Influencing plant growth of lettuce with light schedules in low light conditions on a vertical in-home hydroponic system

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Abstract

This research focuses on the impact of reduced light treatments on the growth and morphology of lettuce (*Lactuca sativa* 'Buttercrunch') plants grown for application in home vertical hydroponic systems. Gardyn Home Inc, producer of a smart home hydroponic system, was the research partner on this project. They requested an investigation into the feasibility of using various light schedules to 'pause' plant growth, which their clients could employ when they are away from home for extended periods. Research has used variations in the intensity and duration of light emitting diodes (LEDs) lighting to influence crops' growth in closed environments, although it has not often been applied under low light conditions with the aim of 'pausing' plant growth. A DLI of 6.5 to 9.7 $mol \cdot m^{-2} \cdot d^{-1}$ is recommended for in home lettuce production (Paz et al., 2019). Low light conditions lead to shade avoidance and etiolated growth, including leaf elongation and thinning. This research applies various lighting schemes to attempt to reduce the incidence of these responses while reducing the total biomass accumulation.

The first experiment investigated the impact of photoperiod and photosynthetic photon flux density (PPFD) under a constant daily light integral (DLI) of 4.3 $mol \cdot m^{-2} \cdot d^{-1}$. Treatments had photoperiods of 5, 7.5 and 10 hours (h) with PPFD of 238, 159 and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The hypothesis was longer photoperiod with lower PPFD would lead to less leaf elongation as the length of continuous darkness was decreased. However, results show that leaf elongation and specific leaf area (SLA) increased with photoperiod. Under the 5 h photoperiod treatment, the dry and fresh yield was larger than the 7.5 and 10 h photoperiod; however, it was still significantly lower than the control. The reduced leaf elongation in SLA indicated the 5 h photoperiod had the best quality crop. The second experiment expanded upon this by applying the results using a 5 h photoperiod with an intensity of 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to intermittent lighting regimes. Light treatments had an intermittent lighting segment and continuous darkness, totaling 24 h. Six reduced light treatments with a total of 5 h photoperiod at a PPFD of 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI = 4.3 $mol \cdot m^{-2} \cdot d^{-1}$) were tested: (1) 5 h continuous, (2) 60 min/60 min over 10 h, (3) 60 min/20 min over 6.7 h, (4) 20 min/60 min over 20 h, (5) 20 min/20 min over 10 h and, (6) 10 min/20 min over 15 h. The control trial was 16 h/8 h with a DLI of 13.75 $mol \cdot m^{-2} \cdot d^{-1}$. The treatment with the greatest reduction in growth was 10/20, although this saw a significant

increase in leaf elongation and SLA, indicating lower quality. 60/60 reduced growth while reducing elongation and SLA. The fresh and dry mass of 20/60 and 60/20 was equal to that of the control, despite the lower total incident light. The length of the continuous dark period and the length of the light interval contributed to this. Overall, the reduced light-limited growth and variation in the intensity and light intervals significantly impacted the growth and morphology of the lettuce grown in the home hydroponic system.

Résumé

Cette recherche se concentre sur l'impact des traitements à éclairage faible sur la croissance et la morphologie des plants de laitue (*Lactuca sativa* 'Buttercrunch') cultivés pour une application dans des systèmes hydroponiques verticaux domestiques. Gardyn Home Inc., est le producteur d’un système intelligent hydroponique destiné au consommateur. Ce dernier est le partenaire de recherche pour l’étendue de ce projet. Les éléments recherchés aux fins des consommateurs par Gardyn Home Inc touchent la possibilité de réalisation de multiples horaires luminaires permettant un arrêt temporaire de la croissance de la plante afin de permettre au consommateur de quitter leur domicile pour des périodes étendues.

La recherche fait l’utilisation de différentes intensités lumineuses et de durées d'éclairage diode électroluminescent (DEL) pour influencer la croissance des cultures dans des environnements contrôlés. Une intégrale de la lumière quotidienne (DLI) de 6.5 à 9.7 $mol \cdot m^{-2} \cdot d^{-1}$ est recommandée pour la production de laitues à domicile (Paz et al., 2019).

Les conditions de faible luminosité provoquent un évitement de l'ombre et une croissance étiolée, y compris l'allongement et l'amincissement des feuilles. Cette recherche applique différents programmes d'éclairage pour tenter de réduire l'incidence de ces réactions tout en réduisant l'accumulation totale de biomasse.

La première expérience a étudié l'impact de la photopériode et de l'intensité de la lumière sous un intégrale de la lumière quotidienne (DLI) constant de 4,3 $mol \cdot m^{-2} \cdot d^{-1}$. Les traitements avaient des photopériodes de 5, 7,5 et 10 heures avec une intensité de 238, 159 et 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectivement. L'hypothèse était qu'une photopériode plus longue avec une intensité plus faible conduirait à une moindre élongation des feuilles puisque la durée de la période d'obscurité était réduite.

Les résultats montrent que l'allongement des feuilles et la surface spécifique des feuilles augmentent avec la photopériode. Sous le traitement de la photopériode de 5 heures, le rendement sec et frais était plus important que sous les photopériodes de 7,5 et 10 heures.

Cependant, il était toujours significativement plus bas que le contrôle. La réduction de l'allongement des feuilles et de la surface foliaire spécifique indique que la photopériode de 5 heures a donné la meilleure qualité de récolte.

La deuxième étude a approfondi ce constat en appliquant les résultats obtenus avec une photopériode de 5 heures et une intensité de 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$ à des traitements d'éclairage intermittents. Les traitements lumineux comportaient un segment d'éclairage intermittent et une obscurité continue, totalisant 24 heures. Six traitements lumineux réduits avec une photopériode totale de 5 heures à une intensité de 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI = 4. 3 $mol \cdot m^{-2} \cdot d^{-1}$) ont été testés : (1) 5 heures en continu, (2) 60 min/60 min sur 10 h, (3) 60 min/20 min sur 6,7 h, (4) 20 min/60 min sur 20 h, (5) 20 min/20 min sur 10 h et, (6) 10 min/20 min sur 15 h. L'essai témoin était de 16 h/8 h avec un DLI de 13,75 $mol \cdot m^{-2} \cdot d^{-1}$. Le traitement avec la plus grande réduction de la croissance était 10/20, bien qu'il y ait eu une augmentation significative de l'élongation des feuilles et de la surface foliaire spécifique, indiquant une qualité inférieure. 60/60 a réduit la croissance tout en réduisant l'élongation et la surface spécifique des feuilles. La masse fraîche et sèche de 20/60 et 60/20 était égale à celle du contrôle, malgré une lumière incidente totale plus faible. La durée de la période d'obscurité continue et la longueur de l'intervalle de lumière y contribuent. En résumé, la réduction de la croissance limitée par la lumière et la variation de l'intensité et des intervalles de lumière ont eu un impact significatif sur la croissance et la morphologie de la laitue cultivée dans le système hydroponique de la maison.

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Contribution of authors

For this thesis, the contribution of the authors is as follows: (1) Rachael Warner – planned and conducted experiments, data collection, analysis, and presentation of results; (2) Dr. Mark Lefsrud — supervised experiment design, provided guidance and knowledge, and reviewed thesis; (3) Dr. Bo-Sen Wu — provided guidance and reviewed thesis; (4) Dr. Sarah MacPherson — reviewed thesis.

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Abbreviations

CCI	Chlorophyll content index
CEA	Controlled environment agriculture
d	day
DAS	Days after seeding
DIF	Temperature differential
DLI	Daily light integral
ETR	Electron transport rate
h	Hour(s)
LCP	Light compensation point
LED	Light emitting diode
LHC	Light harvesting complex
LMA	Leaf mass area
LSP	Light saturation point
min	Minute(s)
nm	Nanometers
NPQ	Non-photochemical quenching
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PMF	Proton motor force
PPFD	Photosynthetic photon flux density
PS	Photosystem
RH	Relative humidity
Rubisco	Ribulos-1,5-bisphosphate carboxylase/oxygenase
RuBP	Ribose 1,5-bisphosphate
SD	Standard deviation
SLA	Specific leaf area
VPD	Vapour pressure deficit
µmol	Micro mole

Chapter 1 General introduction

1.1 Thesis motivation

The genesis of this project comes from Gardyn Home Inc., developer of a vertical in-home hydroponic system. They requested a light schedule to provide their clients with a 'vacation mode' option. They asked to 'pause' plant growth as much as possible while maintaining good quality crops. This was done through the manipulation of the light schedule and intensity.

1.2 Research problem

Most current LED studies for closed plant production focus on optimizing the growth of crops while minimizing the inputs to increase overall efficiency. This research aims to 'pause' growth as much as possible while reducing degradation in quality by leaf elongation and thinning. This is done by manipulating the schedule and intensity of the full spectrum white LED lights on the Gardyn Home system.

1.3 Objective/Goal

- Determine if, under constant low DLI, the optimal delivery of light is under long photoperiod with low intensity or short photoperiod with high intensity through grow trials.

- Design intermittent lighting schedules with the same total incident light at the same PPFD.

- Test impact of intermittent lighting schedules on growth and morphology of lettuce.

1.4 Hypotheses

- Plant growth will be reduced under the reduced light treatment and experience some etiolation.

