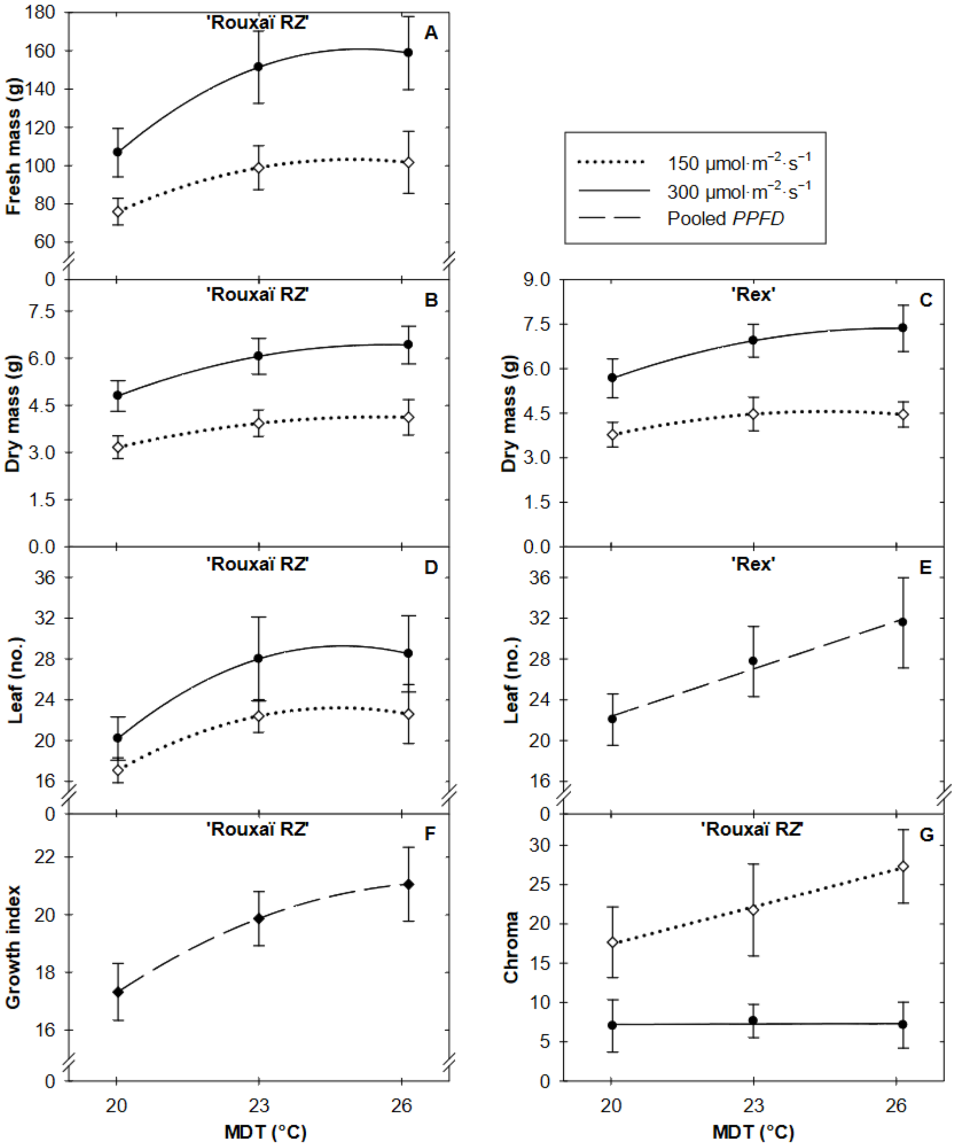

Figure 1. Effects of MDT (20, 23, and 26 °C) and *PPFD* (150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on red oakleaf lettuce (*Lactuca sativa*) 'Rouxaï RZ' shoot fresh (**A**) and dry (**B**) mass, leaf number (**D**), growth index (**F**), and chroma (**G**), and green butterhead lettuce 'Rex' shoot dry mass (**C**) and leaf number (**E**). Model

predictions are represented by lines; error bars represent standard errors; coefficients are in Table 3, and means in Table 2.

