IMPROVING YIELD AND QUALITY OF LEAFY GREENS GROWN INDOORS WITH PRECISE RADIATION, TEMPERATURE, AND CARBON DIOXIDE MANAGEMENT

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ABSTRACT

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Indoor agriculture systems can allow for precise manipulation of the mean daily temperature (MDT), carbon dioxide (CO_2) concentration, and photosynthetic photon flux densities (*PPFD*). Identifying how these environmental parameters interact to influence crop growth, development, yield, and color can assist growers with selecting their desired growing environment. Therefore, the objectives of Expt. 1 and 2 were to quantify and model how *PPFD* and CO_2 concentrations interact with MDT to influence the growth, yield, and quality of hydroponically grown green butterhead 'Rex' and red oakleaf lettuce 'Rouxaï RZ'. In Expt. 3 we developed models to predict growth parameters and cardinal temperatures of lettuce, arugula, and kale from 8 to 33 °C. In Expt. 1, lettuce 'Rex' and 'Rouxaï RZ' were grown in deep-flow hydroponic tanks under a *PPFD* of 150 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 17 $h \cdot d^{-1}$ at MDTs of 20, 23, or 26 °C. *PPFD* and MDT interacted to influence biomass accumulation of both cultivars.
In Expt. 2, lettuce 'Rex' and 'Rouxaï RZ' were grown under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and at the same MDTs as Expt. 1, but with CO_2 concentrations of 500, 800, or 1200 $\mu mol \cdot mol^{-1}$. Dry mass of both cultivars was influenced by the interaction of CO_2 and MDT; biomass accumulation was greatest at 800 $\mu mol \cdot mol^{-1}$ CO_2 at MDTs of 23 and 26 °C. In Expt. 3, 'Rex' and 'Rouxaï RZ', kale 'Red Russian', and arugula 'Astro' were grown at MDTs of 8, 13, 18, 23, 28, or 33 °C. 'Rex' and 'Rouxaï RZ' had similar base and optimal temperature estimates of 8 °C and 26 °C, while arugula and kale were lower at 6 °C and 23 °C.

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Introduction

Leafy green vegetables, such as lettuce (*Lactuca sativa*), arugula (*Eruca sativa*), and kale (*Brassica oleracea*) hold an important place within the U.S. horticulture industry. The wholesale value of lettuce production in the U.S. in 2019 was \$3.5 billion, with leaf, romaine, and head lettuce accounting for \$650 million, \$880 million, and \$2.0 billion, respectively (USDA, 2020a). The majority of lettuce is grown domestically, with California and Arizona accounting for approximately 95% of the field production in 2017 (USDA, 2019). Arugula and kale have grown in popularity over the past several decades, both readily available in many grocery stores as a standalone leafy green or in salad mixes (Enroth, 2018; Satheesh and Workneh Fanta, 2020). California produces the most arugula and kale, accounting for 46% of field grown, U.S. kale in 2017 (USDA, 2019). Leafy greens are well-suited for indoor controlled environment (CE) production give their short harvest cycle, compact growth, and moderate light requirements, alongside seasonality constraints of field production (Gómez et al., 2019). Semi-CEs, such as greenhouses and high tunnels, are already used for leafy green production; total wholesale value of CE-grown lettuce increased by 28% from \$55.5 to \$71.1 million from 2014 to 2019 (USDA, 2015; USDA, 2020b).

Production in CEs has been volatile over the past decade. From 2009 to 2014, the area under protected cultivation increased by 134% to 1,287,000 m^2, then from 2014 to 2019 it decreased by 23% to 993,000 m^2 (USDA, 2010; USDA, 2019; USDA 2020b). CEs, such as greenhouses, shipping containers, and indoor plant factories allow for manipulation of the growing environment to achieve desired conditions for plant growth and development

(McCartney and Lefsrud, 2018; Ahmed et al., 2020). Manipulating the environment can allow for plant production in areas where it otherwise wouldn't be possible, or for improving the production and yield beyond that which can be done in the open field (McCartney and Lefsrud, 2018; Ahmed et al., 2020). CEs enable the control of parameters such as radiation duration, quantity, and quality; mean daily temperature (MDT) and day/night temperature; air flow; vapor pressure deficit (VPD); and carbon dioxide (CO_2) concentration (McCartney and Lefsrud, 2018; Ahmed et al., 2020). Greenhouses and indoor facilities can vary in the amount of control provided. Greenhouses typically offer a lower level of control compared to indoor production facilities, but often allow for greater growing space, alongside allowing for the use of solar radiation. However, maintaining atmospheric conditions in greenhouses can be difficult compared to indoor production, particularly with CO_2 concentrations and VPD.

Leafy green quality attributes

The quality parameters of leafy vegetables are similar as their leaves are the primary product. Quality parameters include physical attributes such as shoot fresh mass (SFM) and shoot dry mass (SDM), and plant size; sensory attributes such as color, flavor, and texture; and chemical attributes including soluble solid content, titratable acidity, nitrate and ascorbic acid content, and volatile aroma compounds such as linalool and estragole (Serna et al., 2012; Pace et al., 2014; Miceli et al., 2019; Walters et al., 2021). Environmental growing conditions can influence these parameters — radiation quantity, quality, and duration promote yield and coloration, while temperature influences the rate of leaf unfolding, color, and plant maturity (Ahmed et al., 2020; Walters, 2020). Growing conditions can be formulated for desired crop quality but must be balanced with the cost of inputs to control the environment.

Flavor is an important quality parameter that varies based upon the genetic chemotype of a plant and the environmental conditions it is grown under (Barrett et al., 2010). In a U.S. hydroponic grower survey conducted in 2017, 90% of respondents responded affirmatively that their customers would pay more for crops with increased flavor (Walters et al., 2020). Additionally, those surveyed reported that managing the growing environment to improve crop flavor was one of the most beneficial research areas for their operations (Walters et al., 2020). Crop flavor is influenced by the concentration and ratios of secondary metabolites, such as volatile organic compounds (Barrett et al., 2010; Walters et al., 2021). Basil (*Ocimum basilicum*), for example, has phenylpropanoids and terpenoids that contribute to its unique aroma and flavor (Walters et al., 2021). Among these include methyl chavicol (estragole), a phenylpropanoid that provides an anise like aroma and flavor, and linalool, a monoterpenoid that has been described as having a floral or spicy aroma akin to Fruit Loops® (Simon et al., 1999; Arena et al., 2006; Walters et al., 2021). Secondary metabolite production can be influenced by environmental parameters, such as temperature, radiation quantity and quality, and CO_2 concentrations (Wang and Bunce, 2004; Walters et al., 2021). Increasing secondary metabolite production does not necessarily improve the flavor profile; for example, glucosinolates are produced in brassicas and have associated human health benefits, but can result in bitter, undesirable flavors (Bell et al., 2018).

A common quality concern for lettuce producers that results in economic losses in CEs is tipburn (Sago, 2016). This is a leaf marginal apex necrosis from a calcium deficiency; however, it often occurs in lettuce while calcium is present in the growing environment (Sago 2016, Ahmed et al., 2020). Tipburn frequently occurs in the inner, younger leaves of lettuce undergoing rapid growth rates, which consequently increases calcium demand for cell wall and membrane expansion (White and Broadley, 2003). Calcium (Ca) is an immobile nutrient, so new plant growth relies on calcium movement through xylem water flow, mediated by transpiration (White and Broadley, 2003). Higher rates of transpiration often occur at the outer leaves of lettuce due to their greater exposure to the surrounding environment, while the inner leaves become enclosed by other leaves during head formation in heading cultivars (Barta and Tibbitts, 1986). Ultimately, Ca is acquired by outer leaves at higher rates than the inner leaves (Sago, 2016). The occurrence of tipburn is cultivar dependent and influenced by environmental parameters such as air temperature, the vapor pressure deficit (VPD), radiation quantity, and air velocity (Lee et al., 2013; Sago, 2016; Lee et al., 2019). Sago (2016) reported that the relative growth rate and total Ca concentration of lettuce increased under increasing photosynthetic photon flux densities (*PPFD*) of 150, 200, 250, and 300 $mol \cdot m^{-2} \cdot s^{-1}$; however, the concentration of Ca within the inner leaves remained similar regardless of *PPFD*. Lee et al. (2019) reported that the increased growth rate of cripshead lettuce under higher temperatures induced greater tipburn incidence. However, Lee et al. (2013) found that temperature did not impact tipburn occurrence, while constant, horizontal airflow over 0.28 $m \cdot s^{-1}$ did reduce tipburn occurrence.

Unique foliage color is another quality attribute that impacts marketability of certain crops, including red and purple cultivars of lettuce and kale (Runkle, 2017). Major contributors to these blue, red, and purple colors are anthocyanins, secondary metabolites that accumulate in response to environmental conditions such as temperature, radiation quantity and quality, and CO_2 concentrations (Christie et al., 1994; Boldt, 2014). Anthocyanins can accumulate in plants in response to low temperatures, increasing blue, red, and purple coloration (Christie et al., 1994). Higher temperatures can inhibit transcription of genes producing anthocyanin, potentially reversing coloration and leading to predominantly green plants (Christie et al., 1994). Increasing the daily light integral (DLI) can increase foliage color (Boldt, 2014; Kelly et al., 2020; Walters, 2020). In red leaf lettuce cultivar 'Rouxaï RZ', increasing DLI from 6.9 to 15.6 mol·m^{-2}·d^{-1} at 22 °C MDT increased foliage redness, blueness, and darkness (Kelly et al., 2020)

Radiation intensity, quality, and duration effect on leafy green production

The three dimensions of light, radiation intensity, quality, and duration, are well documented to influence plant growth, quality, and yield (Faust, 2011). Radiation quality is the spectral distribution of light. The standardized photosynthetically active radiation (PAR) waveband includes blue [B (400-500 nm)], green [G (500-600)], and red [R (600-700)], but plants also respond to radiation wavelengths outside of PAR, ranging from ultraviolet [UV (280-400 nm)] to far-red [FR (700-800 nm)] (Faust, 2011). Radiation intensity is the *PPFD*, or the number of emitted PAR photons in a particular area and time. The radiation duration, or the photoperiod, is the number of hours that PAR is available to a plant.

Plant photosynthesis is driven predominately by the available *PPFD*. Increasing the *PPFD* increases the photosynthetic rate linearly, followed by a quadratic increase until reaching the light saturation point, at which a greater *PPFD* will not further increases photosynthesis for individual leaves or canopies with low leaf area indices (Evans et al., 1992; Runkle, 2015). The total *PPFD* over a day is the daily light integral (DLI), expressed as mol·m^{-2}·d^{-1}. Overall plant growth is impacted by the DLI (Faust, 2011; Torres and Lopez, 2012; Kelly et al., 2020), including shoot (branching, stem diameter, and leaf size) and root growth, foliage coloration, and flowering (Faust, 2011; Torres and Lopez, 2012; Kelly et al., 2020). Continuous increases in DLI by increasing *PPFD* can result in diminishing returns as light saturation is reached, resulting in increased energy inputs without significantly increasing yield (Litvin-Zabal, 2019; Kelly et al., 2020). However, maintaining *PPFD* at or below the saturation point while extending the day length can allow for additional yield increases (Litvin-Zabal, 2019; Kelly et al., 2020). The light saturation point and DLI response is highly species-specific and can vary between cultivars (Evans et al., 1992; Fu et al., 2012; Torres and Lopez, 2012). Crop classification based upon DLI response has been suggested, with classifications ranging from very low, low, medium, high, and very high light with corresponding DLIs of <5, 5 to 10, 10 to 20, and 20 to 30, and >30 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, respectively (Faust, 2011; Litvin-Zabal, 2019).

The response of lettuce to radiation intensity has been recorded in many studies (Kitaya et al., 1998; Fu et al., 2012; Pérez-López et al., 2013; Sago, 2016; Lee et al., 2019; Kelly et al., 2020). Sago (2016) 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 $mol{\cdot}m^{-2}{\cdot}d^{-1}$). SFM and SDM, relative growth rate, leaf number, and tipburn occurrence all significantly increased with increasing *PPFD*s (Sago, 2016). The SDM 35 d after sowing increased 1.12-, 1.32-, and 1.42-fold at 200, 250, and 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, respectively, compared to the lettuce grown at 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 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. Fu et al. (2012) grew romaine lettuce 'Lvling' under radiation intensities of 100, 200, 400, 600, and 800 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ (DLIs of 5, 10, 20, 39, and 40 $mol{\cdot}m^{-2}{\cdot}d^{-1}$) and day/night temperature (14 h/ 10 h) of 20/16 °C (18 °C MDT). *PPFD*s of 200 to 600 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ resulted in high light use efficiency and yield, with 400 and 600 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ having the largest yields and 200 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ having the greatest light use efficiency. Conversely, under 100 and 800 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, lettuce had the lowest light use efficiency and yields. Signs of light stress were present at 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. (2012) recommended radiation intensities of 400 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for lettuce 'Lvling'.

Green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï' had greater SFM and SDM, leaf width and number, and chlorophyll concentration when DLI was increased from 6.9 to 15.6 $mol \cdot m^{-2} \cdot d^{-1}$ at 22 °C MDT, 60% RH, and 380 $\mu mol \cdot mol^{-1}$ CO_2 (Kelly et al., 2020). Additionally, they found a high DLI of 15.6 $mol \cdot m^{-2} \cdot d^{-1}$ composed of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 24 $h \cdot d^{-1}$ resulted in greater lettuce SFM than the same DLI composed of *PPFD*s and photoperiods of 270 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 20 $h \cdot d^{-1}$ and 216 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$. This may be due to reduced light use efficiency under high *PPFD*s, alongside light saturation points being reached, after which increasing photoperiod increased yield while increasing *PPFD* did not.

Literature on the responses of arugula and kale to radiation quantity is limited. Baumbauer et al. (2019) reported that kale SFM was not impacted when DLI increased from 8 to 14 $mol \cdot m^{-2} \cdot d^{-1}$ under a 12 $h \cdot d^{-1}$ photoperiod and a constant temperature of 20 °C, but there was a linear increase in SDM by 47%. Lefsrud et al. (2006) reported linear increases in SFM and SDM for kale 'Winterbor' under DLIs from 10.8 to 43.2 $mol \cdot m^{-2} \cdot d^{-1}$ with a *PPFD* of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, for 6, 12, 16, or 24 $h \cdot d^{-1}$ at an MDT of 20 °C. To our knowledge, there has not been a thorough investigation on the response of arugula to different radiation intensities.

In addition to the influence on yield and biomass, radiation intensity can impact attributes such as foliage texture, size, number, and coloration (Boldt et al., 2014; Kelly et al., 2020; Walters, 2020). Increasing DLI increased leaf number, redness, blueness, and darkness of red lettuce 'Rouxaï' and purple basil 'Dark opal' (Kelly et al., 2020; Walters, 2020). Leaf area increased as DLI increased for sage 'Extrakta', sweet basil 'Nufar', spearmint 'Spanish', and sweet basil 'Improved Genovese Compact' (Dou et al., 2018; Walters, 2020). In a consumer sensory panel evaluation, a *PPFD* of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ resulted in less desirable texture than 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for sweet basil 'Nufar' grown in a 16 $h \cdot d^{-1}$ photoperiod at 23 °C for 2 weeks (Walters et al., 2021).

Radiation quality influences many components of plant quality and growth, including morphology, yield, and secondary metabolite accumulation (Bian et al., 2015; Owen and Lopez, 2015; Naznin et al., 2019; Kelly et al., 2020; Li et al., 2020). Photoreceptors responsible for radiation absorption and certain developmental processes include cryptochromes (B and UV-A radiation) and phytochromes (R, FR, and some B radiation). Phytochromes have two forms, the R absorbing P_R form and the FR absorbing P_{Fr} form (Thomas and Vince-Prue, 1997). These forms are photo-reversible; under a low R to FR radiation ratio (R:FR), P_R absorbs R radiation and converts into P_{Fr}, while a high R:FR induces a P_{Fr} conversion back to P_R (Thomas and Vince-Prue, 1997). The increased presence of P_{Fr} arising from a low R:FR can promote seed germination and flowering in long-day plants while preventing flowering in short-day plant (Thomas and Vince-Prue, 1997). Conversely, the increased P_R form occurring at high R:FR can promote shade avoidance responses and flowering in short-day plants, while preventing seed germination and flowering (Smith, 1982; Thomas and Vince-Prue, 1997). The absorption of B radiation can induce compact stem and leaf growth, anthocyanin accumulation, and stomata opening (Smith, 1982; Li and Kubota, 2009). G radiation can evoke shade avoidance responses, including stem elongation, leaf expansion, and hyponasty; additionally, G radiation can reverse responses from B radiation (Wang and Folta, 2013).

Many studies have considered how leafy greens respond to radiation quality (Li and Kubota, 2009; Loconsole et al., 2019; Meng et al., 2019; Owen and Lopez, 2015). Li and Kubota

(2009) grew ‘Red Cross’ baby leaf lettuce in day/night temperatures of 25/20 °C under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ composed of an all-white light control or supplemented with either UV-A, B, G, R, or FR radiation at *PPFD*s of 18, 130, 130, 130, or 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Compared to the control, supplemental UV-A and B radiation increased anthocyanin concentrations by 11 and 31%, B radiation increased carotenoid concentrations by 12%, and R radiation increased phenolic concentrations by 6%; however, FR decreased anthocyanin, carotenoid, and chlorophyll concentrations by 40, 11, and 14%, respectively (Li and Kubota, 2009). Additionally, compared to the control, the supplemental FR radiation increased SFM and SDM by 28 and 15%, stem length by 14%, and leaf length and width by 44 and 15%, respectively, while UV-A and B radiation decreased stem length by 16 and 33% (Li and Kubota, 2009).