- When varying the intensity and photoperiod under low intensity, longer photoperiod with lower intensity will reduce etiolation as the dark period will be reduced.

- Under intermittent lighting, a medium-length light period and reduced continuous light period will reduce growth and etiolation.

Chapter 2 Literature review

2.1 Overview of controlled environment agriculture

Controlled environment agriculture (CEA) refers to plant production in a structure that partially or entirely isolates production from the outdoors, including greenhouses, closed vertical farms and home production. Greenhouses are currently the most abundant form of CEA in Canada; over 650,000 metric tonnes of fresh produce worth over 1.8 billion CAD were produced in 2020 (Statistics Canada, 2022). The top three crops produced in greenhouses are tomatoes, cucumbers, and peppers, which accounted for 96.1 % of the market share by yield in 2020 (Statistics Canada, 2022). Greenhouses allow for environmental controls of heating, cooling and humidity control, supplemental lighting, or shading installation, in addition to better integrated pest management and overall environmental protection (Ahmed et al., 2020; McCartney et al., 2018). The current wave of innovation in the CEA industry is closed plant production.

Closed plant production is not a new concept, but it has progressed rapidly into large-scale production facilities in recent years. Because of the industry's novelty, there is no current consensus on terminology. Vertical farms (VF), plant factories, plant factories with artificial lighting (PFAL), controlled or closed environment plant production (CEPP), controlled environments with artificial lighting (CEAL), or controlled environments with electrical lighting (CEEL) are all synonymous (Kozai, 2022). Regardless of terminology, these systems are closed from the exterior with controlled input and output, relying solely on electrical lighting, mainly light-emitting diodes (LEDs). Grow systems are exclusively hydroponic and employ the vertical dimension by stacking racks or vertical walls. This utilization of vertical space drastically increases the yield per area (Eaves & Eaves, 2018). This high density is key to the feasibility and profitability of vertical farms, as electrical inputs are higher due to complete reliance on electrical lighting when compared to greenhouses (Graamans et al., 2018). Lighting systems in controlled environments have been evolving, and improvements to LED technology are making closed plant production more feasible. Traditional lighting in greenhouses includes high-intensity discharge (HID) (high-pressure sodium (HPS) or metal halide (MH)) lamps and fluorescent fixtures (Wu et al., 2020). Compared to HPS, LEDs require a higher investment cost at purchase but require less energy input and costs and have a much longer lifespan (Kusuma et al., 2020; Wu et al., 2020). The narrow wavelength spectrum allows for high controllability of spectrum and increases overall efficiency. Ample research into the LED impact on photosynthesis and growth is ongoing, and with it will come further ability to control growth rate, morphology, and any beneficial secondary metabolites (Zhen et al., 2022). Typically, in home systems full spectrum white light is used rather than red and blue to be more visually pleasing for the user in the home.

The increase in home food production was accelerated by the COVID-19 pandemic's impact on supply chains and thus, food availability (Mullins et al., 2021). This trend includes the increase in home hydroponic growing systems, displayed by the prevalence in non-specialty stores (Canadian Tire 2022; The Home Depot 2022). Ranging from countertop systems to large vertical growing, they all encompass the use of LEDs and hydroponics. White LEDs are preferred for in-home systems, as they are more pleasant and add ambiance, whereas red and blue lights are harsh for the user. The complexity of the systems varies in terms of irrigation, instrumentation, and crop selection. Many systems rely on wicking materials for irrigation, while others have pump systems installed. The level of instrumentation lends to the usability and the complexity of these systems. Some systems simply run LEDs on a timer, while others can be controlled through applications on mobile devices. The implementation of machine vision and artificial intelligence is being applied by some through the installation of cameras on devices. The size and complexity determine what crops can grow in a system. Most promote the production of leafy and microgreens, although more encourage flowers and fruiting crops such as cherry tomatoes. Regardless of the system or environment, the fundamental plant science remains the same and can be applied to ensure high quality and efficient crop growth.

2.2 Photosynthesis

Plants and photoautotrophic organisms use the process of photosynthesis to transform sunlight into chemical energy in a series of reduction-oxidation (REDOX) chemical processes.

The process transforms water and carbon dioxide into glucose and oxygen (Whittingham, 1952). The wavelengths of light utilized in the process are photosynthetically active radiation (PAR), defined as 400–700 nm (McCree, 1971).

$$6\ CO_2 + 6\ H_20 \rightarrow C_6H_{12}O_6 + 6\ O_2$$

Plant photosynthesis takes place within the chloroplast, located in mesophyll cells. Within the chloroplast, the thylakoid membrane separates the stroma (outside the membrane) and the lumen (inside the membrane) (Reviewed by Taiz et al. (2018)). Five protein complexes are embedded in the thylakoid membrane and transport electrons into the lumen to accumulate the proton motor force (PMF). The PMF powers the synthesis of high-energy molecules NADPH and ATP from low-energy products of the Calvin cycle (Mitchell, 1972).

Photosystem I (PSI) and photosystem II (PSII) are two of the integral membrane protein complexes that capture energy through redox reactions (Duysens et al., 1961; Witt et al., 1961). These photosystems are comprised of reaction centers and light-harvesting complexes. Light-harvesting complexes one and two (LHCI and LHCII), associated with the PS for which they are named, are an assembly of proteins, including chlorophyll a, b and carotenoid pigments (Thornber et al., 1967). Chlorophylls are the dominant pigment in photosynthetic material, absorbing maximally in the red and blue ranges (600-700 nm and 400-500 nm). The primary pigment in light-harvesting is chlorophyll a, referred to as P-680 in PSII and P-700 in PSI as those are the maximum absorption peaks (reviewed by Taiz et al. (2018)).

2.3 Factors impacting plant growth

2.3.1 Environmental factors: Temperature and relative humidity

The influence of environmental factors, including temperature and relative humidity (RH), is well established (Reviewed by Went (1953) and Kubota (2016)). Temperature increases lead to an increase in growth until a maximum ideal point is reached, after which growth is limited (Kubota, 2016). The day and night temperature, as well as the temperature differential (DIF), which is the difference between them, influences growth, but the average day temperature is the main driving factor (Slack & Hand, 1983). Zhou et al. (2022) compared lettuce (*Lactuca* *sativa*) growth at 15, 23, and 30 °C with intensities varying from 100–600 μmol·m^{-2}·s^{-1}; the highest yield overall was at 23 °C with 500 μmol·m^{-2}·s^{-1}, while it was 600 μmol·m^{-2}·s^{-1} for 30°C. The total yield was lower for all light treatments at 30°C compared to 23°C. There is an interaction effect between the light intensity and temperature. Optimal temperatures for lettuce are 22–26 °C during the light period and 15–20 °C during the dark (Ahmed et al., 2020). The relative humidity is the amount of water in the air divided by the amount of water in the air at saturation at the same temperature (Hernández, 2022). As the temperature influences RH, it is hard to compare trials directly. Vapour pressure deficit (VPD) represents the actual environment the plant senses and is a measure that accounts for both RH and temperature (Amitrano et al., 2019). VPD is the difference between saturation vapour pressure (found inside the leaf) and the ambient vapour pressure in the air (Hernández, 2022). VPD drives transpiration through the gradient formed between the vapour pressure in and outside of the leaf, although stomata add controls to this rate (Amitrano et al., 2019; Wheeler & Stroock, 2008). As VPD increases, more moisture is pulled from the leaves, so stomata close for protection under high VPD to preserve moisture in the plant (Niu et al., 2020; Sulman et al., 2016). VPD is especially important in managing calcium deficiency and the resulting incidents of tip burn in leafy plants (Kubota, 2016). The ideal VPD in closed environments is 0.8–0.95 kPa (Niu et al., 2020).

2.3.2 Light overview

Light drives photosynthesis, and adequate levels are vital for producing high yields and good quality crops. Photosynthetically active radiations (PAR) are the region of the electromagnetic spectrum contributing to plant growth and range from 400–700 nm (Wu et al., 2019). Daily light integral (DLI) is the total amount of PAR delivered to an area over a day, expressed as mol·m^{-2}·d^{-1}. Light intensity, known as photosynthetic photon light intensity (PPFD) and the length of the light period, the photoperiod, contribute to the total DLI. In closed plant production, photoperiods are generally between 12 and 18 h, and PPFD ranges from 200–600 μmol·m^{-2}·s^{-1} (Ahmed et al., 2020). The ideal DLI for leafy greens is 8–14 mol·m^{-2}·d^{-1}, delivered at a PPFD of 150–250 μmol·m^{-2}·s^{-1} across a 16 h photoperiod (Albright et al., 2000; Dou & Niu, 2020).