Table 3. Regression analysis equations and r^2 or R^2 for mean leaf number, dry and fresh mass, growth index, and chroma in response to MDT (20, 23, and 26 °C) and *PPFD* (150 and 300 µmol·m^{-2}·s^{-1}) of green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï RZ'. All models are in the form of: $f = y_0 + a{\cdot}\mathrm{MDT} + b{\cdot}\mathrm{MDT}^2$.

Parameter	*PPFD*	y_0	(a) MDT	(b) MDT^2	R^2 or r^2
		'Rouxaï RZ'			
Fresh mass (g)	150	-6.06×10^{2} [y]	5.68×10	-1.14	0.462
		1.91×10^{2} [w]	1.67×10	3.61×10^{-1}	
	300	-1.14×10^{3}	1.03×10^{2}	-2.06	0.634
		2.70×10^{2}	2.36×10	5.10×10^{-1}	
Dry mass (g)	150	-1.75×10	1.70	-3.30×10^{-2}	0.444
		7.09	6.20×10^{-1}	1.30×10^{-2}	
	300	-2.72×10	2.62	-5.10×10^{-2}	0.607
		8.86	7.74×10^{-1}	1.70×10^{-2}	
Leaf number	150	2.12	1.30×10^{-2}	-4.10×10^{-2}	0.834
		5.11×10^{-1}	6.32×10^{-4}	1.10×10^{-2}	
	300	-2.30×10^{2}	2.10×10	-4.25×10^{-1}	0.553
		5.31×10	4.64	1.00×10^{-1}	
Growth index	*	-3.63×10	4.26	-7.90×10^{-2}	0.673
		1.19×10	1.04	2.30×10^{-2}	
Chroma	150	-1.44×10	1.59	[x]	0.383
		6.00	2.59×10^{-1}		
		'Rex'			
Leaf number	*	-8.67	1.55		0.540
		3.06	1.32×10^{-1}		
Dry mass (g)	150	-1.94×10	1.96	-4.00×10^{-2}	0.317
		7.60	6.65×10^{-1}	1.40×10^{-2}	
	300	-2.52×10	2.52	-4.90×10^{-2}	0.524
		1.07×10	9.36×10^{-1}	2.00×10^{-2}	

* *PPFD* not significant [y] Coefficients for model equations were used to generate Figure 1 [x] Blank cells = 0 [w] Standard error (se).

For both cultivars, MDT and *PPFD* interacted to influence SDM (Table 2; Figure 1B–D); increasing either parameter increased SDM. Under 150 and 300 µmol·m^{-2}·s^{-1}, increasing the MDT from 20 to 23 °C increased the SDM of 'Rouxaï RZ' by 24 and 26% (by 0.76 and 1.25 g), respectively, and 'Rex' by 18 and 22% (by 0.69 and 1.26 g), respectively. However, SDM did not further increase at an MDT of 26 °C. The SDM of 'Rouxaï RZ' increased by 51, 54, and 56% (1.58, 2.25, and 2.36 g) and 'Rex' by 50, 55, and 65% (1.89, 2.47, and 2.90 g) as the *PPFD* was raised from 150 to 300 µmol·m^{-2}·s^{-1} at MDTs of 20, 23, and 26 °C, respectively.

3.2. Plant Morphology

Leaf unfolding of 'Rouxaï RZ' was influenced by interactions between MDT and *PPFD* (Table 2; Figure 1D). As the *PPFD* increased from 150 to 300 µmol·m^{-2}·s^{-1} at MDTs of 20, 23, and 26 °C, 'Rouxaï RZ' unfolded 3, 6, and 6 more leaves (increases of 18, 25, and 26%), respectively. As MDT increased from 20 to 23 °C under a *PPFD* of 150 and 300 µmol·m^{-2}·s^{-1}, five and eight more leaves unfolded (increases of 31 and 39%), respectively, while additional leaves did not unfurl at an MDT of 26 °C. 'Rex' leaf number increased linearly as MDT increased (Table 2; Figure 1E). From an MDT of 20 to 23 °C, leaf number increased from 22 to 28 leaves (by 26%), while increasing from 23 to 26 °C did not increase leaf number.