Meng et al. (2019) compared green butterhead and red oakleaf lettuce ‘Rex’ and ‘Rouxaï’ and kale ‘Siberian’ grown at a 20 °C MDT with a 20 $h \cdot d^{-1}$ photoperiod under controls of warm white and equalized-white LEDs to treatments of a R radiation background (peak =664 nm) of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 8 combinations of B (peak =449 nm), G (peak =526 nm), and FR (peak=733 nm) LEDs with *PPFD*s of 0, 20, 40, or 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with $B_{60}R_{120}$ (60 and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B and R radiation, respectively), $B_{40}G_{20}R_{120}$, $B_{20}G_{40}R_{120}$, $G_{60}R_{120}$, $B_{40}R_{120}FR_{20}$, $B_{20}R_{120}FR_{40}$, $R_{120}FR_{60}$, $B_{20}G_{20}R_{120}FR_{20}$, WW_{180}, and EQW_{180}. Substituting B radiation with G and/or FR radiation under a fixed R-radiation background increased shoot mass, leaf expansion, and radiation interception in kale and lettuce but reduced chlorophyll concentrations (Meng et al., 2019). Additionally, increasing B radiation caused darker, redder foliage for the red leaf cultivar. Under a *PPFD* of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B radiation with 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of either FR or G radiation, foliage was redder with FR radiation (Meng et al., 2019). Similarly, Dou et al. (2020)

grew green and purple leaf basil ‘Improved Genovese Compact’ and ‘Red Rubin’, green and red kale ‘Siberian’ and ‘Scarlet’, green mustard *Brassica carinata* ‘Amara’, and red mustard *B. juncea* ‘Red Giant’ under LED fixtures providing 224 μmol·m^{-2}·s^{-1} for 16 h·d^{-1} of 88% R and 12% B radiation ($R_{88\%}B_{12\%}$), $R_{76\%}B_{24\%}$, $R_{51\%}B_{49\%}$, $R_{44\%}B_{12\%}G_{44\%}$, or $R_{35\%}B_{24\%}G_{41\%}$ at day/night temperatures of 28/18 °C. For the R and B radiation treatments, increasing B radiation proportion from $R_{88\%}B_{12\%}$ to $R_{51\%}B_{49\%}$ resulted in the lowest height, leaf area, and yield for green and purple basil, green and red kale, and green mustard (Dou et al., 2020).

Temperature effect on leafy green production

Temperature influences the rate of plant development, 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 (Christie et al., 1993; Sage and Kubien, 2007; Hatfield and Prueger, 2015). The base temperature (T_b) is the temperature at which plant development halts. Above the T_b, development increases linearly until an optimal temperature (T_{opt}) is reached and the rate of development is the highest. As the temperature increases further, the development rate could plateau or decrease until the maximum temperature (T_{max}) is reached, at which development stops (Sage and Kubien, 2007). Response curves can be created from these cardinal temperatures, assisting growers in determining temperatures for hastening or slowing crop developmental rate, alongside identifying temperatures that are detrimental to crop development, biomass accumulation, or quality.

There are few studies estimating T_b, T_{opt}, and T_{max} for leafy greens. Imler (2020) grew arugula for 21-d and kale ‘Starbor’ for 28-d at constant temperatures of 8, 13, 18, 23, 28, or

33 °C under a *PPFD* of 250 μmol·m^{-2}·s^{-1} for 16 h·d^{-1}. The arugula SFM T_b was estimated at 7.2 °C and T_{opt} at 23.9 °C, while kale T_b was 7.3 °C and T_{opt} was 22.6 °C (Imler, 2020). CE studies on lettuce frequently work to refine temperature responses in conjunction with other environmental parameters (Lee et al., 2019; Carotti et al., 2021), while older studies estimating T_b and T_{opt} were conducted in the field where variance is high or vital information on the growing conditions is lacking (Kristensen et al., 1987; Marsh and Albright, 1991; Seginer et al., 1991; Wheeler et al., 1993).

Vapor pressure deficit and relative humidity effect on leafy green production

Plant water consumption and CO_2 uptake are mediated by stomatal aperture — open stomata increase transpiration rates and CO_2 uptake while closed stomata reduces transpiration and decreases CO_2 uptake (Mortensen and Gislerød, 1990). Stomatal aperture and response to CO_2 are influenced by the relative humidity and VPD. The relative humidity is the amount of water vapor in the air relative to the maximum at a given temperature. The VPD is the difference between the water vapor in the air and the total vapor that can be held until saturation. Under high VPD conditions, stomata conductance lowers, reducing transpiration related water loss while inhibiting CO_2 intake, potentially limiting growth as photosynthesis slows (Merilo et al., 2018). Conversely, a continuously low VPD around 0.11 kPa can cause newly formed stomata to malfunction, failing to close under increased VPDs and allowing excess water loss through transpiration (Fanourakis et al., 2011). The response to VPD varies by species, growth stage, the stability of the VPD, and other environmental conditions, such as airflow (Mortensen and Gislerød, 1990; Ahmed et al. 2020; Inoue et al., 2021).

Inoue et al. (2021) compared lettuce 'Romana' grown under moderate VPD fluctuations (1.32 kPa for 7 min., followed by 0.86 kPa for 3 min.) to large VPD fluctuations (1.63 kPa for 6 min. followed by 3 min. at 0.63 kPa) for 3 weeks at an MDT of 24 °C, *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$, and a CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$. The large VPD fluctuations resulted in 15 and 29% lower SDM and leaf area, respectively, compared to moderate VPD fluctuation (Inoue et al., 2021). Additionally, Inoue et al. (2021) compared how VPD fluctuation duration and speed over a 400 min. interval, slowly fluctuating VPD condition (0.63 kPa for 5 min., 1.27 kPa for 4 min., then 0.95 kPa for 3 min.) had a greater reduction in stomatal conductance and CO_2 assimilation than in rapidly fluctuation VPD conditions (0.48 kPa for 3 min., 1.74 kPa for 3 min., then a VPD of 0.95 kPa for 3 min.).

There is limited information on species specific responses to VPD; however, recommendations have been made to maintain a moderate VPD around 0.3 kPa for rooting cuttings and 0.5-1.0 kPa for finishing plants. Additionally, avoiding long-term, low VPDs; rapidly shifting VPDs; or severe VPD swings can prevent VPD related CO_2 assimilation issues and stomatal malfunction (Fanourakis et al., 2011).

Carbon dioxide effect on leafy green production

CO_2 concentration influences the efficiency of photosynthetic reactions in C_3 plants. Increasing the concentration of atmospheric CO_2 decreases the potential of photorespiration occurring, raising the photosynthetic rate and the light saturation point (He et al., 2009; Runkle, 2015; Dusenge, 2018). The benefits of increased CO_2 concentrations on growth occur until a species-specific saturation point is reached. The increased growth is initially great as concentrations are raised from relatively low concentrations but has a decreasing benefit as more

CO_2 is added (Runkle, 2015). Additionally, radiation quantity, temperature, and VPD interact with CO_2, influencing plant responses (Ainsworth and Rogers, 2007; He et al., 2009; Runkle, 2015). At greater CO_2 concentrations, the T_{opt} for photosynthesis increases (Runkle, 2015). Beyond the T_{opt}, the rate of carboxylation to oxygenation decreases, reducing photosynthetic efficiency due to photorespiration (Ainsworth and Rogers, 2007).

The CO_2 saturation point for lettuce has been investigated previously with mixed results, possibly due to cultivar and growing environment differences (He et al., 2009; LeCaplan, 2018; Esmaili et al., 2020). Butterhead lettuce 'Fairly' grown at an MDT of 22 °C under *PPFD*s ranging from 156 to 330 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and CO_2 concentrations from 400 to 1300 $\mu mol \cdot mol^{-1}$ had the greatest SFM and SDM at 850 $\mu mol \cdot mol^{-1}$ CO_2, while reduced yields occurred at greater CO_2 concentrations (LeCaplan, 2018). Conversely, the SFM of lettuce 'Partavousi' increased by 6 and 55% as CO_2 concentrations increased from 400 to 800 and 800 to 1200 $\mu mol \cdot mol^{-1}$, respectively, under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and at an MDT of 25 °C for 40 d, without additional biomass accumulation at CO_2 concentrations of 1200 to 1600 $\mu mol \cdot mol^{-1}$ (Esmaili et al., 2020). He et al. (2009) grew lettuce 'Buttercrunch' under identical conditions for 27 d, then transferred three seedlings into low pressure plant growth systems for three days under 400 W metal halide lamps providing a *PPFD* of 240 or 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 12 $h \cdot d^{-1}$ and at day/night temperatures of 26/20 °C. The specific CO_2 saturation points of 'Buttercrunch' was estimated at 1,150 and 1,500 $\mu mol \cdot mol^{-1}$ under the *PPFD*s of 240 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, alongside higher CO_2 assimilation and lower CO_2 compensation points occurring under a *PPFD* of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (He et al., 2009).

The influence of environmental parameters on leafy green growth has been characterized previously but there are gaps in the literature when considering the interaction of environmental parameters and the cardinal temperatures.

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SECTION II

INFLUENCE OF DAY AND NIGHT TEMPERATURE AND PHOTOSYNTHETIC PHOTON FLUX DENSITY ON GROWTH, YIELD, AND QUALITY OF GREEN BUTTERHEAD AND RED OAKLEAF LETTUCE

Influence of day and night temperature and photosynthetic photon flux density on growth, yield, and quality of green butterhead and red oakleaf lettuce

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Abstract

Lettuce (*Lactuca sativa*) is among the most consumed vegetables world-wide and is primarily field grown; however, it is increasingly produced indoors as controlled environments enable year-round production and increased control by growers. Through precise manipulation of the mean daily temperature (MDT) and photosynthetic photon flux density (*PPFD*), growers can influence lettuce color, yield, and size. Therefore, our objective was to 1) to quantify how MDT and *PPFD* interact to influence lettuce growth, development, quality, and yield; and 2) to develop models to predict growth and development under various *PPFD*s and MDTs. Green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï RZ' seeds were sown and placed in a growth chamber with an MDT set point of 22 °C, CO_2 concentration of 500 µmol·mol^{-1}, and a total photon flux density of 180 µmol·m^{-2}·s^{-1}. At 11-d, seedlings of each cultivar were transplanted into 6 deep flow hydroponic tanks in growth chambers with day/night temperatures and MDT set points of 22/15 °C (MDT 20 °C), 25/18 °C (23 °C), or 28/21 °C (26 °C), under a 17-h photoperiod from light-emitting diodes providing a *PPFD* of 150 or 300 µmol·m^{-2}·s^{-1}. 'Rex' fresh mass was influenced by the *PPFD*, increasing by 29% (33.4 g) from 150 to 300 µmol·m^{-2}·s^{-1}. Fresh mass of 'Rouxaï RZ' and dry mass of both cultivars was influenced by the interaction of MDT and *PPFD*. The greatest 'Rouxaï RZ' fresh (151.4 g) and dry (6.05 g) mass occurred at an MDT of 23 or 26 °C under a 300 µmol·m^{-2}·s^{-1} *PPFD*, while the lowest fresh (76.0 g) and dry (3.17 g) mass occurred at 20 °C and under a *PPFD* of 150 µmol·m^{-2}·s^{-1}. Similarly, 'Rex' dry mass was the greatest (6.05 g) and lowest (3.17 g) under the aforementioned MDTs and *PPFD*. Increasing the *PPFD* from 150 to 300 µmol·m^{-2}·s^{-1} resulted in an increased incidence of tipburn on 'Rouxaï RZ' from 0 to 25% and 'Rex' from 47 to 100%, while MDT did not impact tipburn incidence. The red-leaf cultivar 'Rouxaï RZ' had darker yellow-red foliage at

lower MDTs under the high *PPFD*, while foliage at the high MDT and low *PPFD* was lighter green. Increasing the *PPFD* from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ altered the hue angle from 110.7 (more green) to 84.4° (more yellow/red) and the CIE L* value from 38.7 (lighter color) to 29.8 (darker color). Additionally, the chroma, or the departure from grey towards chromatic color, increased linearly with MDT from 20 to 26 °C while under 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPFD*, but was not impacted by MDT under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Therefore, we suggest growing 'Rex' and 'Rouxaï RZ' under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and MDT of 23 °C, in conjunction with tipburn mitigation practices.

Keywords: mean daily temperature, controlled environment agriculture, daily light integral, leafy greens, vertical farming

Introduction

Lettuce (*Lactuca sativa*) is an economically significant specialty crop around the world. In the United States (U.S.) alone, wholesale lettuce production in 2019 was \$3.5 billion, with leaf, romaine, and head lettuce accounting for \$650 million, \$880 million, and \$2.0 billion, respectively (USDA, 2020a). Over 95% of leaf and romaine lettuce grown in the U.S. is field grown in California and Arizona (USDA, 2019). However, there is an increasing market for locally grown leafy greens within controlled environments (CE). From 2014 to 2019, total sales of lettuce produced under protection increased by 28% from \$55.5 to \$71.1 million (USDA, 2015; USDA, 2020b). Indoor CE production in vertical farms, warehouses, and containers can enable local and urban production during the off season, and a more consistent price point year-round (Beacham et al., 2019).

With the rise in CE lettuce production, there is value in co-optimizing the mean daily temperature (MDT), carbon dioxide (CO_2) concentration, photosynthetic photon flux density (*PPFD*), and photoperiod to improve aspects including time to harvest, yield, flavor, color, nutrient content, and post-harvest quality. 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 (Christie et al., 1994; Sage and Kubien, 2007; Hatfield and Prueger, 2015). 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 photosynthetic photon flux density (*PPFD*) (Faust, 2011; Torres and Lopez, 2012; Kelly et al., 2020).

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 (Evans et al., 1993; Runkle, 2015). 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 because energy inputs increase without proportional yield responses. However, by maintaining the *PPFD* at or below the saturation point while extending the day length can allow for yield increases (Litvin-Zabal, 2019; Kelly et al., 2020). The light saturation point and DLI response is highly species-specific and can also vary between cultivars (Evans et al., 1993; Fu et al., 2012; Torres and Lopez, 2012) and depend on leaf area index and other environmental factors, such as temperature and CO_2 concentration.

The response of lettuce to *PPFD* has been recorded in many studies (Kitaya et al., 1998; Fu et al., 2012; Pérez-López et al., 2013; Sago, 2016; Lee et al., 2019; Kelly et al., 2020). Sago (2016) 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 $mol \cdot m^{-2} \cdot d^{-1}$). Shoot fresh and dry mass, relative growth rate, leaf number, and tipburn occurrence all increased with increasing *PPFD*. Dry mass 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 dry mass between plants under 250 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating light saturation from 250 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Sage, 2016). Fu et al. (2012) 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). 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, 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. (2012) recommended maintaining a *PPFD* of 400 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for lettuce.

Kelly et al. (2020) found that green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï RZ' increased in shoot fresh mass (SFM) and dry mass (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. Additionally, the SFM under a

DLI of 15.6 mol·m^{-2}·d^{-1} was greatest under a *PPFD* of 180 µmol·m^{-2}·s^{-1} for 24 h·d^{-1} compared to the same DLI composed of *PPFD*s of 216 and 270 µmol·m^{-2}·s^{-1} with shorter photoperiods of 20 and 16 h·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 point increasing photoperiod may increase yield while greater *PPFD*s would not.

In addition to the influence on yield and biomass, *PPFD* can impact produce quality attributes such as foliage texture, size, number, and coloration (Boldt et al., 2014; Kelly et al., 2020; Walters, 2020). For example, greater DLIs increased leaf number, redness, blueness, and darkness of red lettuce 'Rouxaï RZ' (Kelly et al., 2020).

The interaction of MDT and DLI on lettuce growth has been investigated in a few studies (Lee et al., 2019; Carotti et al., 2021). For instance, Lee et al. (2019) 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). 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, 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 and 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*.

Considering the impacts of MDT and *PPFD* on growth, development, and quality, co-optimizing the growing environment can allow for improved resource-use efficiency and yield in

CEs. Therefore, the objectives of this study were 1) to quantify how MDT and *PPFD* interact to influence lettuce growth, development, quality, and yield; and 2) to develop models that predict growth and development 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.

Materials and methods

Plant material and propagation conditions

On 28 Apr. and 09 June 2020, seeds of red oakleaf lettuce 'Rouxaï RZ' and green butterhead lettuce 'Rex' (Rijk Zwaan USA; Salinas, CA) were sown into 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Gordan, Milton, ON, Canada). 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). 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) with an MDT of 22 °C, CO_2 concentration of 500 $\mu mol \cdot mol^{-1}$, and RH of 60%. Light-emitting diode (LED) fixtures (Ray66 Indoor PhysioSpec; Fluence Bioengineering, Austin, TX) provided a total photon flux density (*TPFD*) of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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) 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 $mg \cdot L^{-1}$): 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). The pH and electrical conductivity (EC) were adjusted to 5.6 and 1.6 $dS \cdot m^{-1}$, respectively, as determined with a pH/EC probe (HI 991301 pH/TDS/Temperature Monitor; Hanna Instruments, Smithfield, RI). 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 09 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) 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 $mg \cdot L^{-1}$) 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 $g \cdot L^{-1}$ magnesium sulfate (Pennington Epsom salt; Madison, GA). The EC and pH were adjusted daily to maintain an EC of 1.7 $dS \cdot m^{-1}$ and pH of 5.6 as described previously. Air pumps (Active Aqua 70 $L \cdot min^{-1}$ commercial air pump; Hydrofarm) connected to air stones (Active Aqua air stone round 10.2 cm × 2.5 cm; Hydrofarm) were used to increase 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) and logged by a C6 controller (Environmental Growth Chambers, Chagrin Falls, OH). *PPFD*s of 150 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were provided for 17 h $\cdot d^{-1}$ by LED fixtures (Ray66; Fluence Bioengineering) providing a DLI of 9.2 and 18.4 $mol \cdot m^{-2} \cdot d^{-1}$, respectively, averaged over several measurements (Table II-1). The LEDs were mounted ~130 and 95 cm above the crop canopy for the 150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ treatments, respectively. Every 15 s, water temperature, leaf temperature, and *PPFD* were measured using a thermistor (ST-100; Apogee Instruments, Logan, UT), infrared thermocouple (OS36-01-T-80F; Omega Engineering, INC. Norwalk, CT), and quantum sensor (LI-190R; LI-COR Biosciences, Lincoln, NE), respectively, with means logged every hour by a CR-1000 datalogger (Campbell Scientific, Logan, UT). A CO_2 concentration of 500 $\mu mol \cdot mol^{-1}$ 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 SE).