The photosynthetic light response curve demonstrates the relationship between light intensity and net photosynthetic rate. When light levels are zero or very low, the net carbon assimilation rate is zero, as dark respiration is happening. The light compensation point is the light intensity where respiration and photosynthesis are equal. The PPFD that produced the maximum photosynthetic rate is the light saturation point. This is influenced by the total amount of photosynthetic loci in the plant tissue, and so will shift as a plant grows. At this intensity, light is no longer limiting the rate of photosynthesis, but the rate of the carbon cycle is (Wareing et al., 1968). At intensities much greater than the light saturation point, photoinhibition occurs, causing damage to the photosynthetic apparatus and a reduction in plant growth. Under excessive light, the rate of energy transfer to PSII is faster than the utilization rate, so excess energy must be dissipated through heat via non-photochemical quenching (NPQ) (Taiz et al., 2018). Without dissipating excess energy, photo-oxidative stress accumulates in the thylakoid membrane leading to photoinhibition of PSII and ultimately inhibiting photosynthesis (Ruban et al., 2012; Shafiq et al., 2021). Plants adapt to adverse light conditions (Kami et al., 2010). The light saturation point is higher in younger plants as light intensity increases (Fu et al., 2012). Light compensation point was lowest at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when compared to treatments up to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, although the highest was at 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Zhou et al., 2022).

As DLI increases, so does yield regardless of the photoperiod and PPFD (Ahmed et al., 2020; Gao et al., 2020; Lefsrud et al., 2006). Increasing photoperiod while maintaining a DLI of 400 $mol \cdot m^{-2} \cdot d^{-1}$ affected a linear increase for lettuce yield, leaf number and area between 12 and 18 h, while a 24 h photoperiod had unchanged dry yield but reduced leaf number and area (Silva et al., 2022). Low light induces various adaptations to optimize the use of available light, similar to a shade response (Shafiq et al., 2021). This happens under an increase in the red:far-red (R:Fr) ratio, inducing a shade avoidance response (Bailey et al., 2001; Cheng et al., 2021). At the photosynthetic level, the number of PSI increases while PSII decreases (Bailey et al., 2001). Inversely, there is an increase in LCHII and a decrease in LCHI. It is hypothesized that the decrease in PSII is to conserve resources while an increase in PSI supports the production of ATP and NADPH through cyclic electron flow (Bailey et al., 2001; Walters, 2004). These low light or shaded environments produce thinner leaves with high specific leaf area (SLA), the ratio of area to dry mass of leaves (Evans & Poorter, 2001). Low light produces larger leaves to increase the photosynthetic area in lettuce (Galieni et al., 2016). Other crops respond by elongating stems to find light (Wu et al., 2017).

2.4 Light tuning in closed environment plant production

2.4.1 Photoperiodic and intensity effects under constant daily light integral

Total DLI is a top priority for production in closed environments, but both the variable photoperiod and PPFD determine this. Ample research into optimizing all aspects of lighting in closed plant production, including investigating whether, under constant DLI, higher intensity and shorter photoperiods are advantageous over lower intensity longer photoperiods. Research shows that constant DLI comprised of various photoperiod and PPFD lead to changes in yield, leaf and plant morphology, and photosynthetic efficiency (Elkins & van Iersel, 2020a; Milford & Lenton, 1976; Weaver & van Iersel, 2020). Examining the potential to use lower intensity reduces the number of light fixtures required in a vertical farm establishment, reducing capital costs.

Plants' sessile nature causes them to react to the light environment around them to maximize light capture and efficiency with changes in growth, morphology, and photosynthetic capabilities. Rudbeckia (*Rudbeckia fulgida* ‘Goldstrum’) seedlings' leaf area increased with a 15 and 18 h photoperiod but decreased under 12 and 21 h photoperiods with a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ (Elkins & van Iersel, 2020a). Cucumber (*Cucumis sativus*) seedlings' leaf area and stem diameter were maximized with a 16 h photoperiod (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) compared to photoperiods from 7 to 22 h with a DLI of 11.5 $mol \cdot m^{-2} \cdot d^{-1}$ (Yan et al., 2021). Lettuce leaf area increased under an extended photoperiod and lower intensity with a DLI of 17 $mol \cdot m^{-2} \cdot d^{-1}$ (Weaver & van Iersel, 2020). However, no difference in total or individual leaf area was found in lettuce or mizuna when increasing the photoperiod under a DLI of 17 $mol \cdot m^{-2} \cdot d^{-1}$ (Palmer & van Iersel, 2020). Typically, leaf area will increase under lower light conditions to increase light interceptions. Cho et al. (2020) reported that the leaf area was larger under 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD compared to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for photoperiods ranging from 12–24 hours. Although, as the photoperiod increased, the difference in area between the intensities increased, indicating that the total light or photoperiod has an interaction effect with leaf area and intensity.

When testing the impact of extended photoperiod with lower light intensity, morphology and yields are generally greater. Under constant DLI, longer photoperiods/lower intensity increased fresh and shoot dry mass in lettuce (Palmer & van Iersel, 2020; Zhang et al., 2018), mizuna (*Brassica rapa* ‘japonica’) (Palmer & van Iersel, 2020), nasturtium (*Tropaeolum majus*) (Xu et al., 2021), Rudbeckia seedlings (Elkins & van Iersel, 2020a), strawberry (*Fragaria x ananassa*) (Tsuruyama & Shibuya, 2018) and sugar beets (*Beta vulgaris*) (Milford & Lenton, 1976). There was a decrease of SLA in rudbeckia (Elkins & van Iersel, 2020a) and cucumber seedlings (Yan et al., 2021) as photoperiod increased, and PPFD decreased. However, increased light intensity typically leads to a lower SLA, indicating a thicker leaf (Evans & Poorter, 2001). Lettuce grown at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for photoperiods varying from 12–24 h had decreasing SLA as the photoperiods increased (Silva et al., 2022). Gao et al. (2020) reported that spinach grown under a 16 h photoperiod and increasing intensity showed a decrease in SLA as intensity and DLI increased. These results indicate that total DLI has a more substantial influence on SLA than how it is delivered.

Constant photoperiod with increasing PPFD increases yield and chlorophyll content while decreasing SLA. In contrast, under constant DLI, these trends are opposite, and a decrease in PPFD causes increases in yield and chlorophyll content and decreases in SLA. This indicates that under constant DLI, an extended photoperiod strongly influences growth. Chlorophyll production is light-regulated, so the extended photoperiod allows for more chlorophyll production, even as PPFD decreases (Palmer & van Iersel, 2020). Higher chlorophyll content leads to an increase in photosynthetic rate and overall growth (Emerson, 1929). Research by Kelly et al. (2020) reported the impact of increasing the DLI from 6.9 to 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ by increasing the PPFD or lengthening the photoperiod; lettuce dry mass was significantly higher under the extended photoperiod but not higher light intensity. SLA measures the leaf area available for light capture per unit of dry mass (Yan et al., 2021). A decrease in SLA under high light intensity and increasing DLI is partially attributed to an increase in the number and/or size of palisade cells (Evans & Poorter, 2001), while under constant DLI, the decrease in SLA with longer photoperiods/lower intensity may increase in chlorophyll content (Yan et al., 2021). A summary of the results is presented in Table 2-1.

Table 2-1 | Overview of grow trials with the same daily light integral (DLI) and variable photosynthetic photon flux density (PPFD) and photoperiod (↑: increase, Δ: same as control, ↓: decrease)

Photoperiod (h) & PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$), respectively	**DLI ($mol \cdot m^{-2} \cdot d^{-1}$)**	**Crop cultivar**	**Light spectrum**	**Response to longer photoperiod – lower intensity**	**Reference**
10, 12, 14, 16, 18, 20 h 444, 370, 318, 278, 247, 222 $\mu mol \cdot m^{-2} \cdot s^{-1}$	16	Lettuce (*Lactuca sativa* 'Little gem' Mizuna (*Brassica rapa* 'japonica')	Full-spectrum white	Light interception ↑ Chlorophyll content index ↑ Quantum yield of PSII ↑ Aerial biomass (dry) ↑ Instantaneous CO2 assimilation ↓ total leaf area Δ area per leaf Δ	Palmer and van Iersel (2020)
12, 15, 18, 21 h 278, 222, 185, 159 $\mu mol \cdot m^{-2} \cdot s^{-1}$	12	Rudbeckia fulgida 'Goldsrum' seedlings	Sunlight and supplemental cool white LED	Shoot dry mass ↑ Root try mass ↑ Leaf area ↑ Chlorophyll content index ↑ Specific leaf area ↓	Elkins and van Iersel (2020a)
8, 12, 16, 24 h 350, 230, 175, 115 $\mu mol \cdot m^{-2} \cdot s^{-1}$	10	Strawberry (*fragaria x ananassa*) 'Elan' and 'Yotsuboshi'	Sunlight or LED	Dry weight ↑ Leaf area ↑ Time to flower bud initiation (cv. Elan only) ↓	Tsuruyama and Shibuya (2018)
12, 16 h 200, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$	8.64	Lettuce (*Lactuca sativa* cv Ziwei)	Fluorescent and LED (R:B 1.2, 1.8, 2.2)	Root fresh weight ↑ Root dry weight ↑ Soluble sugar content Δ	Zhang et al. (2018)
7, 10, 13, 16, 19, 21 h 457, 320, 246, 200, 168, 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$	11.5	Cucumber (*Cucumis sativus* 'Tianjiao No 5') seedlings	Full-spectrum white	Pigment content ↑ Fresh and dry weight ↑ Cellulose content ↑ Plant height ↓ Hypocotyl length ↓ Specific leaf area ↓ Sucrose content ↑, peaked at 16 h	Yan et al. (2021)
16, 20, 24 h 144, 180, 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$	10.4	Lettuce (*Lactuca sativa* 'Rex' and 'Rouxai')	Warm white and red LEDs	Leaf length (cv. Rex only) ↓ SPAD (cv. Rouxai only) ↑	Kelly et al. (2020)
7, 10, 13, 16, 19, 21 h 595, 416, 230, 260, 219, 189 μmol·m-2·s-1 (DLI 15 $mol \cdot m^{2-} \cdot d^{-1}$) 794, 555, 427, 347, 292, 252 $\mu mol \cdot m^{-2}$·s-1 (DLI 20 $mol \cdot m^{2-} \cdot d^{-1}$)	15 20	Lettuce (*Lactuca sativa* 'Green towers')	Cool white LED	Quantum yield of PSII ↑ Daily photochemical integral (DPI) ↑	Elkins and van Iersel (2020b)

2.4.2 Cyclic lighting regimes

A novel factor of closed plant production is the unlinking from the solar circadian cycle, allowing growers to utilize various lighting regimes. Cyclic lighting regimes use more than one light-dark cycle over 24 h, with light and dark periods on the hours' scale. A light-dark cycle refers to one alternation of light and dark, which is naturally 24 h. Generally, experiments examining different light/dark cycle trials have the same amount of light delivered over 24 h, usually with the same total hours of light spread differently throughout the day. These light treatments provide the potential to utilize off-peak electricity rates while maintaining high-quality crops.