GI was influenced by the MDT for 'Rouxaï RZ' (Table 2; Figure 1F). Increasing the MDTs from 20 to 23 °C increased the GI by 15%; increasing from 23 to 26 °C did not further increase GI. In contrast, the GI of 'Rex' was influenced by the *PPFD*, decreasing by 8% from 150 to 300 µmol·m^{-2}·s^{-1} (Table 2). As *PPFD* increased from 150 to 300 µmol·m^{-2}·s^{-1}, the leaf length of 'Rex' was reduced by 12% (1.6 cm), and the leaf width of 'Rex' and 'Rouxaï RZ' increased by 9 and 8% (1.2 and 1.4 cm), respectively (Table 2).

Tipburn incidence was influenced by *PPFD* for both cultivars (Table 2). From 150 to 300 μmol·m^{-2}·s^{-1}, tipburn incidence increased from 0 to 25% and from 47 to 100% for 'Rouxaï RZ' and 'Rex', respectively.

3.3. Relative Chlorophyll Concentration, F_v/F_m, and Pigmentation

For both cultivars, *PPFD* influenced RCC (Table 2); RCC was 21 and 31% greater for 'Rouxaï RZ' and 'Rex', respectively, when *PPFD* increased from 150 to 300 μmol·m^{-2}·s^{-1} (Table 2). The chlorophyll fluorescence, estimated and reported as F_v/F_m, stayed within a range of 0.830 and 0.869, not entering ranges associated with stress.

For the red-leaf cultivar 'Rouxaï RZ', h° was influenced by *PPFD*. As *PPFD* increased from 150 to 300 μmol·m^{-2}·s^{-1}, the h° decreased from 110.7 (green) to 84.4° (yellow/red). The C*, the degree of departure from gray toward a chromatic color, was influenced by the interaction of *PPFD* and MDT (Table 2; Figure 1G). Under a *PPFD* of 300 μmol·m^{-2}·s^{-1}, the MDT did not influence C*, with an average value of 7.2 (very gray). Under 150 μmol·m^{-2}·s^{-1}, the C* values at 20, 23, and 26 °C were 17.7, 21.8, and 27.3 (more chromatic), respectively. The foliage lightness, L*, decreased from 38.7 (lighter) to 29.8 (darker) under 150 and 300 μmol·m^{-2}·s^{-1}, respectively (Table 2).

3.4. Economic Analysis

Increasing the *PPFD* from 150 to 300 μmol·m^{-2}·s^{-1} had a multiplier effect on the cost of electricity during production. For 'Rex' and 'Rouxaï RZ' grown at an MDT of 26 °C the sum of variable production costs reached a maximum of \$122 and \$174 per m^{-2} and \$121 and \$175 per m^{-2} at a *PPFD* of 150 and 300 μmol·m^{-2}·s^{-1}, respectively (Table 4). Compared to 'Rouxaï RZ', 'Rex' incurred higher variable production costs as the growth cycle was utilized in the model as a multiplier of the growing area required to allow daily harvest (Table 4). Consumables and labor are associated with plant density and size of the growing area; therefore, they were constant through experiments. Electricity costs are directly associated with DLI and PPFD, with a 30% load of HVAC costs. Finally, packaging costs include labor and the units of packages used, therefore directly associated with SFM. Normalizing costs on a per m^{-2} basis, variable production costs follow increases in SFM, driven by electricity and packaging costs. Similarly, revenue increases are proportional to SFM, which followed increases in the MDT and PPFD. For 'Rex' grown at a *PPFD* of 300 μmol·m^{-2}·s^{-1}, SFM was 29% greater than at 150 μmol·m^{-2}·s^{-1}, but electricity costs were 100% higher. In this case, electricity costs averaged 40% of total variable production costs when *PPFD* was 150 μmol·m^{-2}·s^{-1} and 55% at 300 μmol·m^{-2}·s^{-1}.