Growth data collection and analysis

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 (1992). The relative chlorophyll concentration (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). One leaf of ten plants per treatment was then dark acclimated for >15 minutes using three of the manufacturer-supplied clips and then exposed to 3,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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, U.K.).

‘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) was measured on ten plants of each cultivar per treatment. Plant height from the roots to the highest point of the foliage, and the widths at the widest point and perpendicular from the widest point were measured with a ruler and recorded. Presence, 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) (Krug et al., 2010). The plant material was placed in a forced-air drier maintained at 75 °C for at least 3 d, weighed, and SDM was recorded.

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 randomized. Data were analyzed separately by cultivar with SAS (version 9.4; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) for analysis of variance (ANOVA), pairwise comparisons were performed with Tukey-Kramer difference test ($P \leq 0.05$). SigmaPlot (version 14.5, Systat Software, Inc., San Jose, CA) was used for regression analysis.

Results

Shoot fresh and dry mass

The SFM of 'Rouxaï RZ' was influenced by the interaction of MDT and *PPFD* (Table II-2; Fig. II-1A). Increasing the MDT from 20 to 23 °C under a *PPFD* of 150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased SFM by 29% (33.4 g) among all MDT treatments.

For both cultivars, MDT and *PPFD* interacted to influence SDM (Table II-2; Fig. II-1B-D); increasing either parameter increased SDM. Under 150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at MDTs of 20, 23, and 26 °C, respectively.

Plant morphology

Leaf unfolding of 'Rouxaï RZ' was influenced by interactions between MDT and *PPFD* (Table II-2; Fig. II-1D). As the *PPFD* increased from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 5 and 8 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

II-2; Fig. II-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 II-2; Fig. II-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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table II-2). As *PPFD* increased from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the leaf length of 'Rex' was reduced by 12% (1.6 cm), and leaf width of 'Rex' and 'Rouxaï RZ' increased by 9 and 8% (1.2 and 1.4 cm), respectively (Table II-2).

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

Relative chlorophyll concentration, F_v/F_m, and pigmentation

For both cultivars, *PPFD* influenced RCC (Table II-2); RCC was 21 and 31% greater for 'Rouxaï RZ' and 'Rex', respectively, when *PPFD* increased from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table II-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 (data not shown).

For the red-leaf cultivar 'Rouxaï RZ', h° was influenced by *PPFD*. As *PPFD* increased from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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 II-2; Fig. II-1G). Under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the MDT did not influence C*, with an average value of 7.2 (very gray). Under 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Table II-2).

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 other quality 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Sago, 2016; Zhang et al., 2018; Lee, 2019; Kelly et al., 2020). 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 (Zhang et al., 2018). Kelly et al. (2020) 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.

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 (Fig. II-1A). Similarly, Choi et al. (2000) 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. This suggests that the impact of MDT on SFM may depend on the CO_2 concentration (Tarr, 2022), stage of growth, 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 μmol·m^{-2}·s^{-1} and an MDT of 20 °C, to those under 150 μmol·m^{-2}·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. (2019), where the SFM of 'Sensation' was lower under a *PPFD* of 250 μmol·m^{-2}·s^{-1} and MDT of 17 °C than under a *PPFD* of 200 μmol·m^{-2}·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 20 °C under a *PPFD* 150 μmol·m^{-2}·s^{-1}, they reported that SFM under 150 μmol·m^{-2}·s^{-1} was greater at 17 °C than at 20 °C. This may be due to cultivar or other environmental or 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 μmol·m^{-2}·s^{-1}, leaf width for both cultivars increased; however, the GI and leaf length of 'Rex' decreased at the higher *PPFD* (Table II-2). Compact growth of 'Rex' and greater leaf width of both cultivars under higher *PPFD*s aligns with the findings of Kelly et al. (2020). Greater leaf area and stem lengths, and reduced leaf thickness, has been observed in many species in response to elevated *PPFD*s, including lettuce (Kitaya et al., 1998; Poorter et al., 2009; Walters et al., 2020; Carotti et al., 2021). An increase in leaf area, coupled with a reduction in leaf thickness, can improve light interception without increased assimilate demand (Poorter et al., 2009; Carotti et al., 2021).

The increase in leaf number in response to MDT is consistent with the understanding that developmental rates are primarily dependent on temperature (Hatfield and Prueger, 2015). Interestingly, 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}) (Blanchard et al., 2011). The T_b, T_{opt}, and T_{max} vary by cultivar and are influenced by other environmental conditions, including the DLI (Blanchard et al., 2011; Walters, 2020).

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. (2020), 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 (2016) suggest that the influence of *PPFD* on leaf number may be dependent on the duration of the harvest cycle. 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 appears to be cultivar-specific responses for leaf number in lettuce (Lee et al., 2019; Kelly et al., 2020). 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 (Lee et al., 2019).

A common quality concern for lettuce producers is tipburn, which results in economic losses. Tipburn is a leaf marginal apex necrosis that is associated with calcium deficiency even when calcium is available in the nutrient solution (Frantz et al., 2004; Lee et al., 2013; Sago, 2016; Ahmed et al., 2020). Tipburn frequently occurs in the inner, younger leaves of lettuce undergoing rapid growth rates, which consequently increases calcium demand in the leaves for cell wall and membrane expansion (White and Broadley, 2003).

In our study, tipburn incidence in both cultivars was only influenced by *PPFD*, with ‘Rex’ having greater incidence than ‘Rouxaï RZ’ (Table II-2). The cultivar difference may be attributed to morphological differences; ‘Rex’ forms compact heads that decreases 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 (Frantz et al., 2004; Sago, 2016). Sago (2016) 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. Additionally, 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* (Sago, 2016).

We did not find a relationship between tipburn incidence and MDT. Similarly, Lee et al. (2013) 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. Conversely, Lee et al. (2019) 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, similar to the incidence in lettuce ‘Batavia Othilie’ at higher MDT observed by Carotti et al. (2021). A greater VPD can increase transpiration rates, potentially reducing tipburn occurrence (Lee et al., 2013; Ahmed et al., 2020). 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 severity was not recorded in our study.

The RCC was influenced by *PPFD* in both (Table II-2). Similarly, Kelly et al. (2020) reported the RCC of 'Rex' and 'Rouxaï RZ' increased by 13 and 23%, respectively, as *PPFD* increased from 150 to 270 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a 16-h photoperiod. However, Baumbauer et al. (2019) reported no difference in RCC for 'All Star' lettuce under *PPFD*s of 185, 231, 278, and 324 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over a 12-h photoperiod and at an MDT of 20 °C. Kang et al. (2013) also reported the RCC of lettuce 'Hongyeom Jeockchukmyeon' was unaffected by *PPFD* from 200-290 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under 18-h photoperiods and a 21 °C MDT. Interestingly, very high *PPFD*s have been reported to lower RCC (Fu et al., 2012). For instance, the RCC of romaine lettuce 'Lvling' grown at 18 °C was lower under a *PPFD* of 800 than under 100-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over a 14-h photoperiod when grown at 18 °C (Fu et al., 2012).

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 (Runkle, 2017). MDT and *PPFD* affect anthocyanin biosynthesis and accumulation (Christie et al., 1994; Owen and Lopez, 2015). Anthocyanins can accumulate in plants in response to low temperatures, increasing blue, red, and purple coloration, while higher temperatures can prevent the transcription of anthocyanin producing genes, potentially reversing coloration, leading to predominantly green plants (Christie et al., 1994). Anthocyanin concentration and foliage coloration is also influenced by *PPFD* as anthocyanins fill a role as photoprotectants (Boldt, 2014).

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 II-2; Fig. II-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 towards 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating darker foliage at a higher *PPFD*. Increasing the *PPFD* from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ caused a lower C* regardless of MDT, indicating that the foliage became less colorful and more gray. However, at a *PPFD* of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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.

In conclusion, increasing the *PPFD* from 150 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased many crop attributes, such as fresh and dry mass, 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, and leaf length for 'Rex' decreased. 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* was influenced by the further increase from 23 to 26 °C, creating more vibrant greens. Ultimately, we recommend a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and MDT of 23 °C, assuming tipburn can be mitigated (e.g., by increasing the VPD).

APPENDIX

Table II-1. Mean (± SD) day and night air, 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*	VPD (kPa)		CO_2
Air day	Air night	Canopy	Water	($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Day	Night	($\mu mol \cdot mol^{-1}$)
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

Table II-2. Influence of mean daily temperature (MDT; 20, 23, and 26 °C) and photosynthetic photon flux density (*PPFD*; 150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) 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' (*Lactuca sativa*) and green butterhead lettuce 'Rex'. Data represent the mean of two replications and cultivars 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's honestly significant difference (HSD) 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
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, *, **, *** represent non-significant or significant difference at $P \leq 0.05$, 0.01, and 0.001, respectively.

[y] Data not collected.

Table II-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 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of green butterhead lettuce ‘Rex’ (*Lactuca sativa*) and red oakleaf lettuce ‘Rouxaï RZ’. All models are in the form of: $f = y_0 + a \cdot MDT + b \cdot MDT^2$

Parameter	*PPFD*	y0	(a) MDT	(b) MDT^2	R^2 or r^2
		‘Rouxaï RZ’			
Fresh mass (g)	150	-6.06E2[y]	5.68E1	-1.14	0.462
		1.91E2[w]	1.67E1	3.61E-1	
	300	-1.14E3	1.03E2	-2.06	0.634
		2.70E2	2.36E1	5.10E-1	
Dry mass (g)	150	-1.75E1	1.70	-3.30E-2	0.444
		7.09	6.20E-1	1.30E-2	
	300	-2.72E1	2.62	-5.10E-2	0.607
		8.86	7.74E-1	1.70E-2	
Leaf number	150	2.12	1.30E-2	-4.10E-2	0.834
		5.11E-1	6.32E-4	1.10E-2	
	300	-2.30E2	2.10E1	-4.25E-1	0.553
		5.31E1	4.64	1.00E-1	
Growth index	*	-3.63E1	4.26	-7.90E-2	0.673
		1.19E1	1.04	2.30E-2	
Chroma	150	-1.44E1	1.59	[x]	0.383
		6.00	2.59E-1		
		‘Rex’			
Leaf number	*	-8.67	1.55		0.540
		3.06	1.32E-1		
Dry mass (g)	150	-1.94E1	1.96	-4.00E-2	0.317
		7.60	6.65E-1	1.40E-2	
	300	-2.52E1	2.52	-4.90E-2	0.524
		1.07E1	9.36E-1	2.00E-2	

* *PPFD* not significant

[y] Coefficients for model equations were used to generate Fig. II-1

[x] Blank cells = 0

[w] Standard error (SE)

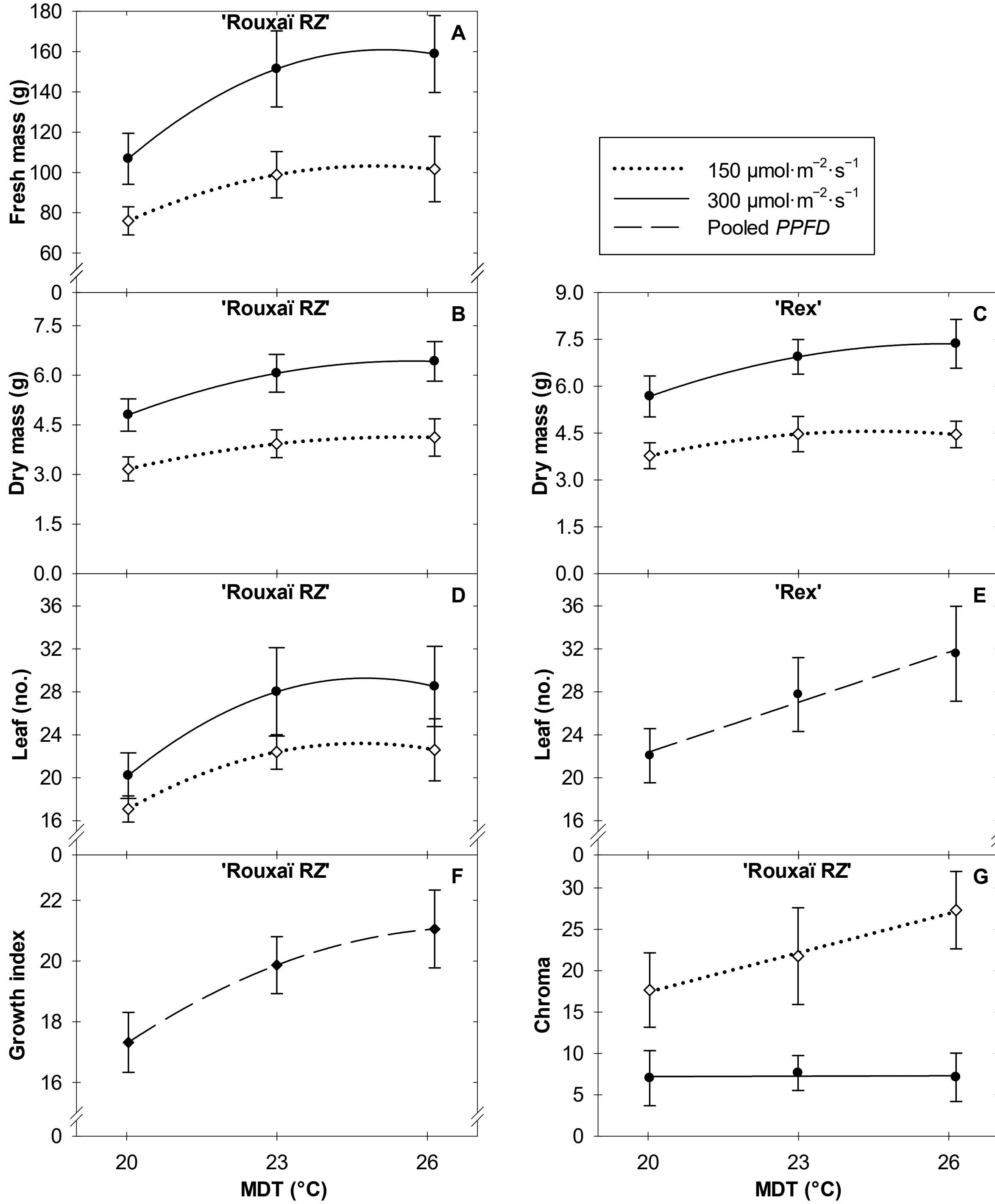

Figure II-1. Effects of MDT (20, 23, and 26 °C) and *PPFD* (150 and 300 µmol·m^{-2}·s^{-1}) on red oakleaf lettuce (*Lactuca sativa*) ‘Rouxaï RZ’ shoot fresh (A) and dry (B) mass, leaf number (D), growth index (F), and chroma (G), and green butterhead lettuce ‘Rex’ shoot dry mass (C) and leaf number (E). Model predictions are represented by lines; error bars represent standard errors; coefficients are in Table II-3 and means in Table II-2.

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SECTION III

INFLUENCE OF DAY AND NIGHT TEMPERATURE AND CARBON DIOXIDE CONCENTRATION ON GROWTH, YIELD, AND QUALITY OF GREEN BUTTERHEAD AND RED OAKLEAF LETTUCE

Influence of day and night temperature and carbon dioxide concentration on growth, yield, and quality of green butterhead and red oakleaf lettuce (*Lactuca sativa*)

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Abstract

Production of food-crops such as lettuce (*Lactuca sativa*), within vertical farms, warehouses, and shipping containers, is an expanding area in agriculture. These facilities have potential for improved water-use efficiency and space-utilization, alongside reduced pesticide applications compared to field-grown crops. The precise manipulation of environmental parameters such as mean daily temperature (MDT) and carbon dioxide (CO_2) concentration within these facilities enables year-round production without restrictions from seasonality, in addition to regulation of color, yield, and crop size. Our objectives were to 1) quantify how MDT and CO_2 concentration interact to influence growth, development, quality, and yield of lettuce; and 2) model predicted lettuce growth and development under several MDTs and CO_2 concentrations. Green butterhead lettuce 'Rex' and red oakleaf lettuce 'Rouxaï RZ' seeds were sown in a growth chamber with an MDT set point of 22 °C, CO_2 concentration of 500 $\mu mol \cdot mol^{-1}$, and a total photon flux density of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seedlings of each cultivar were transplanted into deep flow hydroponic tanks under a photosynthetic photon flux density of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 17-h photoperiod in growth chambers with CO_2 concentrations of 500, 800, or 1200 $\mu mol \cdot mol^{-1}$ and day/night temperatures and MDT set points of 22/15 °C (MDT 20 °C), 25/18 °C (23 °C), or 28/21 °C (26 °C). Fresh mass of 'Rex' increased linearly with MDT, increasing by 18% from 20 to 26 °C. 'Rouxaï RZ' fresh mass increased quadratically with MDT, with a 32% (41.6 g) increase from 20 to 23 °C, then a 7% (12.9 g) increase from 23 to 26 °C. Elevating CO_2 concentration from 500 to 800 $\mu mol \cdot mol^{-1}$ increased 'Rouxaï RZ' and 'Rex' fresh mass by 33 and 16% (46.5 and 24.4 g), while fresh mass did not increase from 800 to 1200 $\mu mol \cdot mol^{-1}$. Both cultivars had the greatest dry mass at 800 $\mu mol \cdot mol^{-1}$ CO_2 (7.1 g for 'Rouxaï RZ' and 7.9 g for 'Rex') and the least at 20 °C at a CO_2 concentration of 500 or 1200

$\mu mol \cdot mol^{-1}$. A high MDT caused 'Rouxaï RZ' foliage to be slightly more light, vibrant, and green, while a low MDT caused slightly darker, grayer, and more yellow/red foliage. From 20 to 26 °C, hue angle increased from 71.4 to 89.2 ° (greener), chroma increased from 6.6 to 9.1 (less gray), and CIE L* value increased from 29.9 to 32.5 (lighter). Tipburn occurred on 'Rex' regardless of CO_2 concentration or MDT, while 25% of 'Rouxaï RZ' were afflicted at 500 $\mu mol \cdot mol^{-1}$ CO_2 and 67% were afflicted at 1200 $\mu mol \cdot mol^{-1}$ CO_2. At the light intensity studied, we recommend growing 'Rex' and 'Rouxaï RZ' at a CO_2 concentration of 800 $\mu mol \cdot mol^{-1}$ and MDT of 23 °C for greatest biomass and leaf number, and slightly redder foliage in 'Rouxaï RZ' than at a 26 °C MDT.