Overall, the highest yields and quality are reached by maintaining one long cycle. Lettuce yield was highest for 1 cycle (16/8) compared to 2 (9/3) and 3 (6/2) cycles (Kang et al., 2013), for 1 cycle of 8/16 compared to 4/8 and 2.67/5.33 (Ishii et al., 1995) and light/dark cycle of 12/12 compared to 6/6 and 3/3. Decreasing the light and dark period length from 6/6 to 3/3 (Hang et al., 2019) and 4/4 (Zhou et al., 2020) reduced biomass accumulation, leaf area, and maximum photosynthetic rate. SPAD was higher in 12/12 compared to 6/6 and 3/3 (Zhou et al., 2020); however, no change in SPAD was reported by (Hang et al., 2019) (6/6/ vs 4/4) and (Kang et al., 2013) (18/6, 9/3, 6/2). In contrast, lettuce yield increased under 4, 6 and 8 light-dark cycles with light periods ranging from 2–4 h and dark periods of 0.5–2 h, and total chlorophyll content was higher in 2, 6 and 8 light-dark cycles compared to one cycle of 16/8, which is inconsistent with other lettuce studies (Chen & Yang, 2018). It is possible that discrepancies between studies reflect data collected with different plant species. Chi (2003) reported the impact of 12/12 and 6/6 on tomato (*Lycopersicon esculentum* 'Momotarou') and hot pepper (*Capsicum annuum* 'Nockkwang'), observing a decrease in dry yield, plant height and leaf area in tomato under the reduced photoperiod, but no impact in peppers. Both tomato and pepper experienced a reduction in chlorophyll content under the 6 h cycle. García-Caparrós et al. (2020) reported no impact on tomato and cucumber seedling growth, while cucumber yield decreased as the number of cycles increased from 18/6 to 9/3 and 6/2. Both also experienced a decrease in chlorophyll concentration.

Once illuminated, the rate of photosynthesis of dark-adapted leaves has a lag period, referred to as photosynthetic induction, which is rate limited due to the initiation of enzyme activity in the carbon cycle and stomatal opening (Hang et al., 2019; Kimura et al., 2020). Approximately 10 min is needed to reach 80 % of the maximum photosynthetic rate (Hang et al., 2019). Stomatal opening limits the increase in rate from 80–100% (Kimura et al., 2020; Pearcy, 1990). The stomatal aperture further limits stomatal conductance; when examining the photosynthetic induction rate of wild-type and *flacca* mutant (stomata always open) tomatoes, wild-type stomatal conductance and thus photosynthetic limitation was significantly decreased when compared to the mutant (Kaiser, Morales, et al., 2020). Stomatal conductance in lettuce was lower in a 4/4 light-dark cycle compared to 6/6, indicating a slowing of photosynthetic induction upon illumination for shorter light cycles (Hang et al., 2019). Increasing the number of light cycles increases the total photosynthetic lag time across the total light period over 24 h, reducing the net photosynthesis over a grow cycle. The light response curve of three light-dark cycles (C12-12/12, C6-6/6 and C3-3/3) were the same net photosynthetic rate from 0–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, above which the net photosynthetic rate of C3 became significantly reduced compared to C6 and C12 (Zhou et al., 2020). The net photosynthetic rate, light saturation point (LSP) and light compensation point (LCP) were reduced in C3 and C6 compared to C12. This indicates that shorter light cycles have lower photosynthetic capacity overall. Zhou et al. (2020) hypothesize this may be because of the increased time with reduced photosynthesis rate due to photosynthetic induction.

Plants have a circadian clock which synchronizes physiological processes with natural fluctuations in daily light and influences gene expression, stomatal opening, and other physiological processes (Dodd et al., 2005). When grown in synch with the circadian rhythm, Arabidopsis (*Arabidopsis thaliana)* had enhanced chlorophyll levels and growth (Dodd et al., 2005). Lettuce grown under photoperiods of 22–24 h had enhanced biomass accumulation under a 22 and 23 h photoperiod compared to 23 and 24 h (Urairi et al., 2017). Under continuous lighting (24 h photoperiod), lettuce with bioluminescent markers indicated gene expression under circadian control was significantly reduced within 72 h (Higashi et al., 2014). This research suggests that it is possible to entrain lettuce's circadian rhythm to maintain high quality if grown under different circadian rhythms. Although in regards to increasing light-dark cycles, some hypothesize that intermittent treatments closest to the natural cycle will provide the best growth (Zhou et al., 2020). A hypothesis Chen et al. (2022) presents is that under the right balance of light and dark periods, dark-induced regulations improve growth. These results are summarized in Table 2-2.

Table 2-2 | Overview of plant response to cyclic lighting schedule compared to control (↑: increase, Δ: same as control, ↓: decrease)

Light/Dark cycles (h/h)	Intensity & Spectrum	Crop cultivar	Response to longer light cycles/fewer L/D cycles	Reference
12/12, 6/6, 3/3	250 μmol·m^{-2}·s^{-1} R:B (83:17)	Romaine lettuce (*Lactuca sativa*)	*Response to a longer light cycle* Electron transfer potential ↑ Chlorophyll content ↑ Leaf area ↑ Biomass ↑ Root/shoot ratio ↓ Specific leaf area ↓ Light saturated net photosynthetic rate ↑ Light saturation point ↑ Light compensation point↑	Zhou et al. (2020)
18/6, 9/3, 6/2	732 μmol·m^{-2}·s^{-1}	Tomato (*Solanum lycopersicum* 'Caniles') Cucumber (*Cucumis sativys* 'Litoral')	*To longer light cycle:* Tomato: Chlorophyll content ↓ Cucumber: Dry yield ↓ Chlorophyll content ↓	García-Caparrós et al. (2020)
8/16, 4/8, 2.67/5.33	360 μmol·m^{-2}·s^{-1} Full spectrum white	Lettuce (*Lactuca sativa*)	*To longer total cycle length/light cycle length* Number of leaves ↑ Fresh and dry weight ↑ Leaf area ↑ Photosynthetic rate ↑ Water and mineral uptake ↑	Ishii et al. (1995)
12/12, 6/6, 3/3	200 μmol·m^{-2}·s^{-1} R:B (83:17)	Lettuce (*Lactuca sativa* 'Adriana' & 'Coastal Star')	*Longer light-dark cycle:* Biomass ↑ Leaf area ↑ Leaf L:W ratio ↑ Photosynthetic rate ↑ Stomatal conductance ↑	Hang et al. (2019)
18/6, 9/3, 6/2	200-290 μmol·m^{-2}·s^{-1} LED R:B:W (8:1:1)	Lettuce (*Lactuca sativa* 'Hongyeom Jeockchukmyeon')	*Low intensity, response to longer photoperiod:* Dry yield ↑ Anthocyanin content ↑ Leaf length ↓ Photosynthetic rate ↑ *High intensity, response to longer photoperiod:* Anthocyanin content ↓ Photosynthetic rate ↑	Kang et al. (2013)
12/12, 3/3	200 μmol·m^{-2}·s^{-1} Full spectrum white	Tomato (*Solanum lycopersicum* 'Momotarou')	*To longer light cycle* Plant height ↑ Leaf area ↑ Total dry weight ↑ Leaf chlorophyll content ↑ Leaf chlorosis ↓	Chi (2003)

2.4.3 Intermittent and fluctuating lighting regimes

Intermittent lighting and fluctuating lighting include short changes in light intensity on the scale of milliseconds to about an hour. Fluctuating light has large shifts in intensity while intermittent turns on and off. Intervals at the rate of < 1 s are known as pulsed lighting and are an application to reduce the required energy for LED, but this review will not investigate this concept further. This review focuses on intervals from 60 s to 60 min. Much of the research into intermittent and fluctuating lighting has focused on the impact of sun specs, and natural variation in light intensity, on plant growth. More recently, research has begun investigating the potential application of these lighting regimes in closed plant production. Overall, intermittent and fluctuating lighting has found a decrease in the overall growth of the crops exposed when they are grown in closed environments.