The economic analysis indicates that at an MDT of 20 °C variable production costs represent ~24% of revenue for 'Rex' and ~30% for 'Rouxaï RZ' regardless of *PPFD*, while at an MDT of 26 °C the costs were reduced to ~20% of revenue (Table 4). For 'Rex', a *PPFD* of 150 μmol·m^{-2}·s^{-1} had a better economic return than under 300 μmol·m^{-2}·s^{-1}, regardless of the MDT (Table 4). However, the economic return for 'Rouxaï RZ' improved under a *PPFD* of 300 μmol·m^{-2}·s^{-1} compared to 150 μmol·m^{-2}·s^{-1}. For 'Rex', from a perspective of cost minimization, the best economic result of 18.6% occurred when plants were grown under a *PPFD* of 150 μmol·m^{-2}·s^{-1} and an MDT of 26 °C, while 'Rouxaï RZ' had the best economic results of 20.8% under a *PPFD* of 300 μmol·m^{-2}·s^{-1} and an MDT of 26 °C.

Table 4. Summary of results from the economic analysis for yield of red oakleaf lettuce 'Rouxaï RZ' and green butterhead lettuce 'Rex' at MDTs of 20, 23, or 26 °C and under a *PPFD* of 150 or 300 μmol·m^{-2}·s^{-1}. Estimates account for variable production costs.

	'Rouxaï RZ'						**'Rex'**					
Parameters	20:150 *	23:150	26:150	20:300	23:300	26:300	20:150	23:150	26:150	20:300	23:300	26:300
Fresh mass (g)	76.0	98.9	101.7	106.8	151.4	158.8	99.6	121.1	129.2	133.1	149.9	167.1

Table 4. *Cont.*

	'Rouxaï RZ'						'Rex'					
Annual harvest (kg)	54,720	71,208	73,224	76,896	109,008	114,336	71,712	87,192	93,024	95,832	107,928	120,312
Costs of consumables ('000$)	46.806	46.80	46.80	46.80	46.80	46.80	46.80	46.80	46.80	46.80	46.80	46.80
Packaging costs ('000$)	17.16	22.33	22.96	24.11	34.18	35.85	22.49	27.34	29.17	30.05	33.84	37.72
Annual wages ('000$)	77.85	77.85	77.85	77.85	77.85	77.85	80.47	80.47	80.47	80.47	80.47	80.47
Annual electricity costs ('000$)	96.21	96.21	96.21	191.69	191.69	191.69	100.03	100.03	100.03	199.32	199.32	199.32
Annual revenue ('000$)	808.8	1052.6	1082.4	1136.6	1611.3	1690.1	1060.0	1288.8	1375.0	1416.5	1595.3	1778.4
Total production space (m^2)	2000	2000	2000	2000	2000	2000	2080	2080	2080	2080	2080	2080
Total propagation space (m^2)	11	11	11	11	11	11	11	11	11	11	11	11
Total growing area (m^2)	2011	2011	2011	2011	2011	2011	2091	2091	2091	2091	2091	2091
Daily harvest (kg)	152	197	203	213	302	317	199	242	258	266	299	334
Daily labor (h)	14	14	14	14	14	14	15	15	15	15	15	15
Total variable production costs ('000$)	238.02	243.19	243.82	340.45	350.52	352.19	249.79	254.64	256.47	356.64	360.42	364.32
Variable production costs per area ($\$/m^2$)	118.36	120.93	121.25	169.29	174.30	175.13	119.46	121.78	122.65	170.56	172.37	174.23
Revenues per area ($\$/m^2$)	402.22	523.42	538.24	565.23	801.27	840.43	506.96	616.39	657.62	677.47	762.98	850.53
Economic results [z]	29.43	23.10	22.53	29.95	21.75	20.84	23.56	19.76	18.65	25.18	22.59	20.49

* MDT: *PPFD.* [z] Variable production cost per square meter as a proportion of revenues per square meter.