Introduction

Vertical farms, warehouses, and shipping containers, collectively known to create controlled environments (CE) provide the opportunity to cultivate produce year-round where growing seasons, land access, or food system infrastructure are limiting (Beacham et al., 2019; Gomez et al., 2019). CE facilities typically enable precise control of environmental conditions, improving production and yield beyond that possible under field conditions (McCartney and Lefsrud, 2018; Ahmed et al., 2020). In recent years, there has been increasing interest in producing leafy greens in CEs (Gomez et al., 2019). Lettuce (*Lactuca sativa*) is particularly well-suited for CE production due to its compact growth, short production time, and high market demand, enabling frequent harvests with efficient space utilization (Beacham et al., 2019).

Parameters influential to plant growth, such as radiation duration, quantity, and quality; day and night temperatures; air flow; vapor pressure deficit (VPD); and carbon dioxide (CO_2) concentration can all be manipulated in CEs (McCartney and Lefsrud, 2018; Beacham et al., 2019; Gomez et al., 2019, Ahmed et al., 2020). Mean daily temperature (MDT) and CO_2

concentration 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 tip-burn (Ahmed et al., 2020).

The rate of plant development increases linearly with increasing temperature, from a species-specific base temperature (T_b) up to an optimum temperature (T_{opt}), after which the developmental rate declines (Blanchard et al., 2011). Like many plants, lettuce uses C_3 carbon fixation, which is responsive to atmospheric CO_2 concentration. Increasing CO_2 decreases photorespiration, raising the photosynthetic rate and light saturation point (Dusenge, 2018; Mattson and Holley, 2021). Growth increases until a species-specific CO_2 concentration saturation point is reached. Because of this initially high biomass impact, only a small increase in CO_2 beyond typical atmospheric levels can greatly increase yield (Dusenge, 2018; Mattson and Holley, 2021). CO_2 and MDT interact to influence growth and development (Ainsworth and Rogers, 2007; Mattson and Holley, 2021). At greater CO_2 concentrations, the T_{opt} for photosynthesis typically increases (Mattson and Holley, 2021). Beyond the T_{opt}, the rate of carboxylation to oxygenation decreases, reducing photosynthetic efficiency due to photorespiration (Ainsworth and Rogers, 2007).

The influence of MDT on lettuce development, morphology, growth, and metabolism has been investigated previously (Heins et al., 1998; Ahmed et al., 2020; Ouyang et al., 2020). For example, Ouyang et al. (2020) grew 'Grand Rapids TBR' at 16, 18, and 20 °C under a continuous photosynthetic photon flux density (*PPFD*) of 210 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 30 days after transplant. Lettuce shoot fresh mass (SFM) and height was 38 and 18% (9.9 g and 1.9 cm) greater at 20 °C than at 16 °C, and shoot dry mass (SDM) was 14% (0.5 g) greater at 18 or 20 °C than at 16 °C (Ouyang et al., 2019).

The influence of CO_2 concentration on lettuce growth and development in CEs has also been investigated previously (Kitaya et al., 1998; Frantz, 2011; Park et al., 2012; Pérez-López et al., 2015; Becker and Kläring, 2016). For example, SFM of lettuce 'Blonde of Paris Batavia' and 'Oak Leaf' increased by 55 and 77% (46 and 34 g), respectively, when the CO_2 concentration was raised from 400 to 700 $\mu mol \cdot mol^{-1}$ under a *PPFD* of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at day/night (14 h/10 h) temperatures of 25/18 °C (Pérez-López et al., 2015). Additionally, photosynthetic rate, apparent quantum yield, antioxidant capacity, and water-use efficiency increased for both cultivars (Pérez-López et al., 2015).

Given the strong influence of MDT and CO_2 concentration on lettuce growth, development, and quality, identifying the environmental parameters for improved resource-use efficiency and yield in CEs is needed. The objectives of this study were to 1) quantify how MDT and CO_2 concentration influence lettuce growth, development, quality, and yield; and 2) create models for predicting growth and development under various MDTs and CO_2 concentrations. We hypothesized that increasing CO_2 concentration would increase biomass production of lettuce across all temperatures, but there would be less of an effect shifting from the moderate to the highest tested CO_2 concentration compared to the shift from low to medium CO_2 concentration.

Materials and methods

Plant culture, environmental control, and data collection and analysis were the same as in Section II. Seeds were sown on 28 Apr. 2020, 09 June 2020, 27 July 2020, 16 Sept. 2020, 12 Nov. 2020, 07 Jan. 2021, and 20 Feb. 2021. Eleven days after sowing, the seedlings were transplanted into walk-in growth chambers under 17-h photoperiods, a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-}$

[1] and a relative humidity setpoint of 60%. Day/night temperature and CO_2 treatments were 22/15 °C (MDT 20 °C), 25/18 °C (23 °C), or 28/21 °C (26 °C) and 500, 800, or 1200 $\mu mol \cdot mol^{-1}$ respectively, and were maintained from transplant until harvest (Table III-1).

Results

Shoot fresh and dry mass

The SFM of 'Rouxaï RZ' increased quadratically with MDT; from 20 to 23 °C, SFM increased by 32% (41.6 g), then 7% (12.9 g) from 23 to 26 °C (Table III-2, 3; Fig. III-1 A). 'Rex' SFM increased linearly by 18% (28.0 g) from 20 to 26 °C (Table III-2, 3; Fig. III-1 C). Both cultivars showed quadratic increases in SFM as CO_2 concentration increased (Table III-2). Elevating CO_2 from 500 to 800 $\mu mol \cdot mol^{-1}$ increased SFM by 33 and 16% (46.5 and 24.4 g) for 'Rouxaï RZ' and 'Rex', respectively, without additional biomass accumulation as CO_2 increased from 800 to 1200 $\mu mol \cdot mol^{-1}$ (Table III-2, 3; Fig. III-1 B, D).

MDT and CO_2 concentration interacted to influence SDM of both cultivars (Table III-2, 3; Fig. III-2 A, B). Regardless of MDT, the greatest SDM (7.1 and 7.9 g for 'Rouxaï RZ' and 'Rex', respectively) was recorded at a CO_2 concentration of 800 $\mu mol \cdot mol^{-1}$, while the least was at 20 °C and a CO_2 concentration of 500 or 1200 $\mu mol \cdot mol^{-1}$ (5.0 and 6.2 g for 'Rouxaï RZ' and 'Rex', respectively). 'Rex' SDM increased from 20 to 23 °C at 500 and 1200 $\mu mol \cdot mol^{-1}$ CO_2. Similarly, the SDM of 'Rouxaï RZ' at 500 $\mu mol \cdot mol^{-1}$ CO_2 peaked at 23 °C, but at 1200 $\mu mol \cdot mol^{-1}$ CO_2, the SDM was greatest at 26 °C.

Morphology

Growth index (GI), an integrated measurement of plant size (GI = {plant height + [(diameter 1 + diameter 2)/2]}/2), of 'Rouxaï RZ' increased linearly with MDT, while the GI of

‘Rex’ showed an interaction of CO_2 and MDT (Table III-2, 3; Fig. III-1 E, III-2 E). ‘Rouxaï RZ’ GI increased by 19% as MDT increased from 20 to 26 °C. GI of ‘Rex’ increased by 20% as MDT and CO_2 concentration increased from 20 °C and 500 $\mu mol \cdot mol^{-1}$ CO_2 to 23 °C and 1200 $\mu mol \cdot mol^{-1}$ CO_2, respectively, or 26 °C MDT.

Leaf number was influenced by the interaction of MDT and CO_2 concentration for both cultivars (Table III-2, 3; Fig. III-2 C, D). The leaf number of ‘Rex’ was primarily influenced by the MDT, and as MDT increased from 20 to 23 °C and 23 to 26 °C, an average of 5 and 8 more leaves unfolded, respectively. The fewest number of leaves (22 leaves) was observed for ‘Rex’ at a CO_2 concentration of 500 or 1200 $\mu mol \cdot mol^{-1}$ and MDT of 20 °C while the greatest number of leaves (43) was at a CO_2 concentration of 1200 $\mu mol \cdot mol^{-1}$ and MDT of 26 °C. However, ‘Rouxaï RZ’ unfolded 21 leaves at an MDT of 20 °C and an additional 8 leaves at 23 °C, regardless of CO_2 concentration. Only 4 additional leaves unfolded as the MDT increased from 23 to 26 °C at a CO_2 concentration of 1200 $\mu mol \cdot mol^{-1}$.

Leaf length of both cultivars and leaf width of ‘Rouxaï RZ’ were influenced by the interaction of MDT and CO_2, while leaf width of ‘Rex’ was influenced only by MDT (Table III-2, 3; Figure III-2 F). Leaves of ‘Rouxaï RZ’ were 22% (2.7 cm) longer and 15% (2.9 cm) wider at an MDT of 26 °C and CO_2 concentration of 1200 $\mu mol \cdot mol^{-1}$ than those grown at 20 °C and 500 $\mu mol \cdot mol^{-1}$ CO_2. At 500 $\mu mol \cdot mol^{-1}$ CO_2, ‘Rex’ leaves were 10% longer at an MDT of 26 °C than at 20 °C, and were 4% wider at 20 °C than at 26 °C.

Tipburn incidence, recorded as the percentage of plants affected, was 25% and 67% at CO_2 concentrations of 500 and 1200 $\mu mol \cdot mol^{-1}$ for ‘Rouxaï RZ’ (data not shown). Tipburn was present on all of ‘Rex’ irrespective of MDT or CO_2 concentration.

F_v/F_m, relative chlorophyll concentration, and pigmentation

The maximum photosystem II quantum yields (F_v/F_m) ranged from 0.813 to 0.861, suggesting minimal impacts of stress on photosynthetic reactions (data not shown). For 'Rex', the relative chlorophyll concentrations were 7% lower at 23 °C (30.6) than at 20 or 26 °C (32.6; data not shown).

Foliage pigmentation of 'Rouxaï RZ' was influenced by MDT (Table III-2). As MDT increased from 20 to 26 °C, h° increased from 71.4 to 89.2, C* from 6.6 to 9.1, and L* from 29.9 to 32.5. These greater h°, C*, and L* values correspond to slightly lighter and more vibrant green foliage than the darker-gray, more yellow/red foliage with lower values.

Discussion

Lettuce yield is primarily determined by marketable fresh mass, with time to harvest and quality parameters such as color and tipburn incidence being of particular importance to growers. Lettuce biomass accumulation varies by cultivar (Fu et al., 2012; Kelly et al., 2020; Tarr, 2022) and depends on stage of growth and time to harvest (Choi et al., 2000; Esmaili et al., 2020) and environmental conditions, including *PPFD* (Sago, 2016; Kelly et al., 2020; Tarr, 2022), MDT (Choi et al., 2000; Lee et al., 2019; Tarr, 2022) and CO_2 concentration (Kitaya et al., 1998; Pérez-López et al., 2013; Esmaili et al., 2020). In the present study of 'Rouxaï RZ' and 'Rex', SFM was influenced by CO_2 and MDT independently, while SDM was influenced by the interaction of CO_2 and MDT.

SFM and SDM of both cultivars increased as MDT increased from 20 to 26 °C at a PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table III-2; Fig. III-1 A, C; Fig. III-2 A, B). Conversely, in Section II the SFM of 'Rex' was not influenced by MDT when grown at a CO_2 concentration of

500 μmol·mol^{-1} and *PPFD* of 150 or 300 μmol·m^{-2}·s^{-1} (Table II-2). SFM of 'Rouxaï RZ' and SDM of both cultivars increased quadratically with MDT in Section II and III; however, in Section II, SFM and SDM did not increase from 23 to 26 °C (Table II-2, 3; Fig. II-1 A).

In the present study, SFM of both cultivars only increased as the CO_2 concentration was raised from 500 to 800 μmol·mol^{-1}, with no further increases from 800 to 1200 μmol·mol^{-1} (Table III-2). Caplan (2018) reported a similar response: butterhead lettuce 'Fairly' grown at an MDT of 22 °C under *PPFD*s ranging from 156 to 330 μmol·m^{-2}·s^{-1} and CO_2 concentrations from 400 to 1300 μmol·mol^{-1} had the greatest SFM and SDM at 850 μmol·mol^{-1} CO_2, while yields decreased as CO_2 concentration increased. Conversely, the SFM of lettuce 'Partavousi' increased by 6 and 55% as CO_2 concentration increased from 400 to 800 and 800 to 1200 μmol·mol^{-1}, respectively, under a *PPFD* of 300 μmol·m^{-2}·s^{-1} and at an MDT of 25 °C for 40 d, without additional biomass accumulation at CO_2 concentrations of 1200 to 1600 μmol·mol^{-1} (Esmaili et al., 2020).

SDM was influenced by the interaction of CO_2 and MDT for both cultivars (Table III-2, 3; Fig. III-2 A, B). Regardless of MDT, the greatest SDM occurred at a CO_2 concentration of 800 μmol·mol^{-1}, while at higher or lower CO_2 concentrations, SDM was only influenced by MDT. Esmaili et al. (2020) observed that the SDM of 'Partavousi' increased by 31 and 147% as CO_2 concentration increased from 400 to 800 and 800 to 1200 μmol·mol^{-1}, respectively, with similar SDM at 1200 and 1600 μmol·mol^{-1}.

The GI, a measure of plant size that integrates plant height and width, was greatest at an MDT of 26 °C for both cultivars, while 'Rex' was marginally influenced by the interaction of CO_2 and MDT (Table III-2, 3; Fig. III-1 E, III-2 E). In Section II, MDT only influenced the GI of 'Rouxaï RZ' as it increased from 20 to 23 °C (Table II-2; Fig. II-1 F). The size of plants can impact recommended planting density and packaging; understanding that size increases within MDTs of 20 to 26 °C enables growers to adjust conditions based upon market preferences. Leaf size was influenced by the interaction of MDT and CO_2, but primarily by MDT (Table III-2). MDT has been suggested to have a greater impact on leaf mass area (leaf dry mass per leaf area) than CO_2 concentration (Poorter et al., 2009). Both cultivars had the shortest leaves at 20 °C, while 'Rouxaï RZ' leaves were narrowest and 'Rex' leaves were widest at 20 °C.

The interaction of CO_2 concentration and MDT impacted leaf unfolding rate for both cultivars (Table III-2, 3; Fig. III-2 C, D). Leaf unfolding increased from 20 to 26 °C when CO_2 concentrations were pooled, as expected with developmental rates increasing up to the T_{opt} (Hatfield and Prueger, 2015). The influence of CO_2 and MDT on the leaf unfolding rate of lettuce is not well documented. Lettuce 'Grand Rapids' grown at an MDT of 16.7 °C and ~500 $\mu mol \cdot mol^{-1}$ CO_2 unfolded 3 more leaves than at 18.3 °C and at CO_2 concentrations of 200–400 $\mu mol \cdot mol^{-1}$ (Frantz, 2011). In CO_2-limited conditions, leaf unfolding rate may be restricted along with the photosynthetic rate as photorespiration occurs (Dusenge et al., 2019).

A major concern for CE lettuce producers is tipburn, the necrosis on a leaf margin induced by calcium deficiency (Sago, 2016; Ahmed et al., 2020). Lettuce undergoing rapid growth with limited transpiration at the growing point is susceptible to tipburn. In the current study, tipburn incidence of 'Rouxaï RZ' was greatest under a PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a CO_2 concentration of 1200 $\mu mol \cdot mol^{-1}$ (Table III-2). When grown at an MDT of 22 °C and under a *PPFD* of 330 $\mu mol \cdot m^{-2} \cdot s^{-1}$, tipburn occurrence in butterhead lettuce 'Fairly' was not observed at CO_2 concentrations of 400 and 550 $\mu mol \cdot mol^{-1}$, but 10, 10, 25, and 33% of plants had tipburn at 700, 850, 1000, and 1300 $\mu mol \cdot mol^{-1}$ CO_2, respectively (Caplan, 2018). The increased incidence of tipburn at elevated CO_2 concentrations is likely due to a reduction in stomatal conductance

(Caplan, 2018; Dusenge et al., 2019) as stomata close in elevated CO_2 concentrations, reducing transpiration and, consequently, calcium movement to the growing point. MDT did not influence tipburn occurrence in Section II or III (Table II-2, III-3). Literature is mixed on the influence of MDT on lettuce tipburn, varying by cultivars and environmental conditions (Lee et al., 2013; Lee et al., 2019; Carotti et al., 2021). The VPD may have influenced tipburn incidence in our study. We maintained a constant 60% RH at 20, 23, and 26 °C, which translates to VPDs of ~0.9, 1.1, and 1.3 kPa, respectively (Table III-1). Transpiration rate increases with VPD, potentially increasing calcium access at the growing point and reducing tipburn incidence (Lee et al., 2013; Ahmed et al., 2020). Tipburn occurrence may have been suppressed at the higher MDTs due to greater VPDs, suppressing how MDT may influence tipburn.

Marketability of crops is influenced by foliage color (Runkle, 2017), with green foliage being undesirable in red-leaf cultivars. Most red, blue, and purple coloration of foliage is primarily caused by anthocyanins (Boldt et al., 2014). At low temperatures, anthocyanins can accumulate in leaves, inducing a darker, more pigmented foliage. The color of 'Rouxaï RZ' foliage at an MDT of 26 °C was a lighter, more vibrant green than the darker, grayer yellow/red foliage at 20 °C, which is consistent with Section II and other studies (Table II-2, III-2; Marin et al., 2015; Walters, 2020).