Fluctuating light research originally aimed to reproduce natural variations in outdoor lighting environments and to compare research results in natural light (greenhouse) to constant light (growth chambers) environments. The impact of sizeable light intensity fluctuations of at least 60 sec on horticultural crops has shown a reduced dry yield in rice (*Oryza sativa)* (Wei et al., 2021) and basil (*Ocimum basilicum* 'Aroma 2'*)* and lettuce 'Galiano' (Bochenek & Fällström, 2016). Similar results are reported for Arabidopsis in fluctuating light with decreased yield (Vialet-Chabrand et al., 2017), and leaf area (Kaiser, Walther, et al., 2020; Vialet-Chabrand et al., 2017). Fluctuating conditions reduced rice's chlorophyll content and electron transport rate (Wei et al., 2021). In Arabidopsis, ETRII (Kaiser, Walther, et al., 2020) and light absorption (Vialet-Chabrand et al., 2017) were significantly reduced; however, the net photosynthetic rate/leaf area was not significantly changed. In a review and meta-analysis of six studies investigating photosynthetic acclimation to fluctuating light, Morales and Kaiser (2020) reported that many trends observed were similar to shade acclimated plants including increased SLA and chlorophyll a/b ratio. Bhuiyan and van Iersel (2021) examined the impact of fluctuating high light intensity every 15 min over a 16 h period on lettuce growth. Each trial received the same DLI over the same photoperiod but shifts in intensity ranged from the control (no change in intensity), 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, minimal change (240/160, 280/120, 320/80 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and extreme fluctuation between 360 and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 400 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ every 15 min for 16 h. There was a significant impact on growth, morphology, and photosynthetic capacity on the most extreme fluctuations (400/0, 360/40), although slightly lesser so on 360/40 than 400/0, indicating intermittent light impact growth differently than fluctuating.

Intermittent lighting has been shown to both increase and decrease yield and photosynthetic apparatus. These trends are likely impacted by the duration of the light interval and potentially dark interval. For lettuce grown under intermittent lighting with the same DLI, the 15 min/15 min intervals over 16 h (Bhuiyan & van Iersel, 2021) resulted in a decrease in yield, while 60 min/30 min intervals over 24 h (Chen & Yang, 2018) increased yield. This could be attributed to the time for photosynthetic induction, discussed in section 2.4.1. During the 15-minute light period, the net photosynthetic rate was slow to increase and did not reach a steady state, indicating that a 15-minute light period is too short to support quality growth. Avgoustaki et al. (2020) grew basil in a growth chamber with 4-h of continuous light followed by 4-h of intermittent lighting (10 min light/50 min dark), repeating this cycle 3 times daily (no continuous dark period provided). Compared to the standard 16/8 lighting regime, there was no significant impact on growth. The net assimilation rate during the 10 minutes light interval was lower than control but similar during the 4 h light period, although the overall average was lower for the intermittent treatment. Although the total illuminated time for intermittent lighting was 14 h, compared to 16 h, the total dry biomass was higher for intermittent. These results suggest that intermittent lighting mixed with longer cycles does not decrease the photosynthetic ability of plants. Results are summarized in Table 2-3.

Table 2-3 | Overview of impact intermittent and fluctuating lighting with light intervals of at 1 minute to 60 minutes and at least one dark period on horticultural crops and model plant Arabidopsis (↑: increase, Δ: same as control, ↓: decrease)

	Light treatment	DLI ($mol \cdot m^{-2} \cdot d^{-1}$), quality and duration	Crop	Impact of fluctuating treatment compared to control	Reference
Intermittent	· 15 min light/dark intervals over 16 h: 200/200 (control), 240/160, 280/120, 320/80, 360/40, 400/0 $\mu mol \cdot m^{-2} \cdot s^{-1}$	11.5 $mol \cdot m^{-2} \cdot d^{-1}$ 6 weeks	Lettuce 'Little Gem' & 'Green Salad'	Chlorophyll content index ↓ Leaf area ↓ Dry mass ↓ SLA ↑ Net assimilation rate ↓	Bhuiyan and van Iersel (2021)
Intermittent	· [4 h light x1, 10 min light/ 50 min dark x 4] x 3 · 16/8 control	25 (*intermittent)*, 29 *(control)* $mol \cdot m^{-2} \cdot d^{-1}$ R:B = 2:1 24 days	Genovese basil	Net photosynthetic rate ↓ Dry mass ↑ CCI Δ	Avgoustaki et al. (2020)
Intermittent	· 60 min light 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$/30 min dark over 24 h ·16/8 @ 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ control	11.5 $mol \cdot m^{-2} \cdot d^{-1}$ R:B = 9:1 60 days	Lettuce 'Green Oak Leaf'	Fresh mass ↑ Dry mass↑ Plant height ↑ Leaf number Δ Chlorophyll content Δ Soluble sugar Δ	Chen and Yang (2018)
Fluctuating	· [4 min 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 min 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$] over 16 h · [4 min 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 min 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$] over 16 h *Control* · [300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 16/8]	17 $mol \cdot m^{-2} \cdot d^{-1}$ *Spectrum not specified* 7 days	Rice 'Nipponbare'	Fresh mass ↓ Leaf mass area (LMA) ↓ Chl a & b ↓ ETR ↓ Stomatal conductance ↓	Wei et al. (2021)
Fluctuating	*Low & high intensity dynamic fluctuations (mimic daylight)* · low: 100 – 750 (mean 230) $\mu mol \cdot m^{-2} \cdot s^{-1}$, over 12 h · high: 100 – 1500, (mean 460) $\mu mol \cdot m^{-2} \cdot s^{-1}$ over 12 h *control:* · 460 $\mu mol \cdot m^{-2} \cdot s^{-1}$ constant 12 h · 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$ constant 12 h	10 (*low*), 20 (*high*) $mol \cdot m^{-2} \cdot d^{-1}$ Full spectrum white LED 27 days	Arabidopsis	SLA ↑ Light absorption ↓ Leaf area ↓ Dry mass ↓ Photosynthetic rate/leaf area Δ	Vialet-Chabrand et al. (2017)
Fluctuating	· [1 min 900, 4 min 90 (mean 252 $\mu mol \cdot m^{-2} \cdot s^{-1}$)] over 12 h · 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over 12 h	10 $mol \cdot m^{-2} \cdot d^{-1}$ Full-spectrum white LED 14 days	Arabidopsis	Projected leaf area (PLA) ↓ Operating efficiency of PSII ↓	Kaiser, Walther, et al. (2020)
Fluctuating	· Random 3-6 min ranging from 90 – 420 (avg 180) $\mu mol \cdot m^{-2} \cdot s^{-1}$ over 12 h · 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over 12 h	10.4 $mol \cdot m^{-2} \cdot d^{-1}$ 28 days	Basil 'Aroma 2' Lettuce 'Galiano'	*Lettuce* Fresh and dry mass ↓ Leaf number ↓ *Basil* Dry mass ↓	Bochenek and Fällström (2016)

2.4.4 Influence on consumer experience and taste of lettuce grown in various lighting environments

Light environment can have an impact on flavour producing compounds, including soluble sugars. Consumers reported having a higher likeness for lettuce with higher sweetness and lower bitterness in a taste panel (Chadwick et al., 2016). Specifically, they found the ratio of bitter:sweet compounds was a driving determinant in level of perceived bitterness and overall liking by consumers. Bitter compounds are sesquiterpenoid lactones while sugars are the sweet compounds, with glucose being the closest related to the overall interpretation of sweetness by panelists. (Chadwick et al., 2016).

The influence of variable photoperiod under constant DLI on soluble sugar levels in lettuce was reported by Zhang et al. (2018) and showed no significant differences under a DLI of 8.64 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ with photoperiods of 12 and 16 h. Yan et al. (2021) reported an increase in sucrose content in cucumber seedlings as photoperiod increased from 7 to 22 h under a DLI of 11.5 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, with the highest sucrose content being under a 16 h photoperiod. More significantly, Chen and Yang (2018) reported an increase in glucose for all intermittent light treatments compared to the control, while the total soluble sugars increased under treatments with 2 and 3 light/dark cycles. More extensive reporting on soluble sugar levels should be done to form a conclusion but results indicate it is possible to influence soluble sugar accumulation under different lighting regimes with constant DLI and light spectrum. Future research can be done to further improve the quality of the experience for consumers and allows for a higher sale price, which can contribute to improving the revenue of the enterprise.

2.4.5 Impact on capital and operational expenditure

Impact on capital expenditure

LED light investment is one of the largest capital expenditures incurred while establishing a vertical farm. Economic analysis indicates lighting equipment constitutes approximately 30% of all startup costs of a vertical farm (Van Iersel & Gianino, 2017; Zeidler & Schubert, 2014). If a farm requires lower light intensity for production, the number of lights required is lower leading to lower capital costs (Elkins & van Iersel, 2020a). With sufficient planning the lighting system can be designed to use lower intensity, longer photoperiod and a farm can reduce the number of lights required.