4. Discussion

Plant responses to temperature, *PPFD*, and their interaction are species- and cultivar-specific. Therefore, the specificity of environmental responses, coupled with the tight profit margins of many vertical farm operations, emphasizes the need for crop modeling to predict yield and economic parameters. In the present study, SFM for 'Rouxaï RZ' and SDM for both cultivars were influenced by the interaction of MDT and *PPFD,* while only the SFM of 'Rex' was influenced by *PPFD* alone. Similar to other studies, the greatest SFM for both cultivars occurred under a relatively high *PPFD* (~300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$) [11,18,19,30]. For instance, after 18 d at day/night temperatures (16 h/8 h) of 22/18 °C and 800 $\mu mol{\cdot}mol^{-1}$ CO_2, SFM, and SDM of 'Ziwei' increased by approximately 30 and 60% as the *PPFD* was raised from 150 to 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, respectively [30]. Kelly et al. reported a 50 and 50% increase in the SFM and SDM of 'Rex' and 51 and 31% for 'Rouxaï RZ' under *PPFD*s of 150 and 270 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, respectively, at an MDT of 22 °C, 60% RH, and 380 $\mu mol{\cdot}mol^{-1}$ CO_2 [11].

In the current study, we observed the greatest SFM for 'Rouxaï RZ' under a *PPFD* of 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and at MDTs of 23 and 26 °C (Figure 1A). Similarly, Choi et al. reported that the SFM and relative growth rate of butterhead lettuce 'Omega' was greatest at 30/25 °C, compared to 20/15 °C, during the first 25 d, but by 35 d there was no difference in the SFM between plants at 20/15 and 30/25 °C, while the relative growth rate was lowest at 30/25 °C [31]. This suggests that the impact of MDT on SFM may depend on the CO_2 concentration [32], stage of growth, cultivar, plant density, and/or time to harvest.

In contrast, the lowest SFM in this study was under a *PPFD* of 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and MDT of 20 °C. Interestingly, the SFM of 'Rouxaï RZ' was similar between those harvested under a *PPFD* of 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and an MDT of 20 °C to those under 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and MDTs of 23 and 26 °C. This indicates that a greater *PPFD* does not always increase yield or crop quality with a suboptimal MDT. This aligns with the findings of Lee et al. [19], where the SFM of 'Sensation' was lower under a *PPFD* of 250 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and MDT of 17 °C than under a *PPFD* of 200 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and MDT of 20 °C. However, in contrast to our results where the SFM of 'Rouxaï RZ' was greater at 23 and 26 °C than at 20 °C under a *PPFD* 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, they reported that SFM under 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ was greater at 17 °C than at 20 °C. This may be due to cultivar and other environmental and cultural differences, such as the vapor-pressure deficit (VPD) or CO_2 concentration, both of which can influence the photosynthetic rate.

Morphological changes in response to MDT and *PPFD* were observed for both cultivars. As the *PPFD* increased from 150 to 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, leaf width for both cultivars increased; however, the GI and leaf length of 'Rex' decreased at the higher *PPFD* (Table 2). The compact growth of 'Rex' and greater leaf width of both cultivars under higher *PPFD*s align with the findings of Kelly et al. [11]. Greater leaf area and stem lengths, alongside reduced leaf thickness, have been observed in many species in response to reduced *PPFD*s, including lettuce [16,22,33,34]. An increase in leaf area, coupled with a reduction in leaf thickness, can improve light interception without increased assimilate demand [22,34].

The increase in leaf number in response to MDT is consistent with the understanding that developmental rates are primarily dependent on temperature [9]. Interestingly, the leaf unfolding rate only increased from an MDT of 20 to 23 °C, not 23 to 26 °C. This may be indicative of an optimum temperature (T_{opt}) being reached between 23 and 26 °C, given that the rate of development is often characterized by a linear increase from the base temperature (T_b) to the T_{opt}, after which developmental rate plateaus or declines to the maximum temperature (T_{max}) [35]. The T_b, T_{opt}, and T_{max} vary by cultivar and are influenced by other environmental conditions, including the DLI [34,35].