Future studies comparing lettuce growth responses to CO_2 concentrations and temperatures applied at different growth stages is needed to identify when supplemental inputs are most valuable. Esmaili et al. (2020) reported lettuce growth responses 10, 20, 30, and 40 d after sowing, with the greatest growth rate change occurring after 30 d. However, this was using constant environmental conditions over the growth cycle, rather than comparing CO_2 supplementation at different growth stages. Response to MDT may also vary by growth stage;

relative growth rate of butterhead lettuce 'Omega' 25 d after transplant was greater at 30/25 °C than 20/15 °C, but by 35 d after transplant, relative growth rate was lowest at 30/25 °C (Choi et al., 2000). Identifying the growth stage that specific MDT and CO_2 supplementation is most beneficial can reduce inputs and cost of production.

In conclusion, under a PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, we recommend growing 'Rex' and 'Rouxaï RZ' at a CO_2 concentration of 800 $\mu mol \cdot mol^{-1}$ and MDT of 23 °C because this provided the greatest biomass and leaf number, kept plants moderately compact, and, for 'Rouxaï RZ', induced redder foliage than growth at 26 °C.

APPENDIX

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

Temperature (°C)				CO_2	*PPFD*	VPD (kPa)	
Air day	Air night	Canopy	Water	($\mu mol \cdot mol^{-1}$)	($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Day	Night
22.0 ± 0.1	15.3 ± 0.1	22.2 ± 3.3	20.6 ± 1.0	499.2 ± 44.9	307.8 ± 5.8	1.08 ± 0.05	0.67 ± 0.03
22.0 ± 0.2	15.4 ± 0.3	23.1 ± 3.2	21.3 ± 3.2	801.2 ± 12.3	303.4 ± 7.7	1.11 ± 0.08	0.69 ± 0.08
21.9 ± 0.1	15.1 ± 0.0	22.3 ± 3.7	20.5 ± 1.0	1173.2 ± 187.9	295.6 ± 7.0	1.00 ± 0.35	0.51 ± 0.25
24.9 ± 0.4	18.3 ± 0.2	25.7 ± 3.7	23.9 ± 1.0	503.1 ± 51.4	299.9 ± 9.3	1.22 ± 0.06	0.81 ± 0.04
25.4 ± 1.3	18.5 ± 0.8	24.8 ± 3.6	23.9 ± 2.7	798.3 ± 23.0	300.0 ± 5.7	1.34 ± 0.05	0.90 ± 0.06
25.0 ± 0.0	18.2 ± 0.0	25.5 ± 3.1	23.3 ± 2.0	1166.1 ± 204.5	295.6 ± 8.9	1.16 ± 0.23	0.70 ± 0.08
28.1 ± 0.5	21.3 ± 0.4	28.0 ± 3.1	26.4 ± 1.1	497.8 ± 58.4	299.4 ± 10.2	1.73 ± 0.20	1.17 ± 0.13
27.9 ± 0.1	21.2 ± 0.0	27.8 ± 3.4	26.3 ± 2.5	801.5 ± 15.3	297.9 ± 12.5	1.64 ± 0.03	1.09 ± 0.01
27.9 ± 0.1	21.1 ± 0.0	27.5 ± 3.4	26.2 ± 1.2	1167.8 ± 205.1	299.4 ± 5.8	1.25 ± 0.25	0.63 ± 0.06

Table III-2. Influence of mean daily temperature (MDT) and carbon dioxide (CO2; μmol·mol^{-1}) on height and width (cm); growth index; leaf length and width (cm) and number (no.); shoot fresh and dry mass (g); hue angle (h°); chroma (C*); and CIE L* color value of red oakleaf lettuce ‘Rouxaï RZ’ (Lactuca sativa) and green butterhead lettuce ‘Rex’. Data represent the mean of two replications and cultivars with 10 samples. Analyses of variance for the effects of MDT and CO2 and their interaction are included below each cultivar mean. Within-column means of a given treatment with different letters were significantly different according to Tukey’s honestly significant difference (hsd) test (P < 0.05).

MDT	CO_2	Height	Width	Growth index	Leaf length	Leaf width	Leaf (no.)	Fresh mass	Dry mass	h°	C*	L*
						‘Rouxaï RZ’						
20		12.5 c	24.8 c	18.6 c				131.6 c		71.4 b	6.6 b	29.9 b
23		14.3 b	27.5 b	20.9 b				173.3 b		77.5 b	7.8 ab	31.2 ab
26		15.4 a	28.7 a	22.1 a				186.1 a		89.2 a	9.1 a	32.5 a
	500	[z]						139.9 b				
	800							186.4 a				
	1200							169.5 ab				
20	500				12.3 c	18.9 b	20.2 de		4.80 d			
	800				14.3 ab	20.8 ab	24.8 cde		6.70 ab			
	1200				13.4 bc	20.5 ab	19.5 e		5.25 cd			
23	500				14.3 ab	20.4 a	28.0 abc		6.06 abc			
	800				14.4 ab	20.4 ab	31.5 ab		7.31 ab			
	1200				14.2 abc	20.8 ab	27.7 bd		6.24 b			
26	500				14.9 ab	20.9 a	28.5 abc		6.42 abc			
	800				15.0 ab	21.0 ab	33.4 ab		7.26 ab			
	1200				15.0 a	21.8 a	31.3 ac		7.39 a			
MDT		***[y]	***	***	***	***	***	***	***	***	***	***
CO_2		NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
MDT×CO_2		NS	NS	NS	***	*	**	NS	***	NS	NS	NS
						‘Rex’						
20		12.2 b				15.1 a		154.8 c		–[x]	–	–
23		13.1 a				15.0 ab		168.1 b		–	–	–
26		12.8 a				14.6 b		182.8 a		–	–	–
	500							150.0 b		–	–	–
	800							174.4 a		–	–	–
	1200							181.9 a		–	–	–
20	500		19.0 e	15.4 e	11.7 c		21.5 eg		5.68 c	–	–	–
	800		21.7 cd	16.9 d	12.0 abc		24.5 def		7.63 ab	–	–	–
	1200		21.6 cd	17.1 cd	11.6 bc		23.5 fg		6.69 bc	–	–	–
23	500		21.4 d	17.3 bcd	12.6 ab		28.0 cf		6.94 b	–	–	–
	800		21.8 cd	17.5 bcd	12.4 abc		27.8 def		7.68 ab	–	–	–
	1200		23.2 ab	18.1 ab	11.9 abc		28.5 bcde		6.96 b	–	–	–
26	500		22.8 abc	17.7 abcd	12.9 a		32.3 bd		7.26 b	–	–	–
	800		22.6 bcd	18.0 abc	11.8 bc		35.0 bc		8.35 a	–	–	–
	1200		24.1 a	18.5 a	11.6 bc		43.1 a		7.48 ab	–	–	–
MDT		***	***	***	**	*	***	***	***	–	–	–
CO_2		NS	*	*	NS	NS	NS	*	*	–	–	–
MDT×CO_2		NS	***	*	***	NS	***	NS	*	–	–	–

[z] blank cells were not significant

[y] NS, *, **, *** represent non-significant or significant difference at P ≤ 0.05, 0.01, and 0.001, respectively.

[x] Data not collected.

Table III-3. Equations of regression analysis and r^2 or R^2 for mean shoot fresh and dry mass, growth index, leaf number, and leaf length in response to mean daily temperature (MDT; 20, 23, and 26 °C) and carbon dioxide concentration (CO_2; 500, 800, 1200 µmol·mol^{-1}) of green butterhead lettuce 'Rex' (*Lactuca sativa*) and red oakleaf lettuce 'Rouxaï RZ'. All models are in the form of: f = y0 + a*MDT + b*CO_2 + c*MDT^2 + d*CO_2^2 + e*MDT*CO_2.

Parameter	y0	(a) MDT	(b) CO_2	(c) MDT^2	(d) CO_2^2	(e) MDT*CO_2	R^2 or r^2
			'Rex'				
Fresh mass (g)	6.23E1[z]	4.61	[y]				0.15
	1.90E1[x]	8.19E-1					
Fresh mass (g)	7.18E1		2.03E-1		-9.31E-5		0.21
	2.40E1		6.20E-2		3.68E-5		
Dry mass (g)	-3.13	1.68E-1	1.60E-2		-9.49E-6		0.38
	9.98E-1	2.60E-2	2.00E-3		1.23E-6		
Growth index	9.71	2.73E-1	2.00E-3				0.42
	7.11E-1	2.90E-2	2.58E-4				
Leaf (no.)	1.37E2	-1.04E1	-4.50E-2	2.33E-1		2.00E-3	0.58
	4.88E1	4.16	1.30E-2	8.90E-2		5.74E-4	
			'Rouxaï RZ'				
Fresh mass (g)	-9.15E2	8.54E1		-1.66			0.46
	2.37E2	2.08E1		4.51E-1			
Fresh mass (g)	-6.05E1		5.53E-1		-3.05E-4		0.31
	2.73E1		7.10E-2		4.20E-5		
Dry mass (g)	-5.86	2.42E-1	1.70E-2		-9.60E-6		0.55
	8.46E-1	2.20E-2	2.00E-3		1.05E-6		
Growth Index	7.17	5.81E-1					0.51
	9.89E-1	4.30E-2					
Leaf (no.)	-1.45E2	1.25E1	3.00E-2	-2.55E-1	-3.15E-5	1.00E-3	0.59
	3.73E1	3.18	1.40E-2	6.80E-2	5.47E-6	4.20E-4	
Leaf length (cm)	4.47	2.69E-1	9.00E-3		-4.89E-6		0.30
	1.27	3.40E-2	3.00E-3		1.59E-6		

[z] Coefficients for model equations were used to generate Figs. III-1, III-2
[y] Blank cells = 0
[x] Standard error (SE)

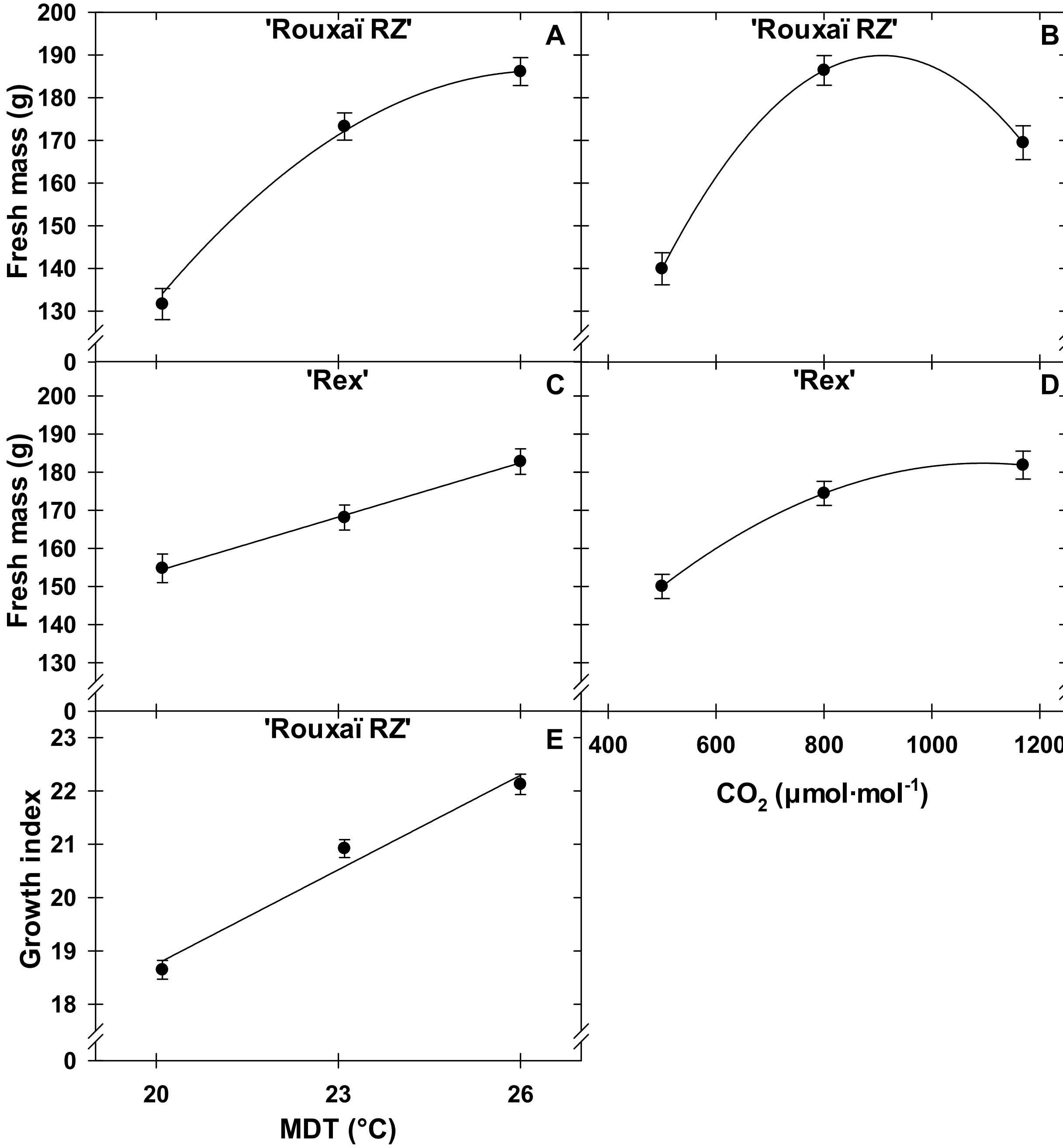

Figure III-1. Effects of mean daily temperature (MDT; 20, 23, and 26 °C) and carbon dioxide concentration (CO_2; 500, 800, 1200 µmol·mol^{-1}) on red oakleaf lettuce (*Lactuca sativa*) 'Rouxaï RZ' fresh mass (A and B) and growth index (E), and green butterhead lettuce 'Rex' fresh mass (C and D). Model predictions are represented by lines; coefficients are in Table III-3; error bars represent standard errors.

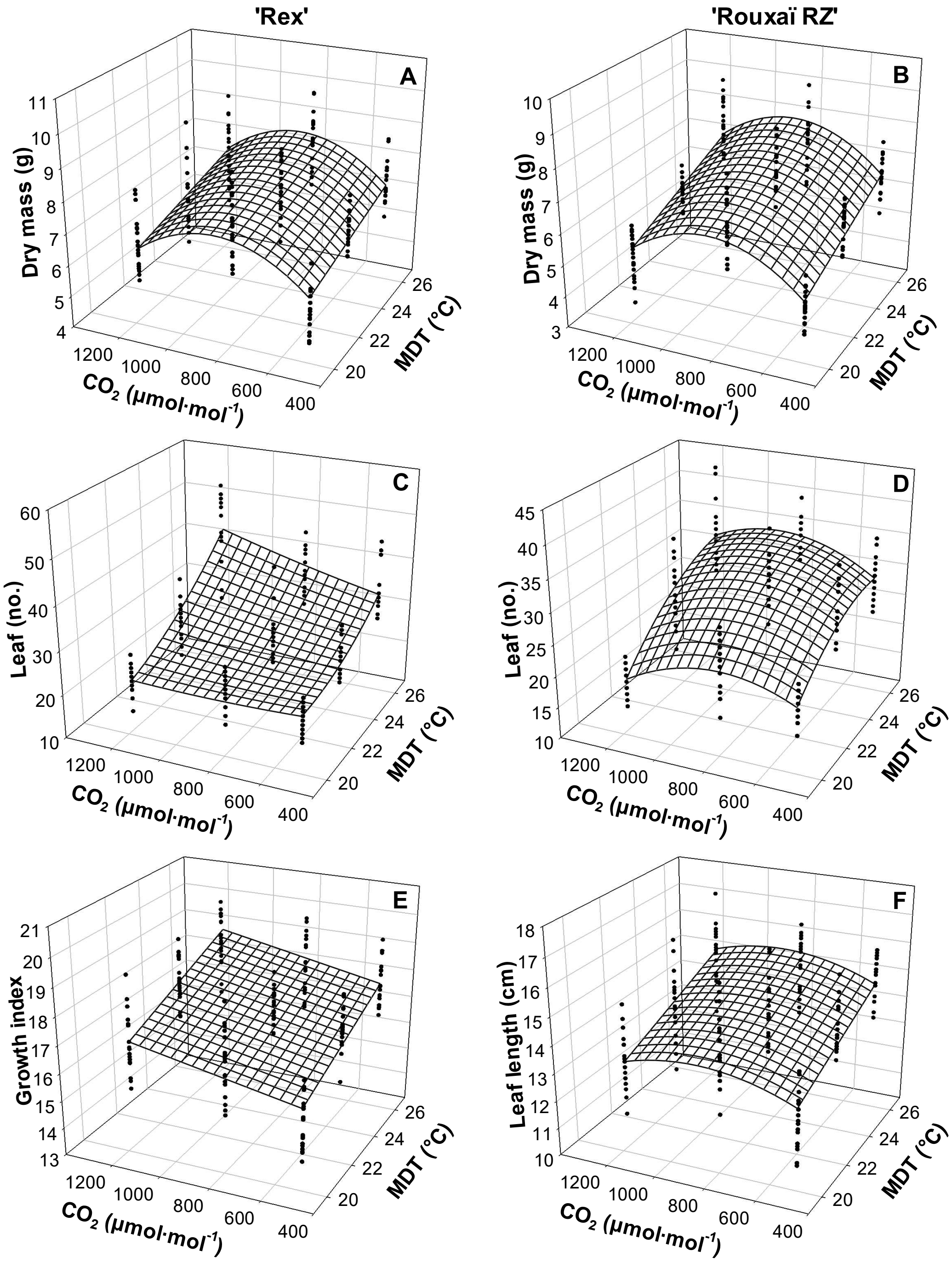

Figure III-2. Effects of carbon dioxide concentration (CO_2; 500, 800, 1200 μmol·mol^{-1}) and mean daily temperature (MDT; 20, 23, and 26 °C) on green butterhead lettuce (*Lactuca sativa*) 'Rex' shoot dry mass (A), leaf number (C), and growth index (E) and red oakleaf lettuce 'Rouxaï RZ' shoot dry mass (B), leaf number (D), and leaf width (F). Model predictions are represented by response surfaces; coefficients are in Table III-3.