Impact on operational expenditure

Lighting constitutes up to 30% of operating costs in greenhouses (Van Iersel & Gianino, 2017). Electricity costs constitute from 40 - 60% in of operational expenses plant factories (Avgoustaki & Xydis, 2021; Zeidler & Schubert, 2014), with lighting accounting for approximately 25% of production costs (Avgoustaki et al., 2020). Optimizing lighting allows for the minimum input necessary to have a profitable yield. Variable electricity costs are offered in many regions as a response to shifts in momentary supply or total electricity used by a consumer. For example, Denmark has a system which switches hourly based on the previous days usage (Avgoustaki & Xydis, 2021). The cost of lighting for basil production in Denmark was reduced by an average of 20 % across the year by using electricity cost adaptive lighting (Avgoustaki & Xydis, 2021). This is an example of how intermittent lighting can be used to reduce the cost of electricity while using the same total light, although it is location dependent.

While in Canada tiered electrical billing is common in most provinces and Ontario uses peak hour pricing (Urban, 2021). Lower light intensity over longer photoperiods, compared to the same DLI with higher intensity and shorter photoperiod allows for fewer LED bars required and the amount of heat produced to be less. If, for example, a farm was in Ontario the lighting would require less total light during peak hours. It is possible to operate lights during off peak hours (7 pm to 7 am in Ontario (Ontario Energy Board)), although producers often want to be on site during production hours to monitor automated processes throughout their work day. Results discussed in section 2.4.1 indicate that lower light intensity over longer photoperiods produce improved crop growth compared to higher intensity and shorter photoperiod, indicating lighting costs can be used with this technique in some areas.

2.5 Conclusion

Overall, there is ample research investigating the impact of varying light delivery to crops in controlled environments. Most findings point toward maintaining a typical circadian rhythm to produce the best results if the minimum DLI requirements are met. These light procedures could allow producers to optimize available lighting and minimize cost by potentially utilizing off-peak hours and reducing the light intensity overall. Future research should focus on the impact of various light regimes on commonly produced species in closed environments. Also, identifying the optimal length of light and dark intervals in intermittent lighting could improve growth and help elucidate how these intervals impact metabolic processes.

Connecting statement to Chapter 3

Chapter 3 covers the methodology, results, and discussion of lettuce (*Lactuca sativa* 'Buttercrunch') growth and morphological response to a reduced light environment, with light delivered over 3 photoperiod and PPFD regimes with constant DLI. This chapter applies methods from similar research in the literature under a reduced total light environment to determine if the results would show the same trends.

Chapter 3 Growth and morphological response of lettuce to varying photoperiod under constant low DLI on a home hydroponic system

Abstract

Ample research in controlled environment agriculture investigates lighting regimes in closed environments to produce the highest and most efficient yields. However, some scenarios may want to minimize or control the growth of crops instead. In the present study, lettuce (*Lactuca sativa* cv 'Buttercrunch') was grown under constant daily light integral (DLI) with three combinations of photoperiod and photosynthetic photon flux density (PPFD), under a DLI of 4.3 $mol \cdot m^{-2} \cdot d^{-1,}$ treatments with photoperiods of 5, 7.5 and 10 h with PPFD of 120, 159 and 238 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Plants were grown on a 'Gardyn Home System,' which includes warm light-emitting diodes (LEDs) to provide light and was in a home environment. Longer photoperiods reduced fresh and dry yield and total and per leaf area while increasing the specific leaf area (SLA) and leaf length-to-width (L:W) ratio. These results trend opposite to most similar research performed under growth promoting DLI levels, indicating different mechanisms could be driving growth response under low light. In summary, under constant low DLI, biomass accumulation in lettuce was reduced, while more leaf elongation and thinner leaves were observed.

3.1 Introduction

The prevalence of home gardening has been steadily increasing for years, with growing interest in indoor systems. In a survey of Canadians who produce at least one type of fruit or vegetable at home, 17.4 % began growing in 2020 during the COVID-19 pandemic lockdown (Mullins et al., 2021). Home hydroponic systems called micro indoor smart hydroponics (MISH) or hydroponic gardening, are included in this trend, demonstrated by their increasing presence in various non-specialty stores (Canadian Tire 2022; The Home Depot 2022). Factors contributing to this increase include reduced materials costs, particularly lighting, increased consumer desire to produce food at home to combat volatility in supply chains, and rising food prices. An attractive potential feature for users of these home systems is to reduce growth while on vacation or when desired growth is achieved but not ready for harvest.

Plant science research in controlled environment agriculture (CEA) focuses on the quality (spectrum) and quantity (amount) of light to influence the plant growth rate and morphology. On in-home systems, neutral or warm white light emitting diodes (LEDs) are typically used to light the systems to appeal to the consumer. So, any light trials focused on home growth systems should focus on manipulating the total amount of light and how it is delivered. Daily light integral (DLI) represents the total number of photons delivered over a day, determined by the photosynthetic photon flux density (PPFD) and photoperiod. A review of optimal lighting for lettuce (*Lactuca sativa)* growth in controlled environments found the optimal reported PPFD to be 200–250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a photoperiod of 16-18 h (DLI ranging from 11.52–16.20 $mol \cdot m^{-2} \cdot d^{-1}$) (Ahmed et al., 2020). Paz et al. (2019) investigated the minimum light requirement for lettuce in-home production with consumer-grade lights. They tested DLI ranging from 6.5–9.7 $mol \cdot m^{-2} \cdot d^{-1}$ and found a minimum DLI of 6.5 $mol \cdot m^{-2} \cdot d^{-1}$ yielded red-leaf lettuce 'Red Salad Bowl' plants with sufficient biomass accumulation and aesthetic quality. Light uniformity can greatly impact the growth of plants in various sections of the hydroponic system, as different light intensities under the same photoperiod impact growth and morphology (Kelly et al., 2020). LED light distribution greatly impacted by the type and layout of lights. In general, for systems using light bars, the middle of the growing area will provide somewhat uniform light distribution which diminishes towards the edges of the growing area. This should be accounted for in the choice of crop position in the system.

Research has shown that growth under constant DLI with varying photoperiod and intensity influences biomass accumulation and allocation, photosynthetic rate, and plant morphology (Elkins & van Iersel, 2020a; Milford & Lenton, 1976; Yan et al., 2021). However, there has not been any investigation into the impact of varying photoperiod and intensity under reduced DLI. Low light, or shade responses, vary between species, but leaf elongation, taller plants, less biomass accumulation, and reduced chlorophyll levels are common responses (Fan et al., 2013; Paz et al., 2019). Varying PPFD and photoperiod at growth promoting DLI can influence these characteristics, so it could be possible to use these light parameters to influence growth under low quality and mitigate some shade responses.

This research investigates the growth and aesthetic quality of lettuce grown in an at-home hydroponic system under constant low light intensity with varying light intensity and photoperiod. Our goal was to determine if crop growth could be minimized while maintaining quality under low DLI. Total DLI remained constant while trials with varying photoperiod and PPFD combinations were applied. Quality is defined by minimal etiolated growth and biomass accumulation. It was hypothesized that under reduced DLI, longer photoperiods with lower intensity lighting will produce higher quality crops as the dark period is reduced.

3.2 Materials and methods

3.2.1 Plant culture

The experiment was conducted in a home environment (temperature 21 ± 2 °C; relative humidity 45 ± 5 %). Three Gardyn Home systems were housed in an office. Each system had two layers of 80% light-blocking shade cloth to reduce the impact of light pollution from neighbouring systems. Small fans were mounted to blow air across the base of the systems to maintain air quality. Two vertically mounted LED light bars (Gardyn Home Inc., Bethesda, MD) supplied light to each growing area. The Gardyn Home central processing unit (CPU) automatically controlled light intensity and photoperiod and was controlled from the app.

Lettuce 'Buttercrunch' was grown from seed. Seeds were germinated in 1-inch Rockwool (Grodan (ROXUL inc); Milton, ON) cubes in black 10" x 20" trays, and the trays were irrigated every 3 days and fertilized every 7 days. During germination, the lighting schedule was 16/8 (light/dark) at an intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 14 days, the seedlings were randomly transplanted to the Gardyn systems at the second true leaf stage. Each system has three towers, divided into 10 growing pods to allow up to 30 plants per system. All transplants were placed onto the middle tower, which had the highest light level uniformity. Fertilizer nutrients were dissolved in tap water with an EC of 1.5 $\mu S \cdot cm^{-1}$ - about 1.25 $g \cdot L^{-1}$ (MaxiGrow 10N-5P-14K, General Hydroponics, Santa Rosa, Ca) in the system 40 L reservoir. During the experiment, the system was irrigated for 5 minutes daily before the beginning of the light cycle. Lettuce was harvested after 14 days. Plants were weighed and measured immediately upon harvest. Samples were dried (80 °C; 32100C, Hamilton Beach, VA, USA) until changes in mass were no longer detectable day-over-day (after 2 days). Leaf area was measured using ImageJ from photos (12 MP wide-angle, Samsung S10) taken immediately after harvest.

3.2.2 Experimental design and data analysis

Plants were grown under three photoperiods (5, 7.5 and 10 h). The PPFD output of the lights in each treatment was adjusted so all treatments received the same DLI of 4.3 $mol \cdot m^{-2} \cdot d^{-1}$ throughout the study (Table 3-1). A control crop was grown at a DLI of 13.75 $mol \cdot m^{-2} \cdot d^{-1}$ over a 16 h photoperiod. PPFD was measured 10 cm above the grow pods under each light fixture using a handheld quantum sensor (LI-250 a meter with LI-193 sensor; LI-COR Environmental, Lincoln, NE).