In the current study, we determined that *PPFD* only influenced leaf number for 'Rouxaï RZ', and to a lesser extent than MDT. This is consistent with the findings by Kelly et al. [11], where leaf number increased by 13% as *PPFD* increased from 150 to 270 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under a 16-h photoperiod. Findings by Sago suggest that the influence of *PPFD* on leaf number may be dependent on the duration of the harvest cycle [18]. Leaf number in 'Pansoma' butterhead lettuce grown at 20 °C increased as *PPFD* increased from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when harvested 30 DAT; however, at 35 DAT, leaf number only increased under *PPFD*s of 150 or 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Additionally, there appear to be cultivar-specific responses for leaf number in lettuce [11,19]. When comparing crisphead lettuce cultivars 'Adam', 'Manchu', and 'Sensation', leaf number was dependent on MDT and cultivar, while only 'Manchu' was impacted by *PPFD* interacting with MDT [19].

In our study, tipburn incidence in both cultivars was only influenced by *PPFD*, with 'Rex' having a greater incidence than 'Rouxaï RZ' (Table 2). The cultivar difference may be attributed to morphological differences; 'Rex' forms compact heads that decrease transpiration at the growing point, while 'Rouxaï RZ' does not produce a head. The influence of *PPFD* on tipburn has been described in several studies [18,36]. Sago reported that the number of leaves exhibiting tipburn increased with *PPFD* from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, concluding tipburn development is proportional to fresh and dry weight, relative growth rate, and leaf number [18]. Additionally, the total calcium concentration of lettuce increased with *PPFD* from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$; however, the concentration of calcium within the inner leaves remained similar regardless of the *PPFD* [18].

We did not find a relationship between tipburn incidence and MDT. Similarly, Lee et al. reported that tipburn occurrence in 'Dambaesangchuesse' and 'Mostcheongssam' was similar at MDTs of 18, 22, and 25 °C and under a *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from day 30–40 after sowing [37]. Conversely, Lee et al. reported that the increased growth rate of crisphead lettuce 'Adam', 'Manchu', and 'Sensation' at MDTs of 18.5 °C brought higher incidence of tipburn when compared to those grown at 16.5 °C [19], similar to the incidence in lettuce 'Batavia Othilie' at higher MDT observed by Carotti et al. [22]. A greater VPD can increase transpiration rates, potentially reducing tipburn occurrence [4,37]. In our study, maintaining a ~60% RH at each MDT calculated into VPDs of ~0.9, 1.1, and 1.3 kPa at 20, 23, and 26 °C, respectively. The greater VPDs at 23 and 26 °C may have reduced tipburn incidence due to greater transpiration compared to the lower VPD at 20 °C, mitigating MDT-influenced tipburn. Additionally, there may have been impacts on tipburn severity by MDT or *PPFD*, but the severity was not recorded in our study.

The marketability of certain crops is influenced by their unique coloration, so a balance between optimal temperature for yield and optimal temperature for coloration must be considered [38]. MDT and *PPFD* affect anthocyanin biosynthesis and accumulation of Compact growth of 'Rex' [7,39]. In our study, h° and L* of 'Rouxaï RZ' foliage was influenced by the *PPFD*, while C* was influenced by the MDT and *PPFD* interaction (Table 2; Figure 1G). Increasing *PPFD* from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ reduced the h°