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SECTION IV

IDENTIFYING THE BASE, OPTIMUM, AND MAXIMUM TEMPERATURE OF ARUGULA, KALE, AND LETTUCE.

Identifying the base, optimum, and maximum temperature of arugula, kale, and lettuce.

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Introduction

Regardless of outdoor climatic conditions, consistent, year-round vegetable production is possible within indoor controlled environments (CE) (Engler and Krarti, 2021). However, the continued growth of the indoor CE industry is constrained by high energy, operational costs, and carbon footprints (Engler and Krarti, 2021). The majority of the energy inputs stem from the precise manipulation of the growing environment, including heating, ventilation, and cooling (HVAC), dehumidification, and sole-source lighting. Although much research has focused on lighting, temperature control in CEs can impact the cost of production, crop timing, and quality (Gómez et al., 2019; Engler and Krarti, 2021). Therefore, identifying how environmental parameters influence crop growth and development can enable growers to balance inputs and yield.

Leafy green vegetables, including lettuce (*Lactuca sativa*), arugula (*Eruca sativa*), and kale (*Brassica oleracea*), are well-suited for indoor CE production given their short harvest cycle, compact growth, moderate light requirement, and the seasonality constraints of field production (Gómez et al., 2019). Leafy greens are already grown inside semi-CEs such as greenhouses and high tunnels; total wholesale value of CE-grown lettuce increased by 28% from $55.5 to $71.1 million from 2014 to 2019 (USDA, 2015; USDA, 2020b).

Arugula has grown in popularity over the past several decades, with it now readily available in many grocery stores as a standalone leafy green or in salad mixes (Enroth, 2018). Arugula is a fast-growing, cool-season crop that will flower under long daylengths and high temperatures (Morales et al., 2006). Most arugula is field grown in California, but it is a specialty green that many CE operations can incorporate if environmental conditions can prevent bolting (Enroth, 2018). Kale has also increased in market value over the past several decades, partly due to its associated health benefits (Satheesh and Workneh Fanta, 2020). In 2017, kale was harvested on 6,201 hectares, an increase of ~145% from 2012 (USDA, 2019).

Temperature influences many aspects of plant development, including leaf unfolding and flowering; anthocyanin biosynthesis and accumulation; and overall quality. Different crops having specific temperature ranges conducive for development (Christie et al., 1993; Sage and Kubien, 2007; Hatfield and Prueger, 2015). The base temperature (T_b) is the temperature at which plant development halts. Above the T_b, development increases linearly until the optimum temperature (T_{opt}) is reached, where the rate of development is greatest. As the temperature increases further, the development rate could plateau, or decrease until the maximum temperature (T_{max}) is reached and development stops (Sage and Kubien, 2007). By identifying the T_b, T_{opt}, and T_{max} of crops, we can create temperature response curves that growers can utilize to increase or slow down the rate of development, and identify temperatures that are detrimental to crop development, biomass accumulation, or lead to undesirable bolting.

Given the economic value of leafy greens in the horticultural industry and the rise in CE production, modeling their growth and development in response to mean daily temperature (MDT) in CEs is needed. Therefore, the objective of this study was to 1) model lettuce, arugula, and kale growth and development in response to MDT. We postulated that MDT influences the growth, development, and quality of lettuce, arugula, and kale to varying extents, with an increasing rate of development from T_b up to an T_{opt}, followed by a decrease or plateau in development rate as temperature moves beyond the optimum temperature depending on the conducive growing temperature range of each genus.

Materials and Methods

Plant material and propagation conditions

On 23 May and 09 July 2021, seeds of arugula 'Astro' (Ball Horticultural Co., West Chicago, IL), kale 'Red Russian' (Ball Horticultural Co.), red oakleaf lettuce 'Rouxaï RZ' (Rijk Zwaan USA, Salinas, CA), and green butterhead lettuce 'Rex' (Rijk Zwaan USA) were sown one seed per cell into 72-cell plug trays (72-cell trays, 28-mL individual cell volume; Landmark Plastics, Akron, OH) filled with a seed sowing mixture composed of 1:1 (v/v) 50% vermiculite (Vermiculite Premium Grade; Sungro Horticulture, Agawam, MA) and 50% substrate mixture of 70% peat, 21% perlite, and 9% vermiculite (Suremix; Michigan Grower Products, Inc., Galesburg, MI). Translucent domes were placed over each plug tray to maintain high humidity during germination. The trays were placed in a walk-in growth chamber (Hotpack environmental room UWP 2614-3; SP Scientific, Warminster, PA) with an MDT of 22 ºC, carbon dioxide concentration of 500 $\mu mol \cdot mol^{-1}$, and VPD of 1.1 kPa. Light-emitting diode (LED) fixtures (Ray66; Fluence Bioengineering, Austin, TX) provided a total photosynthetic photon flux density (*TPFD*) of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height and a light ratio (%) of 19:39:39:3 blue:green:red:far-red for 24 $h \cdot d^{-1}$. After 3 d, the translucent domes were removed from the trays and the photoperiod was reduced to 20 h until transplant at 11 d. The trays were hand-watered as necessary with water-soluble fertilizer (MSU Plug Special 13N–1.8P–10.8K; Greencare Fertilizers, Inc., Kankakee, IL) and a micronutrient supplement (M.O.S.T.; J.R. Peters, Inc., Allentown, PA) that delivered ($mg \cdot L^{-1}$): 61 N, 10 P, 50 K, 28 Ca, 6 Mg, 27 S, 17 Fe, 10 Zn, 17 Mn, 5 Cu, 3 B, and 0.2 Mo.

Growing environment

On 03 June and 20 July 2021, 48 seedlings of each cultivar were transplanted into 15-cm containers (East Jordan Plastics Inc., East Jordan, MI) filled with the previously mentioned commercial medium. The containers were then distributed into two blocks within the three previously described walk-in growth chambers. Air day/night (12 h/12 h) and MDT set points (Table IV-1) of 11/5 °C (8 °C), 16/10 °C (13 °C), 21/15 °C (18 °C), 26/20 °C (23 °C), 31/25 °C (28 °C), or 36/30 °C (33 °C) were measured every 5 s by a resistance temperature detector (Platinum RTD RBBJL-GW05A-00-M 36B; SensorTec, Inc., Fort Wayne, IN) and logged by a C6 controller (Environmental Growth Chambers, Chagrin Falls, OH). A photosynthetic photon flux density (*PPFD*) of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was provided for 12 $h \cdot d^{-1}$ by LED fixtures (Ray66; Fluence Bioengineering) that were mounted ~95 cm above the crop canopy, providing a daily light integral (DLI) of ≈13.0 $mol \cdot m^{-2} \cdot d^{-1}$ averaged over several measurements across the growing area. Every 15 s, substrate temperature, canopy temperature, and *PPFD* were monitored using a thermistor (ST-100; Apogee Instruments, Logan, UT), infrared thermocouple (OS36-01-T-80F; Omega Engineering, INC. Norwalk, CT), and quantum sensor (LI-190R; LI-COR Biosciences, Lincoln, NE), respectively, with means logged every hour by a CR-1000 datalogger (Campbell Scientific, Logan, UT). A CO_2 concentration of 500 $\mu mol \cdot mol^{-1}$ 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.

Plants were overhead fertigated every 4 d with a water-soluble fertilizer (MSU Orchid RO Water Special 13N−1.8P−12.5K; Greencare Fertilizers, Inc.), providing ($mg \cdot L^{-1}$): 126 N, 13 P, 121 K, 78 Ca, 19 Mg, 0.2 B, 0.4 Cu, 2 Fe, 0.9 Mn, 0.2 Mo, and 0.4 Zn. Based on observation and weight of multiple pots within each treatment, plants were irrigated with RO water between fertigation events to maintain similar substrate moisture and provided nutrition among treatments. A vapor pressure deficit (VPD) of 1.0 kPa was targeted with the injection of fine mist and dehumidifiers. At the highest tested MDT of 33 °C, the mean VPD was 2.01 kPa, much higher than the other 5 tested MDTs (Table IV-1).

Growth data collection and analysis

On 28, 31, 34, and 34 d after transplant of 'Rex', 'Rouxaï RZ', arugula 'Astro', and kale 'Red Russian', respectively, a SPAD meter (MC-100 Chlorophyll Meter; Apogee Instruments, Logan, UT) was used to estimate the relative chlorophyll concentration (RCC) of the most recent fully expanded leaf for eight plants in each block in each temperature treatment. On the same day, a portable chlorophyll fluorescence meter (Handy Plant Efficiency Analyzer; Hanstech Instruments Ltd. Norfolk, U.K.) was used to record F_v/F_m by dark acclimating leaves for >15 minutes with manufacturer-supplied clips, then exposing them to 3,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of red radiation (peak wavelength 650 nm) to saturate photosystem II. On the same day, foliage coloration on a fully unfolded leaf for arugula 'Astro', kale 'Red Russian', and red lettuce 'Rouxaï RZ' was averaged across three readings per plant with a tristimulus colorimeter (Chroma Meter CR-400; Konica Minolta Sensing, Inc., Chiyoda, Tokyo). Foliage coloration values were converted from the International Commission on Illumination (CIE) L*a*b* color space values and reported as hue angle (h°), chroma (C*), and lightness (L*), as suggested by McGuire (1992).

Harvest occurred on 29, 32, 35 and 35 d after transplant for lettuce 'Rex', 'Rouxaï RZ', arugula 'Astro', and kale 'Red Russian', respectively. Height from the medium to the highest point of the plant, plant width from the widest point and perpendicular from the widest point, length and width of the sixth fully expanded leaf, leaf number (when >5 cm), and the presence of visible buds were recorded. Plants were excised just above the medium, then total shoot (stems and leaves) fresh mass (SFM) was measured using a digital balance. The growth index (GI = {plant height + [(diameter 1 + diameter 2)/2]}/2) was calculated to obtain an integrated measurement of plant size for lettuce (Krug et al., 2010). As suggested by Frantz et al. (2004), lettuce was scored by tipburn severity: light — only central leaflets show minor necrotic spots; medium — a few older leaves and meristem have small necrotic spots, leaf margins appear blackened/misshapen; severe — occurrence of malformed leaves and meristem death in majority of plants. The severity of tipburn occurrence in relation to the number of plants was used for a tipburn index (TBI = {[(Severe × 5) + (Medium × 3) + (Light × 1)] × 100}/plant number × 5), emphasizing severely affected plants instead of those with minor tipburn symptoms (Frantz et al., 2004). The plant material was then placed at 75 ºC in a forced-air drier for at least 3 d and shoot dry mass (SDM) was recorded.

Experiment design and statistical analysis

The experiment was a randomized complete-block design, with two replications occurring within each temperature treatment (six levels). Each cultivar was analyzed separately with SAS (version 9.4; SAS Institute, Cary, NC). Cardinal temperature parameters were estimated using the nonlinear regression procedure (PROC NLIN) suggested by Blanchard and Runkle (2011), analysis of variance (ANOVA) was assessed with mixed model procedure (PROC MIXED), and pairwise comparisons were performed with Tukey-Kramer difference test ($P \leq 0.05$). SigmaPlot (version 14.5, Systat Software, Inc., San Jose, CA) was used for regression analysis.

Results

Leaf unfolding and biomass accumulation rates.

The T_b for leaf unfolding for arugula, kale, 'Rex', and 'Rouxaï RZ' was estimated to be ≈6.1, 4.2, 7.8, and 7.8 °C, respectively (Table IV-2; Fig. IV-1A–D). Leaf unfolding rate increased linearly up to the estimated T_{opt} of 29.2 °C for arugula, 22.7 °C for kale, and 27.2 and 27.4 °C for 'Rex' and 'Rouxaï RZ', respectively. A T_{max} could not be estimated for arugula or kale from the observed data; therefore, the T_{max} for the nonlinear model was set to 40 °C so estimates could be made for T_b and T_{opt}. The leaf unfolding rate for kale apparently plateaued after the T_{opt} but then increased at 33 °C, accelerating from 0.30 to 0.38 leaves a day. This was deemed supraoptimal and indicative of bolting, so it was not included in the leaf unfolding model. The T_{max} for leaf unfolding of 'Rex' and 'Rouxaï RZ' was estimated to be 34.4 and 34.8 °C, respectively.

We estimated that the T_b for fresh mass accumulation in arugula, kale, 'Rex', and 'Rouxaï RZ' is ≈6.6, 7.0, 8.4, and 8.5 °C, respectively, under a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and CO_2 concentration of 500 $\mu mol \cdot mol^{-1}$ (Table IV-2; Fig. IV-1E–H). The greatest fresh mass accumulation occurred at a T_{opt} of 24.7, 22.9, 24.7, and 26.2 °C for kale, arugula, 'Rex', and 'Rouxaï RZ', respectively. The T_{max} of arugula and kale is estimated to be 39.6 and 37.3 °C, while the T_{max} of 'Rex' and 'Rouxaï RZ' was at 34.1 and 33.6 °C, respectively.

The T_b for dry mass accumulation ranged from 7.5 to 8.1 °C for all genera (Table IV-2; Fig. IV-1I–L). The T_{opt} of arugula and kale was 21.0 and 21.8 °C, respectively, while the T_{max} was 40.9 and 36.9 °C, respectively. 'Rex' has a T_{opt} of 27.0 °C and T_{max} of 33.8 °C, and 'Rouxaï RZ' has a T_{opt} and T_{max} of 26.5 and 34.4 °C, respectively.

Shoot fresh and dry mass

Increasing the MDT from 8 to 18 °C quadrupled the SFM of arugula from 12.1 to 66.7 g (Table IV-2; Fig. IV-2A). Further increasing the MDT from 28 to 33 °C resulted in a 21% reduction (14.1 g) in SFM. SFM of kale increased over 5-fold, from 11.7 to 72.0 g as the MDT increased from 8 to 18 °C (Table IV-2; Fig. IV-3A). Beyond an MDT of 23 °C, SFM of arugula decreased by 41% (28.7 g) to 40.1 g. SDM of arugula and kale was lowest at an MDT of 8 °C (1.4 and 1.3 g, respectively), increased to a maximum at 18 °C (11.3 and 10.0 g, respectively), then decreased until the maximum MDT of 33 °C (4.9 and 3.9 g, respectively) (Table IV-2; Fig. IV-2B; IV-3B).

The SFM of both 'Rex' and 'Rouxaï RZ' was lowest at 8 °C (3.9 and 3.3 g) and increased to a maximum at 23 °C (127.1 and 105.6 g) (Table IV-2; Fig. IV-4A). Further raising the MDT from 23 to 28 °C led to a 26% (32.8 g) reduction in the SFM of 'Rex', The SFM of 'Rex' and 'Rouxaï RZ' decreased by 60% (76.9 g) and 55% (57.6 g) as the MDT increased from 23 to 33 °C. The SDM of both lettuce cultivars increased from a minimum of 0.3 g at an MDT of 8 °C to a maximum of 4.9 and 5.2 g at 23 °C for 'Rex' and 'Rouxaï RZ', respectively. At an MDT of 33 °C, the SDM of 'Rex' and 'Rouxaï RZ' decreased to 2.9 and 3.2 g, respectively.

Morphology

The most compact arugula and kale were observed at an MDT of 8 °C (9.3 and 7.2 cm), while the tallest (19.9 and 21.5 cm), were at 18 °C (Table IV-2). Similarly, 'Rex' and 'Rouxaï RZ' were the shortest at an MDT of 8 °C and largest from 18 to 28 °C (Table IV-2). Leaves generally were longest and widest between MDTs of 18 and 23 °C, and leaves were small at the temperature extremes (Table IV-2).

Tipburn was not present in ‘Rex’ or ‘Rouxaï RZ’ at an MDT of 8 or 13 °C, while moderate tipburn occurred in ‘Rex’ from 18 to 33 °C (Table IV-2). ‘Rouxaï RZ’ had minimal tip burn at all other MDTs and moderate severity at 18 °C.

F_v/F_m, Relative chlorophyll concentration and pigmentation

Chlorophyll fluorescence values (F_v/F_m) were not below 0.8 for either lettuce cultivar and remained between 0.822 and 0.863 (Table IV-2). Conversely, the F_v/F_m of arugula and kale were below 0.8 at an MDT of 8 °C. The RCC of arugula was lowest at 8 and 33 °C (≈44.6) and the RCC of kale was lowest at 33 °C (43.1).

The foliage color of arugula was lightest at 8 and 33 °C (Table IV-2). Kale grown at 18 °C was darker (L* 36.4) and grayer (C* 1.9) than those grown at 33 °C (L* 40.4 and C* 13.8). ‘Rouxaï RZ’ was the darkest (L* 23), grayest (C* 2.3), and reddest (h° 18.1) at an MDT of 8 °C, becoming lighter, greener, and less gray as MDT increased.