The experiment layout was randomized with each Gardyn system acting as a block (3 blocks) and was repeated 4 times in time. Treatments were randomized across Gardyn Home systems for each trial. Data were analyzed using SPSS (IBM, IL, USA). An analysis of variance (ANOVA) test was run at a significance level of $p = 0.05$ with Tukey post hoc to determine the impact of lighting regimes on various growth parameters. A principal component analysis (PCA) was performed in Minitab (Minitab LLC, PA, USA) to determine how growth and morphology were impacted by light intensity and length of photoperiod under constant DLI.

Table 3-1 | Lighting treatment parameters

Trial	Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Photoperiod (h)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
Low intensity, long photoperiod (long/low)	120	10	4.32
Medium intensity, medium photoperiod (med/med)	159	7.5	4.29
High intensity, short photoperiod (short/high)	238	5	4.29
Control	238	16	13.75

3.3 Results and discussion

The total DLI and the delivery of low-level DLI impacted the growth and morphology of lettuce plants. Light reduction led to a decrease in biomass accumulation and drastically impacted leaf morphology. Between treatments of DLI 4.3 $mol \cdot m^{-2} \cdot d^{-1}$, increasing photoperiod leads to a significant increase in moisture content, leaf length to width (L:W) ratio, and specific leaf area (SLA). In contrast, total leaf area and area/leaf trend inversely with increasing photoperiod. We are unaware of any research investigating the influence of the photoperiodic effect under reduced DLI, so comparisons were made with trials using growth-promoting DLI levels. Compared to similar research, the 5 h and 7.5 h treatments had an exceptionally short photoperiod and long dark period.

3.3.1 Impact of total daily light integral

Increases in DLI did not proportionally impact crop fresh and dry yields. Comparing the control and high/short treatment, which use the same light intensity, a 330 % increase in DLI yielded a 555 % and 820 % increase in fresh and dry yield, respectively. These findings support work by Randall and Lopez (2015), who reported an increase of up to 74 % in seedling dry mass with only a 38 % increase in DLI. Kelly et al. (2020) reported the impact of various combinations of PPFD and photoperiods with constant and varying DLI on the growth of two lettuce cultivars. They found that regardless of PPFD and photoperiod, as DLI increases, so does fresh and dry yield, indicating that total light is the primary driver of biomass accumulation. Comparing the control crop to the low DLI treatments found that the average area per leaf of the control was 2.9 to 4.3 times higher, SLA 1.7 to 2 times higher and leaf ratio 52 to 65 % lower (p

< 0.01,Table 3-2**)**. Morphological shade avoidance and acclimation traits include leaf elongation and thinning and increasing specific leaf area (SLA), consistent with the previous results (Evans & Poorter, 2001; Gommers et al., 2013). Increases in DLI have been shown to influence leaf area in a species-dependent manner, with lettuce leaf area decreasing under increasing DLI (Baumbauer et al., 2019; Fu et al., 2017). Under high light intensity, leaves grow thicker, with more layers of palisade and spongy mesophyll to act as a protection mechanism to prevent light damage from excess light energy, which reduces SLA (Fan et al., 2013).

Table 3-2 **|** Growth and morphological characteristics of lettuce grown under reduced light treatments (daily light integral (DLI) = 4.29 mol·m^{-2}·d^{-1}) and control (DLI = 13.75 mol·m^{-2}·d^{-1}). Different letters indicate a significant difference between means within the columns according to Tukey's post hoc test at a significance of $p < 0.05$). SD = standard deviation.

Treatment		Fresh mass (g)	Dry mass (g)	Moisture content (%)	Number of leaves	Total leaf area (cm2)	Leaf ratio (L:W)	Area per leaf (cm^2/leaf)	Specific Leaf Area (cm^2/g)
10 h @ 120 µmol·m^{-2}·s^{-1}	Mean	0.81	0.04	95.51	4.73	33.81	2.93	6.72	932.47
	SD	0.44	0.02	0.55	1.35	17.54	0.47	2.09	142.07
		c	c	a	b	c	a	c	a
7.5 h @ 159 µmol·m^{-2}·s^{-1}	Mean	0.79	0.04	94.72	4.38	28.56	2.70	6.40	722.97
	SD	0.50	0.02	0.45	1.30	14.45	0.15	1.80	98.88
		c	c	ab	b	bc	ab	c	b
5 h @ 238 µmol·m^{-2}·s^{-1}	Mean	1.38	0.08	94.06	5.80	58.12	2.38	9.84	726.15
	SD	0.49	0.01	1.14	1.48	23.83	0.33	2.17	203.90
		b	b	b	b	b	b	b	b
Control 16 h @ 238 µmol·m^{-2}·s^{-1}	Mean	7.64	0.63	91.68	9.40	263.61	1.52	29.00	432.55
	SD	0.96	0.08	0.55	1.07	31.81	0.09	2.93	61.50
		a	a	c	a	a	c	a	c

3.3.2 Impact of light trials

Increasing photoperiod length from 5 to 10 h significantly reduced fresh and dry mass under a constant DLI of 4.3 $mol \cdot m^{-2} \cdot d^{-1}$ (Table 3-2, Figure 3-1). However, other trials have shown an increase in dry mass with longer photoperiods and lower PPFD. Dry mass increased with increasing photoperiods for cucumber seedlings in a DLI of 11.5 $mol \cdot m^{-2} \cdot d^{-1}$ (Yan et al., 2021); rudbeckia 'Goldstrum' seedlings with a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ (Elkins & van Iersel, 2020a), strawberries with a DLI of 10 $mol \cdot m^{-2} \cdot d^{-1}$ (Tsuruyama & Shibuya, 2018); lettuce 'Little Gem' and mizuna with a DLI of 16 $mol \cdot m^{-2} \cdot d^{-1}$ (Palmer & van Iersel, 2020); romaine lettuce 'Coastal Star' with a DLI of 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ (Mao et al., 2019). Kelly et al. (2020) examined the impact of DLI ranging from 6.9 to 15.6 $mol \cdot m^{-2} \cdot d^{-1}$ with various photoperiod and PPFD combinations. At a DLI of 8.6 $mol \cdot m^{-2} \cdot d^{-1}$, with photoperiods of 16, 20 and 24 h, there was no significant difference between the fresh or dry yield of lettuce 'Rex' or 'Rouxai.' However, at a DLI of 15.6 $mol \cdot m^{-2} \cdot d^{-1}$, increasing the photoperiod from 16 to 24 h significantly increased fresh and dry mass. Trials with the same results as Kelly et al. (2020) reporting no change in yield as photoperiod changes under DLI of about 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ (Zhang et al., 2018), while contrasting results show an increased dry yield under a more extended photoperiod (Mao et al., 2019). These results suggest that as total DLI declines, the impact of photoperiod and PPFD change, a trend supported by this research, as the trends are opposite the majority of reported data from higher DLI.

The total leaf area was significantly larger for the 5 h photoperiod than the 10 h photoperiod. The 5 h photoperiod has a significantly larger average area per leaf than the 7.5 and 10 h photoperiods. There is no significant difference in the number of leaves which averages between 4.4 and 5.8. Research has shown that under constant DLI with increasing photoperiod, plant leaf area was increased in strawberries 'Elan' and 'Yotsuboshi' under a DLI of 10 $mol \cdot m^{-2} \cdot d^{-1}$ (Tsuruyama & Shibuya, 2018), while in rudbeckia seedlings, leaf area was largest for 15 and 18 h photoperiod and reduced under 12 and 21 h (Elkins & van Iersel, 2020a). The specific leaf area increased quadratically as the photoperiod increased ($p < 0.01$, Figure 3-2). These findings oppose previous studies showing that increasing photoperiod under constant DLI decreased SLA in cucumber seedlings (Yan et al., 2021) and rudbeckia seedlings (Elkins & van Iersel, 2020a).

Regardless of shifts in PPFD or photoperiod, increasing the total DLI has been shown to decrease the SLA in spinach (Gao et al., 2020), young tomato plants (Fan et al., 2013) and lettuce 'Cheongchima' (Cho et al., 2020), indicating the total incident light influences the SLA more than the PPFD or photoperiod.

Leaf length to width ratio increased linearly ($p < 0.01$, Figure 3-3) from 2.38 to 2.93 (23 % increase) as the photoperiod increased from 5 to 10 h. The leaf L:W ratio for the control plants was 1.52. This finding contradicts Kelly et al. (2020), who displayed leaf length of one cultivar decreased under longer photoperiods with a DLI of 10.4 $mol \cdot m^{-2} \cdot d^{-1}$ and did not change with a changing photoperiod and intensity under a DLI of 8.4 $mol \cdot m^{-2} \cdot d^{-1}$, while width stayed consistent for all.