from 110.7 to 84.4°. On the color wheel, a h° of 0°/360° indicates red, 90° indicates yellow, and 120° indicates green; therefore, increasing the *PPFD* caused foliage to move toward yellow and red values, away from green and blue values. The L*, a scale of lightness (high values) and darkness (low values), decreased from 38.7 to 29.8 under 150 and 300 μmol·m^{-2}·s^{-1}, indicating darker foliage at a higher *PPFD*. Increasing the *PPFD* from 150 to 300 μmol·m^{-2}·s^{-1} caused a lower C* regardless of MDT, indicating that the foliage became less colorful and closer to gray. However, at a *PPFD* of 150 μmol·m^{-2}·s^{-1}, the C* was influenced by the MDT, increasing from 17.7 to 27.3 as MDT increased from 20 to 26 °C. Overall, this indicates that foliage was a darker yellow and red at lower MDTs and high *PPFD*, while high MDT and low *PPFD* had vibrant, light-green foliage.

The economic analysis focused on the variable production costs per area as a percentage of revenue per area. A higher MDT improved the economic results across treatments, while a greater *PPFD* only improved the economic results for 'Rouxaï RZ'. Increasing the *PPFD* up to 300 μmol·m^{-2}·s^{-1} improved overall revenue across cultivars and temperatures, but this was not proportional to the doubling of electricity cost and packaging costs for 'Rex'. Our model was unable to account for the changes in the costs of heating and cooling. The cost of climate control in plant factories varies by location, seasonality, and climate control equipment. Typically, estimated cooling costs are greater in plant factories than in greenhouses, with components such as light fixtures releasing heat [26,40]. Additionally, our economic analysis did not account for the impact on crop quality and marketability, such as foliage coloration and tipburn incidence. Focusing on the economic effect of alternative MDT and PPFD on lettuce growth, the economic analysis considered revenue and costs associated with yield applying constant prices and zero harvest loss and shrinkage costs. However, 'Rex' had the greatest tipburn occurrence under a *PPFD* of 300 μmol·m^{-2}·s^{-1}, which was deemed to have worse economic results than at 150 μmol·m^{-2}·s^{-1} without accounting for product loss from tipburn. Further research is also needed to identify the effect of premium prices paid and specific niche markets for morphological changes such as foliage coloration and leaf texture on revenues.

5. Conclusions

In conclusion, increasing the *PPFD* from 150 to 300 μmol·m^{-2}·s^{-1} increased many crop attributes, such as SFM and SDM, leaf width, and RCC for both cultivars, alongside GI for 'Rex' and leaf number and color for 'Rouxaï RZ'. However, the occurrence of tipburn also increased under the higher *PPFD* for both cultivars, from 0 to 25% for 'Rouxaï RZ' and 47 to 100% for 'Rex', while economic results for 'Rex' worsened. Increasing MDT from 20 to 23 °C improved many crop parameters but increasing it further to 26 °C did not. Increasing the MDT from 20 to 23 °C increased SDM and leaf number for both cultivars, and SFM and GI for 'Rouxaï RZ'. However, only C* in 'Rouxaï RZ' was influenced by the further increase from 23 to 26 °C, creating more vibrant greens. 'Rouxaï RZ' had the greatest SFM (158.8 g) and SDM (6.42 g) at an MDT of 23 or 26 °C under a 300 μmol·m^{-2}·s^{-1} *PPFD*, while the lowest SFM (76.0 g) and SDM (3.17 g) occurred at 20 °C and under a *PPFD* of 150 μmol·m^{-2}·s^{-1}. Similarly, 'Rex' SDM was greatest (7.36 g) and lowest (3.78 g) under the aforementioned MDTs and *PPFD*s. Considering cost minimization, the best economic result for 'Rex' was 18.6% when plants were grown under a *PPFD* of 150 μmol·m^{-2}·s^{-1} and an MDT of 26 °C. 'Rouxaï RZ' had the best economic result of 20.8% under a *PPFD* of 300 μmol·m^{-2}·s^{-1} and an MDT of 26 °C. Ultimately, we recommend a *PPFD* of 300 μmol·m^{-2}·s^{-1} to produce 'Rouxaï RZ' and 150 μmol·m^{-2}·s^{-1} for 'Rex', while maintaining the MDT around 23 to 26 °C depending on the cost of temperature control.

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