Discussion

In this study, we identified the cardinal temperatures for kale, arugula, and lettuce leaf unfolding rate and rate of SFM and SDM accumulation at a *PPFD* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12-$h \cdot d^{-1}$ and CO_2 concentration of 500 $\mu mol \cdot mol^{-1}$. Our estimated T_b and T_{opt} for SFM and SDM of arugula and kale align with the findings of Imler (2020) under constant day and night temperatures. Arugula and kale ‘Starbor’ had an estimated SFM T_b of 7.2 and 7.4 °C, respectively, and T_{opt} of 23.9 and 22.6 °C, respectively, when grown for 21 or 28 d at constant temperatures of 8, 13, 18, 23, 28, or 33 °C and under a *PPFD* of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16-$h \cdot d^{-1}$ (Imler, 2020). Compared to Imler (2020), our estimated T_b and T_{opt} for arugula SFM was 0.6 °C lower and 0.8 °C higher, respectively, while our kale T_b and T_{opt} was 0.4 °C lower and 0.3 °C higher, respectively (Table IV-3). Interestingly, our estimated T_b and T_{opt} for arugula leaf number were both 3 °C warmer than previous estimates of 3.1 and 26.4 °C (Imler, 2020), respectively, while our kale T_b was 1.2 °C warmer and T_{opt} was 4.4 °C cooler than prior estimates of 3.0 and 27.1 °C (Imler, 2020). The difference in leaf number estimates may be associated with the DLI provided, as DLI and MDT can interact to influence leaf unfolding rate (Walters, 2021; Tarr, 2022). Additionally, the growing duration and growth stage can impact relative growth rate (Choi et al., 2000; Esmaili et al., 2020).

Studies estimating lettuce cardinal temperatures are limited. Recent CE studies often focused on refining a small range of temperatures in conjunction with other environmental parameters (Lee et al., 2019; Carotti et al., 2021; Tarr, 2022), while older studies that estimated the T_b and T_{opt} were conducted in the field or without adequate information on the growing conditions (Kristensen et al., 1987; Marsh and Albright, 1991; Seginer et al., 1991; Wheeler et al., 1993). Our estimated T_b of ≈8 °C conflicts with the estimated T_b of 4.0 °C from Kristensen et al. (1987); however, the 4.0 °C T_b was estimated from variety trials of field-grown lettuce and other environmental conditions were not controlled or reported. Our estimated T_{opt} for 'Rex' SFM gain of 24.7 °C aligns with other studies suggesting a T_{opt} of 24 °C for lettuce (Marsh and Albright, 1991; Thompson et al., 1998; Carotti et al., 2021). Conversely, the T_{opt} for 'Rouxaï RZ' SFM gain and leaf unfolding rate and SDM gain for both lettuce cultivars were estimated to be ≈27 °C (Table IV-3). Cultivar differences can account for the difference in T_{opt}, as Frantz et al. (2004) estimated a 30 °C T_{opt} for lettuce 'Grand Rapids'.

In the present study, the height of arugula and kale was the greatest between an MDT of 18 to 28 °C, a range that includes Imler's (2020) estimated T_{opt} for arugula at 27.3 °C and kale at 22.4 °C. Similarly, the GI of both lettuce cultivars in our study was the greatest from an MDT of

18 to 28 °C, and was triple the size of lettuce grown at 8 °C (Table IV-3). In Section II, the GI of ‘Rouxaï RZ’ was 15% greater at an MDT of 23 or 26 °C (GI of 20.5) than at 20 °C (GI of 17.3) (Table II-2), while in Section III both cultivars had the greatest GI at 26 °C (GI of 22.1 for ‘Rouxaï RZ’ and 18.1 for ‘Rex’) (Table III-2).

TBI, an assessment of tipburn severity and incidence, was mild in ‘Rouxaï RZ’ at all MDTs except at 18 °C, where it was moderate. ‘Rex’ had moderate tipburn from 18 to 33 °C, and none at 8 to 13 °C (Table IV-2). In Section II and III, MDT did not influence tipburn occurrence, while *PPFD* and CO_2 concentrations did. The lack of an MDT influence on tipburn in those studies may have to do with the range of temperatures studied, since MDTs of 20 to 26 °C all had some tipburn occurrence in the present study (Table IV-2). Generally, the influence of MDT on lettuce tipburn is inconsistent throughout the literature, varying by cultivars and environmental conditions beyond MDT alone (Lee et al., 2013; Lee et al., 2019; Carotti et al., 2021). Additionally, tipburn can occur more often under high DLIs (Sago, 2016); the present study had a DLI of 13 $mol \cdot m^{-2} \cdot d^{-1}$, while Section II and III had DLIs of 9.2 or 18.4 $mol \cdot m^{-2} \cdot d^{-1}$.

The RCC of arugula was the lowest at MDTs of 8 and 33 °C, contrasting with no difference reported by Imler (2020). Kale RCC in our study was stable across all MDTs except for a lower value at 33 °C, while Imler (2020) reported a linear downward trend in kale RCC as MDT increased. The F_v/F_m of our arugula and kale was lowest at an 8 °C MDT, entering a range associated with stress and reduced photosystem II efficiency (Murchie and Lawson, 2013). Under constant temperatures, Imler (2020) did not report that arugula and kale F_v/F_m readings indicated a reduction in photosystem II efficiency. Lettuce did not enter stress-related ranges of F_v/F_m in this study or in Section II or III.

Many crops are marketed based upon their unique foliage coloration, with purple or red-leaf cultivars being less desirable if their foliage is green or yellow (Runkle, 2017). Plant pigments such as anthocyanins cause many of the red, blue, and purple colors in foliage (Boldt et al., 2014). These anthocyanins can accumulate in leaves, inducing a darker, more pigmented foliage at cooler temperatures. These changes in color can be induced or reversed as the temperature conditions change (Boldt et al., 2014). 'Rouxaï RZ' foliage was the darkest, reddest color at an MDT of 8 °C, becoming a lighter, more vibrant green as MDT increased, coinciding with Section II, III, and other literature (Table IV-2; Marin et al., 2015; Walters, 2020).

APPENDIX

Table IV-1. Average (mean ± SD) day and night air, canopy, and media temperatures; photosynthetic photon flux density (*PPFD*); and vapor pressure deficits (VPD) for container grown arugula (*Eruca sativa*) 'Astro,' kale (*Brassica oleracea*) 'Red Russian,' green butterhead lettuce (*Lactuca sativa*) 'Rex', and red oakleaf lettuce 'Rouxaï RZ.'

Temperature (°C)				*PPFD*	VPD (kPa)		
Day	Night	Canopy	Media	($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Day	Night	Overall
10.9 ± 0.07	5.2 ± 0.03	8.5 ± 0.31	8.8 ± 0.21	304.5 ± 13.3	0.76	0.47	0.60
15.9 ± 0.07	10.1 ± 0.03	13.3 ± 0.39	12.5 ± 0.44	305.7 ± 11.0	1.01	0.64	0.80
21.4 ± 1.12	15.2 ± 0.19	22.3 ± 1.02	19.5 ± 0.51	294.8 ± 17.1	0.90	0.40	0.61
26.1 ± 0.34	20.1 ± 0.04	27.2 ± 0.89	24.3 ± 0.28	298.4 ± 10.3	1.04	0.49	0.73
30.9 ± 0.14	25.1 ± 0.02	31.2 ± 0.23	27.2 ± 0.88	301.4 ± 7.9	1.08	0.49	0.75
35.9 ± 0.02	30.1 ± 0.02	35.6 ± 0.38	33.7 ± 0.54	300.1 ± 10.6	2.01	1.13	1.49

Table IV-2. Influence of mean daily temperature (MDT; 8, 13, 18, 23, 28, and 33 °C) on arugula (*Eruca sativa*) 'Astro', kale (*Brassica oleracea*) 'Red Russian', green butterhead lettuce (*Lactuca sativa*) 'Rex', and red oakleaf lettuce 'Rouxaï RZ' on leaf number; shoot fresh and dry mass (g); size; leaf length and width (cm); maximum photosystem II quantum yields (F_v/F_m); relative chlorophyll concentration (RCC); tipburn index (TBI); hue angle (h°); chroma (C*); and CIE L* color value. Data represents the mean of two replications with 8 samples each. Analyses of variance for the impact of MDT are below each cultivar mean. Within-column and cultivar means with different letters are significantly different according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

MDT	Leaf (no.)	Fresh mass	Dry mass	Size[z]	Leaf length	Leaf width	Fv/Fm	RCC	TBI	h°	C*	L*
						Arugula 'Astro'						
8	5.4 b	12.1 d	1.4 e	9.3 c	15.3 d	5.5 c	0.765 c	43.7 c	–[y]	117.7 bc	17.8 ab	36.5 a
13	15.5 b	34.5 c	4.3 d	13.7 b	21.9 ab	9.2 a	0.835 a	55.0 a	–	124.6 a	12.1 c	31.4 c
18	28.4 a	66.7 a	11.3 a	19.9 a	19.5 bc	6.9 bc	0.839 a	60.8 a	–	121.6 ab	9.8 c	32.5 c
23	28.8 a	67.2 a	8.6 b	21.3 a	23.8 a	8.5 ab	0.838 a	58.7 a	–	121.5 ab	12.9 bc	34.1 b
28	35.5 a	65.8 a	6.7 c	21.4 a	19.2 bcd	6.6 bc	0.833 ab	53.0 ab	–	120.1 bc	14.2 abc	34.7 c
33	32.8 a	51.7 b	4.9 d	15.1 b	16.2 cd	5.8 c	0.814 b	45.4 bc	–	116.0 c	18.2 a	37.1 a
	***[x]	***	***	***	**	**	***	**	_	**	**	***
						Kale 'Red Russian'						
8	4.3 d	11.7 d	1.3 e	7.2 d	13.1 c	7.9 c	0.795 c	55.1 a	–	127.1 ab	6.8 c	33.1 a
13	8.2 c	31.8 c	3.5 d	12.1 c	21.3 b	11.8 b	0.831 ab	54.7 a	–	135.3 ab	3.4 d	34.3 a
18	11.1 ab	72.0 a	10.0 a	21.5 a	28.2 a	13.8 a	0.833 a	52.5 a	–	140.9 a	1.9 d	36.4 a
23	10.5 b	68.8 a	8.6 b	24.3 a	30.1 a	14.3 a	0.837 a	52.6 a	–	141.4 a	7.3 c	40.4 b
28	10.2 bc	56.5 b	6.0 c	22.0 a	27.7 a	13.9 a	0.832 ab	50.8 a	–	128.2 ab	10.5 b	41.9 b
33	13.1 a	40.1 c	3.9 d	16.9 b	24.2 b	10.8 b	0.814 bc	43.1 b	–	121.6 b	13.8 a	40.4 b
	***	***	***	***	***	***	***	**	_	*	***	***
						Green butterhead lettuce 'Rex'						
8	2.3 d	3.9 e	0.3 d	5.8 d	4.9 d	4.4 d	0.822 ab	30.6 ab	0 c	–	–	–
13	10.8 c	27.6 d	1.6 c	10.5 c	9.0 c	7.2 c	0.852 a	32.1 a	0 c	–	–	–
18	26.8 b	96.6 b	3.5 b	16.8 ab	12.7 a	10.6 a	0.862 a	25.8 b	41 ab	–	–	–
23	34.1 a	127.1 a	4.9 a	17.6 ab	13.1 a	9.9 ab	0.863 a	28.4 ab	60 a	–	–	–
28	33.6 a	94.3 b	4.6 a	17.0 ab	12.6 a	9.3 b	0.847 a	30.8 ab	35 b	–	–	–
33	24.9 b	50.2 c	2.9 b	15.7 b	10.8 b	7.1 c	0.844 b	32.4 a	46 ab	–	–	–
	***	***	***	***	***	***	**	*	***	_	_	_
						Red oakleaf lettuce 'Rouxaï RZ'						
8	1.8 d	3.3 e	0.3 d	5.8 d	5.0 e	5.6 c	0.838 c	31.4 a	0 b	18.1 e	2.3 d	23.0 b
13	10.9 c	22.4 d	1.4 d	10.5 c	8.4 d	9.8 b	0.857 a	25.3 ab	0 b	79.0 d	9.3 c	30.2 ab
18	23.4 b	81.8 b	3.8 bc	18.0 a	14.1 ab	16.5 a	0.852 ab	19.4 b	60 a	101.7 bc	13.6 bc	31.9 ab
23	33.2 a	105.6 a	5.2 a	19.0 a	14.4 a	16.2 a	0.861 a	20.5 b	15 b	99.0 c	15.5 ab	32.6 ab
28	31.2 a	93.0 ab	4.5 ab	18.1 a	13.0 b	14.4 a	0.860 a	20.8 b	15 b	105.4 b	19.0 ab	35.0 a
33	24.9 b	48.0 c	3.2 c	16.3 b	10.8 c	10.1 b	0.841 bc	23.9 b	21 b	110.2 a	20.3 a	39.7 a
	***	***	***	***	***	***	**	**	***	***	***	**

[z] Size for arugula and kale is height (cm), for lettuce it is growth index.
[y] Data not collected.
[x] NS, *, **, *** represent non-significant or significant difference at P ≤0.05, 0.01, and 0.001, respectively.

Table IV-3. Parameter estimates and R^2 of nonlinear models for rate of leaf unfolding (no.·d^{-1}) and fresh and dry mass accumulation (g·d^{-1}) in relation to mean daily temperature of arugula (*Eruca sativa*) 'Astro', kale (*Brassica oleracea*) 'Red Russian', green butterhead lettuce (*Lactuca sativa*) 'Rex', and red oakleaf lettuce 'Rouxaï RZ'. Developmental rates are zero at the base (T_b) and maximum temperatures (T_{max}), and maximum development rate occurs at the optimum temperature (T_{opt}).

	Arugula 'Astro'				Kale 'Red Russian'			
Parameter	T_b	T_{opt}	T_{max}	R^{2z}	T_b	T_{opt}	T_{max}	R^2
Leaf unfolding rate	6.1	29.2	–[y]	0.58	4.2	22.7	–	0.89
Fresh mass gain	6.6	24.7	39.6	0.86	7.0	22.9	27.3	0.86
Dry mass gain	7.5	21.0	40.9	0.70	7.5	21.8	36.9	0.79
	Lettuce 'Rex'				Lettuce 'Rouxaï RZ'			
Leaf unfolding rate	7.8	27.2	34.4	0.95	7.8	27.4	34.8	0.93
Fresh mass gain	8.4	24.7	34.1	0.86	8.5	26.2	33.6	0.84
Dry mass gain	7.7	27.0	33.8	0.90	8.1	26.5	34.4	0.86

[z] Generated by performing linear regression analysis on the predicted versus observed data.
[y] T_{max} could not be estimated from observed data and was fixed at 40.0 °C so the nonlinear model could be solved.

Table IV-4. Regression analysis equations and R^2 for mean shoot fresh and dry mass; height; leaf length, width, and number; maximum quantum yield of dark-adapted leaves (F_v/F_m); relative chlorophyll concentration (RCC); hue angle (h°) ; chroma (C*); and L* in response to mean daily temperature (MDT; 8, 13, 18, 23, 28, and 33 °C) of arugula (*Eruca sativa*) 'Astro', kale (*Brassica oleracea*) 'Red Russian', green butterhead lettuce (*Lactuca sativa*) 'Rex', and red oakleaf lettuce 'Rouxaï RZ'. All models are in the form of: $f = y0 + a \cdot MDT + b \cdot MDT^2$.

	Arugula 'Astro'				Kale 'Red Russian'			
Parameter	y0	(a)MDT	(b) MDT^2	R^2	y0	(a) MDT	(b) MDT^2	R^2
Fresh mass (g)	-6.35E1	1.09E1	-2.25E-1	0.86	-7.56E1	1.26E1	-2.77E-1	0.86
	5.21	5.64E-1	1.40E-2		5.37	5.89E-1	1.40E-2	
Dry mass (g)	-1.11E1	1.85	-4.20E-2	0.69	-1.08E1	1.75	-4.00E-2	0.79
	1.21	1.31E-1	3.00E-3		8.98E-1	9.90E-2	2.00E-3	
Height (cm)	-8.80	2.57	-5.50E-2	0.71	-1.61E1	3.28	-6.80E-2	0.82
	1.76	1.90E-1	5.00E-3		1.78	1.94E-1	5.00E-3	
Leaf length (cm)	5.04	1.66	-4.00E-2	0.48	-8.62	3.23	-6.80E-2	0.91
	1.66	1.80E-1	4.00E-3		1.16	1.27E-1	3.00E-3	
Leaf width (cm)	2.32	5.91E-1	-1.50E-2	0.27	-1.51	1.42	-3.20E-2	0.82
	9.71E-1	1.06E-1	3.00E-3		7.11E-1	7.70E-2	2.00E-3	
F_v/F_m	6.74E-1	1.50E-2	-3.40E-4	0.74	7.38E-1	9.00E-3	-2.11E-4	0.65
	9.00E-3	1.00E-3	2.41E-5		7.00E-3	7.25E-4	1.75E-5	
RCC	1.75E1	4.14	-1.01E-1	0.54	5.13E1	5.96E-1	-2.40E-2	0.44
	3.63	3.93E-1	9.00E-3		2.65	2.87E-1	7.00E-3	
H°	1.10E2	1.34	-3.60E-2	0.40	1.01E2	4.12	-1.07E-1	0.39
	1.88	2.06E-1	5.00E-3		5.78	6.33E-1	1.50E-2	
C*	2.86E1	-1.79	4.50E-2	0.52	1.30E1	-1.16	3.70E-2	0.76
	1.71	1.86E-1	4.00E-3		1.37	1.50E-1	4.00E-3	
L*	4.16E1	-9.66E-1	2.50E-2	0.36	2.66E1	7.91E-1	-1.00E-2	0.74
	1.44	1.56E-1	4.00E-3		1.18	1.29E-1	3.00E-1	
	Lettuce 'Rex'				Lettuce 'Rouxaï RZ'			
Fresh mass (g)	-1.71E2	2.44E1	-5.31E-1	0.83	-1.46E2	2.04E1	-4.32E-1	0.79
	1.17E1	1.27	3.00E-2		1.15E1	1.25	3.00E-2	
Dry mass (g)	-5.40	7.97E-1	-1.60E-2	0.86	-5.91	8.48E-1	-1.70E-2	0.83
	4.02E-1	4.40E-2	1.00E-3		4.70E-1	5.10E-2	1.00E-3	
Leaf length	-5.80	1.59	-3.30E-2	0.93	-8.17	1.89	-4.00E-2	0.89
	4.91E-1	5.30E-2	1.00E-3		7.44E-1	8.10E-2	2.00E-3	
Leaf width (cm)	-4.45	1.32	-2.90E-2	0.84	-1.12E1	2.45	-5.40E-2	0.84
	5.73E-1	6.20E-2	1.00E-3		1.05	1.14E-1	3.00E-3	
RCC	-4.45	1.32	-2.90E-2	0.14	4.71E1	-2.36	5.00E-2	0.65
	5.73E-1	6.20E-2	1.00E-3		1.85	2.00E-1	5.00E-3	
H°	–	–	–	–	-5.97E1	1.28E1	-2.39E-1	0.79
	–	–	–		9.98	1.06	2.50E-2	
C*	–	–	–	–	-8.57	1.59	-2.20E-2	0.76
	–	–	–		2.18	2.37E-1	6.00E-3	
L*	–	–	–	–	2.04E1	5.65E-1		0.53
	–	–	–		1.21	5.40E-2		

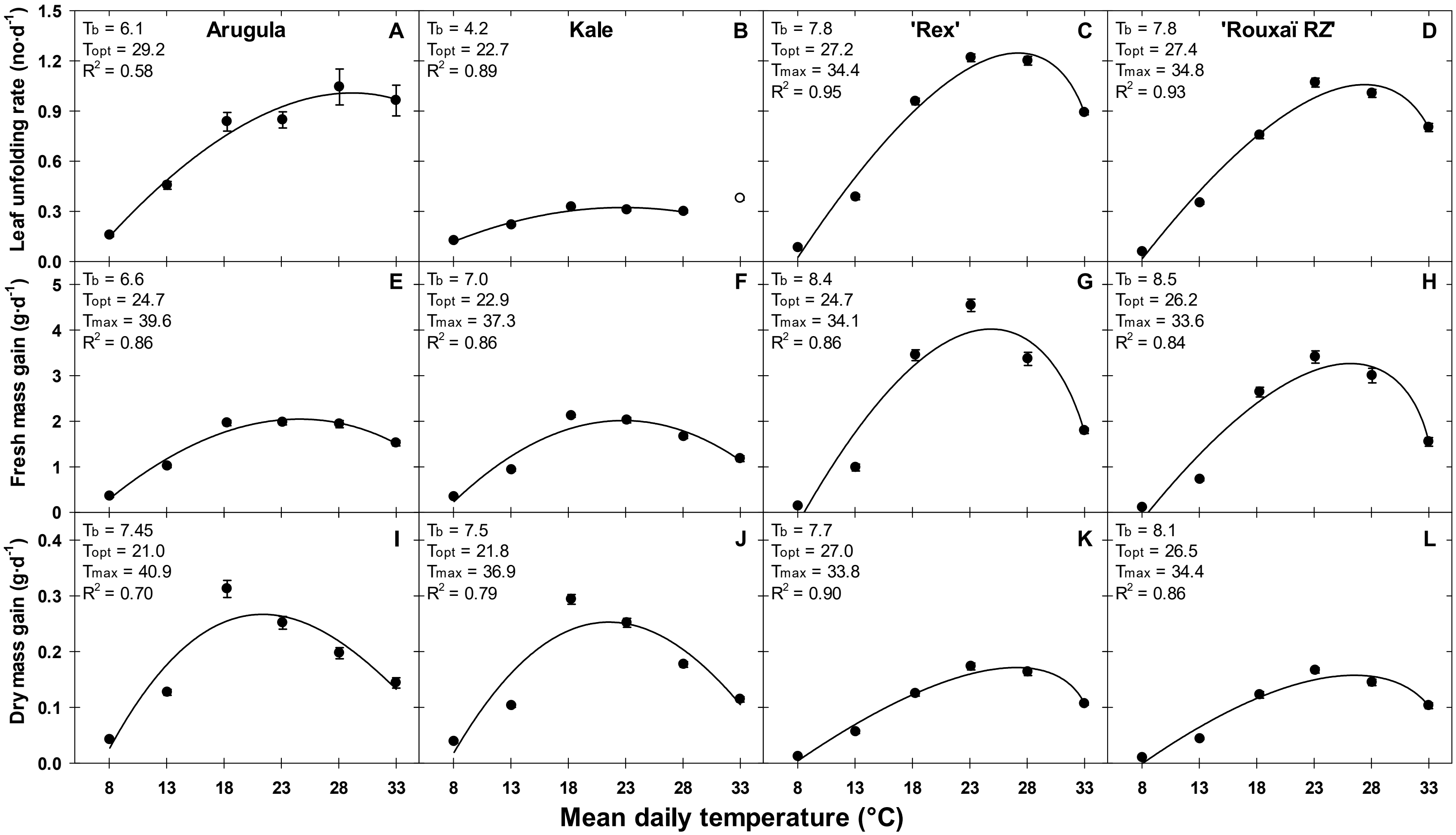

Figure IV-1. Effects of mean daily temperature (8, 13, 18, 23, 28, and 33 °C) on the rate of leaf unfolding and fresh and dry mass gain for arugula (*Eruca sativa*) ‘Astro’ (A, E, and I), kale (*Brassica oleracea*) ‘Red Russian’ (B, F, and J), green butterhead lettuce (*Lactuca sativa*) ‘Rex’ (C, G, and K), and red oakleaf lettuce ‘Rouxaï RZ’ (D, H, and L) after 34, 34, 28, and 31 d from transplant, respectively. Data points represent treatment means (± SE); model predictions are represented by lines; coefficients and parameter estimates are in Table IV-2.

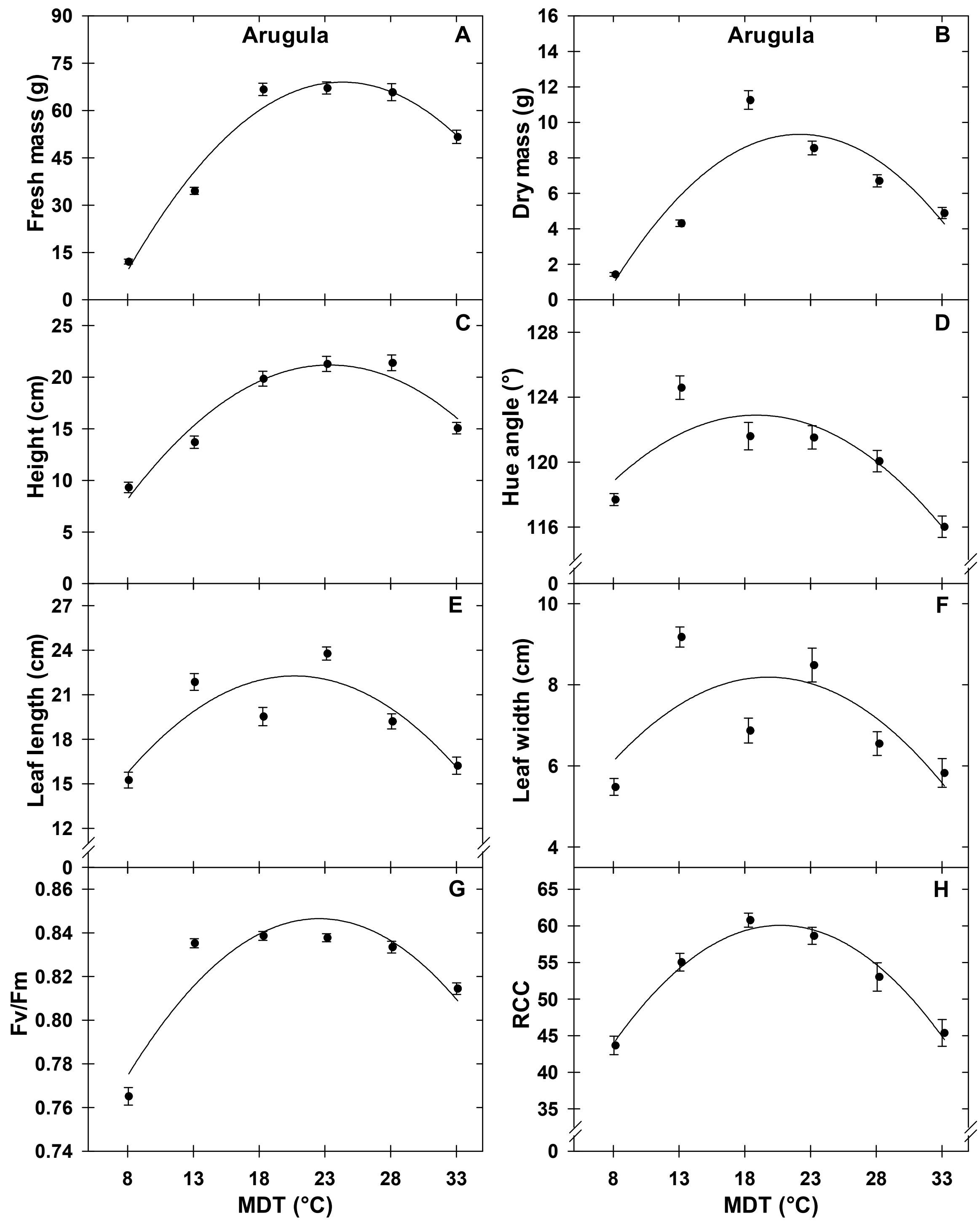

Figure IV-2. Effects of mean daily temperature (MDT; 8, 13, 18, 23, 28, and 33 °C) on kale (*Brassica oleracea*) 'Red Russian' shoot fresh (A) and dry mass (B), height (C), hue angle (D), leaf length (E), leaf width (F), maximum quantum yield of dark-adapted leaves (F_v/F_m; G), and relative chlorophyll concentration (RCC; H). Model predictions are represented by lines; coefficients are in Table IV-2.

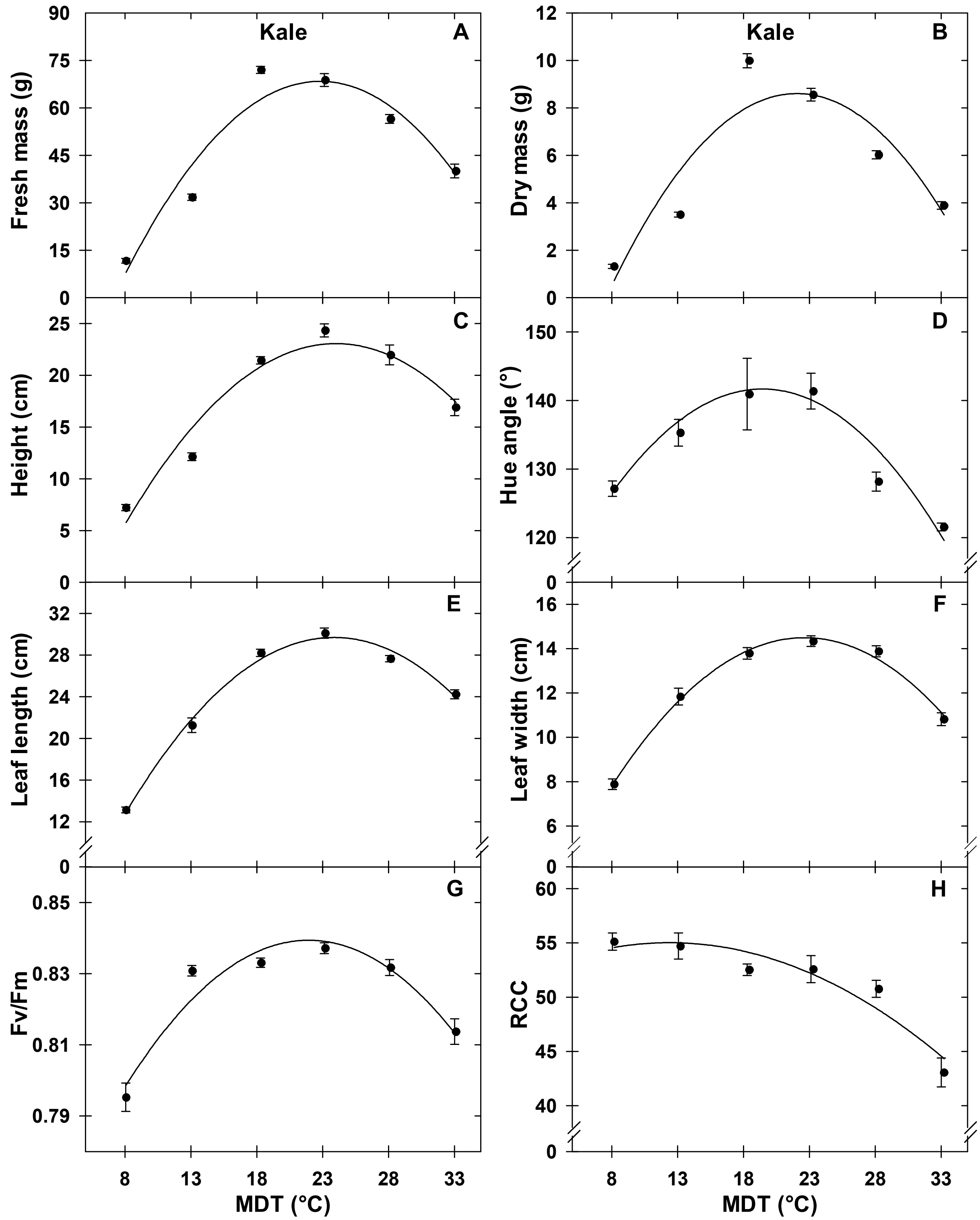

Figure IV-3. Effects of mean daily temperature (MDT; 8, 13, 18, 23, 28, and 33 °C) on arugula (Eruca sativa) 'Astro' shoot fresh (A) and dry mass (B), height (C), hue angle (D), leaf length (E), leaf width (F), maximum quantum yield of dark-adapted leaves (Fv/Fm; G), and relative chlorophyll concentration (RCC; H). Model predictions are represented by lines; coefficients are in Table IV-2.

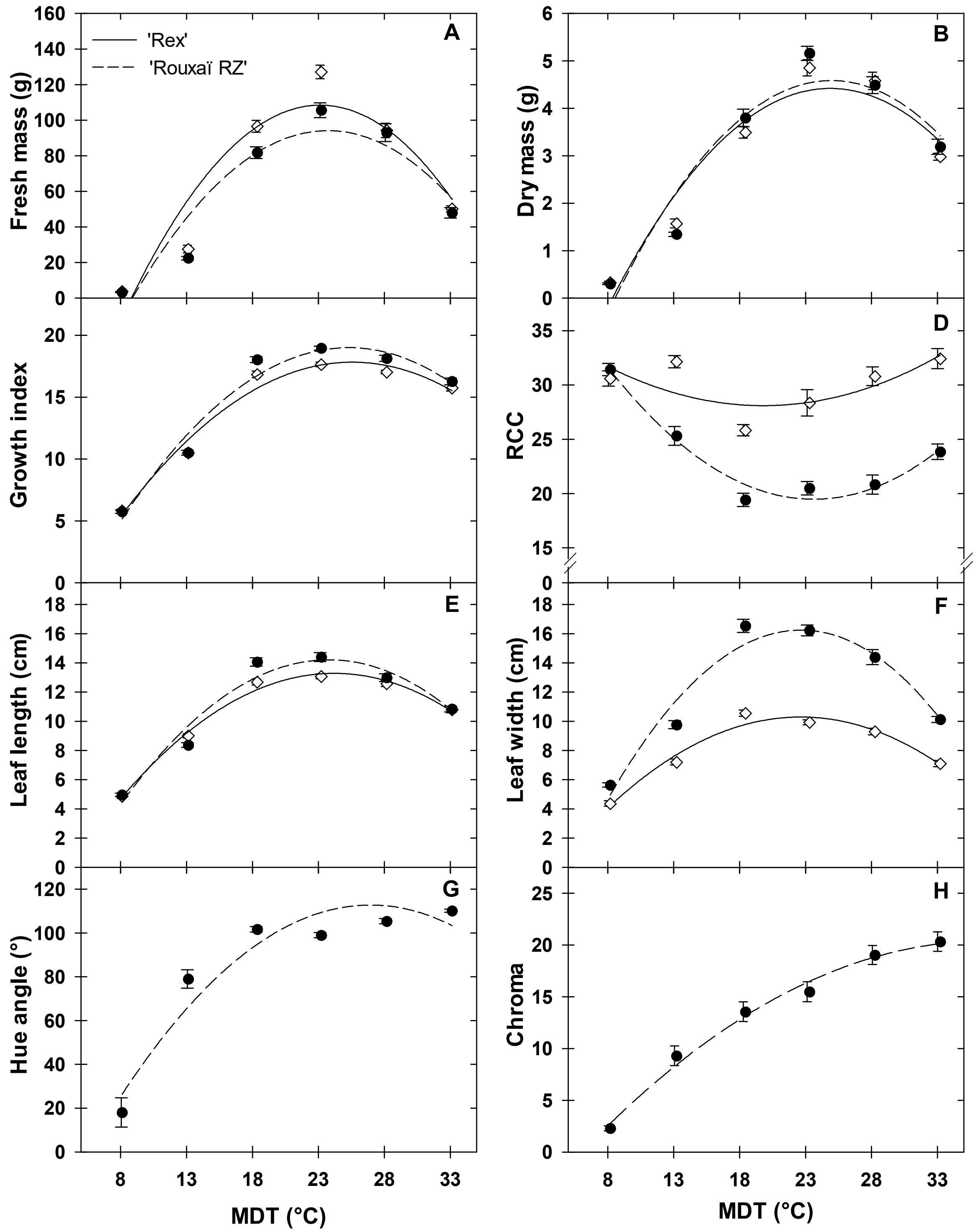

Figure IV-4. Effects of mean daily temperature (MDT; 8, 13, 18, 23, 28, and 33 °C) on red oakleaf lettuce (Lactuca sativa) 'Rouxaï RZ' and green butterhead lettuce 'Rex' shoot fresh (A) and dry mass (B), growth index (C), relative chlorophyll concentration (RCC; D), leaf length (E), leaf width (F); and 'Rouxaï RZ' hue angle (G) and chroma (H). Model predictions are represented by lines; coefficients are in Table IV-2.

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