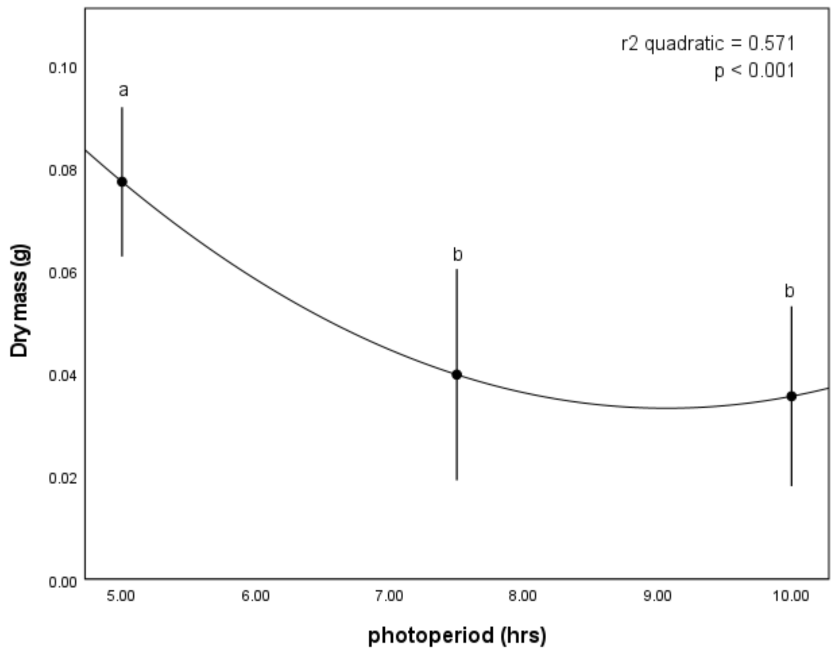

Figure 3-1 | Relationship between dry mass and photoperiod under reduced light treatment treatments (daily light integral (DLI) = 4.29 mol·m-2·d-1). Different letters indicate significant differences in means according to Tukey's post hoc test at $p < 0.05$. Error bars represent ± 1 SD.

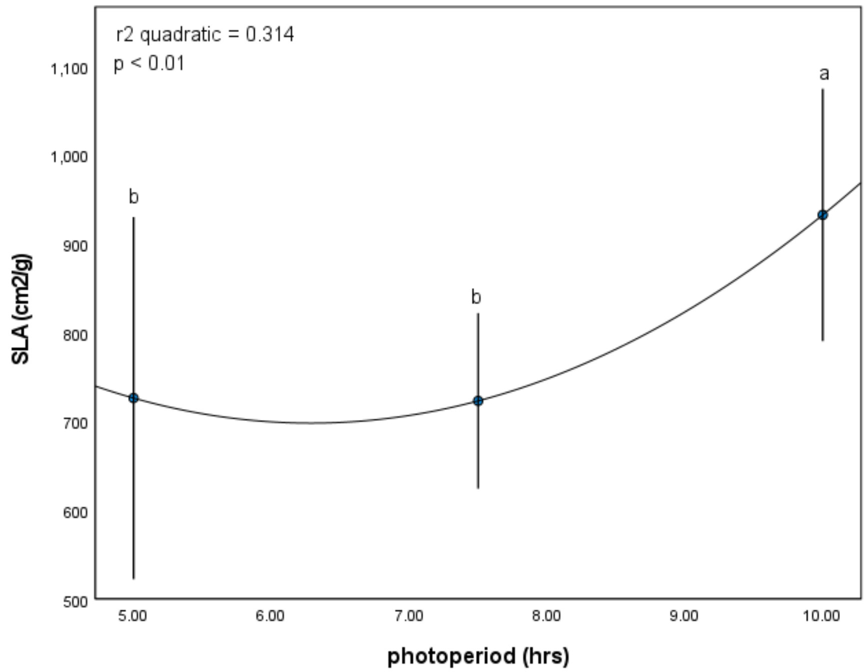

Figure 3-2 **|** Relationship between specific leaf area (SLA) and photoperiod under reduced light treatment treatments (daily light integral (DLI) = 4.29 mol·m-2·d-1). Different letters indicate significant differences in means according to Tukey's post hoc test at $p < 0.05$. Error bars represent ± 1 SD.

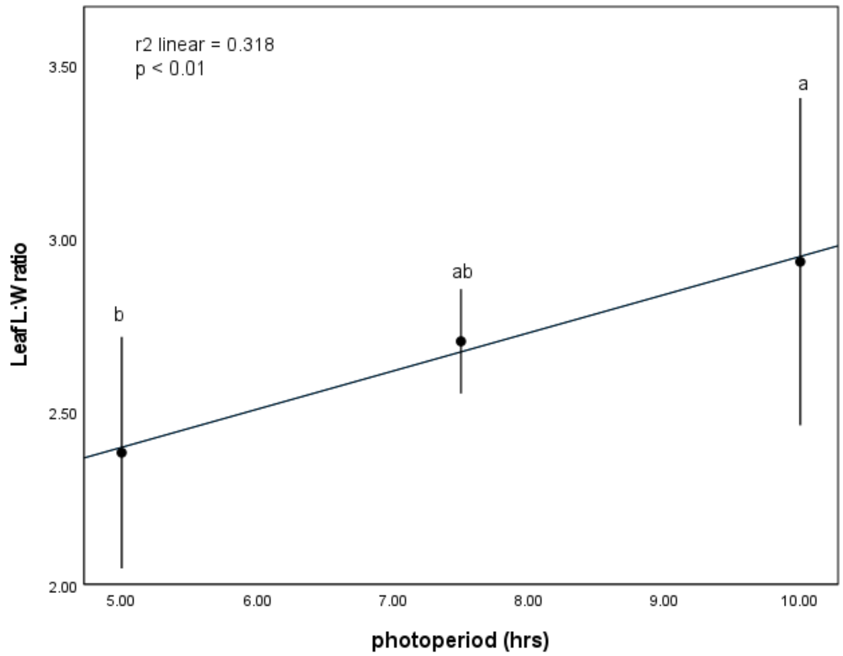

Figure 3-3 | Relationship between leaf length (L) to width (W) ratio and photoperiod under reduced light treatment treatments(daily light integral (DLI) = 4.29 mol·m-2·d-1). Different letters indicate significantly different means according to Tukey's post hoc test at $p < 0.05$. Error bars represent ± 1 SD

3.3.3 Principal component analysis

The first two Eigenvalues were larger than 1 and accounted for 84 % of the variation in the data. The first two principal components (PCs) accounted for 57.1 % and 26.9 %, respectively. PC1 is positively related to fresh and dry mass, the number of leaves, total lead area and area per leaf. PC2 is negatively related to moisture content, leaf length:width ratio and SLA. The 10 and 7.5 h photoperiod treatments were not separated along PC1, but high/low was. This indicates little variation in yield and leaf area or number between the 10 and 7.5 h photoperiod while boost varied significantly from those. All three treatments were separated along PC2, indicating variation in SLA, leaf ratio and moisture content of all 3. This trend indicates that reducing the photoperiod below 7.5 h under constant DLI of 4.29 mol·m^{-2}·d^{-1} is the threshold for inducing more growth and leaf expansion. The 5 h photoperiod treatment position in the top right quadrant indicates it induces positive variation in the yield, leaf area and number (Figure 3-4). The opposite is shown for the 10 h photoperiod in the bottom left quadrant, leading to increases in SLA, moisture, and leaf ratio.

In day greenhouse extension trials yielding constant DLI over different photoperiods, Weaver and van Iersel (2020) reported strong correlative relationships between dry mass and leaf area and dry mass and chlorophyll content. These findings are consistent with our data (Figure 3-4), suggesting that high-intensity lighting over a 5-h period result in higher chlorophyll production and more dry mass accumulation. Although SLA is defined by the ratio of dry mass to leaf area, we do not see SLA related to either in the PCA chart; this implies it is most closely related to leaf thickness (Galieni et al., 2016). Yan et al. (2021) reported a quadratic relationship between photoperiod and SLA, with the longer photoperiods resulting in thinner leaves, with shorter photoperiods having thicker leaves. Our results show the inverse trend, with SLA being lowest with the high-intensity short photoperiod (Figure 3-2). SLA correlated with PC2 in this study, implying that variation is driven by the shortest and longest photoperiods, while the medium does not greatly impact PC2.

As the longest and medium treatment cluster alone with PC2, variation is not seen between yield and area, while there is significant variation with the short high intensity light treatment. When comparing 16 h, 20 h and 24 h photoperiods, Kelly et al. (2020) found that the dry yield of lettuce ‘Rex’ for the 20 h and 24 h photoperiods was not significantly different, while the 16 h photoperiod was significantly less. This displays the same trends as our results, although with much longer photoperiods. When increasing the DLI from 6.9 to 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ by extending the photoperiod or increasing intensity, lettuce growth was not influenced in the same manner. Increasing photoperiod significantly increased lettuce ‘Rex’ and ‘Rouxai’ dry mass, while increasing light intensity did not, despite having the same total DLI for both (Kelly et al., 2020). Iqbal et al. (2022) similarly investigated the impact of light intensity and photoperiod on lettuce ‘Gustav’; photoperiod had a higher influence on morphology (including leaf area, dry yield, and fresh yield) over light intensity. This indicates that photoperiod has a stronger influence on growth than intensity when total light delivered is comparable. As the variation between the 10 and 7.5 h photoperiod is low with respect to yield and area, it indicates there is a threshold between 5 and 7.5 h in the length of light (or darkness) and intensity increasing growth.

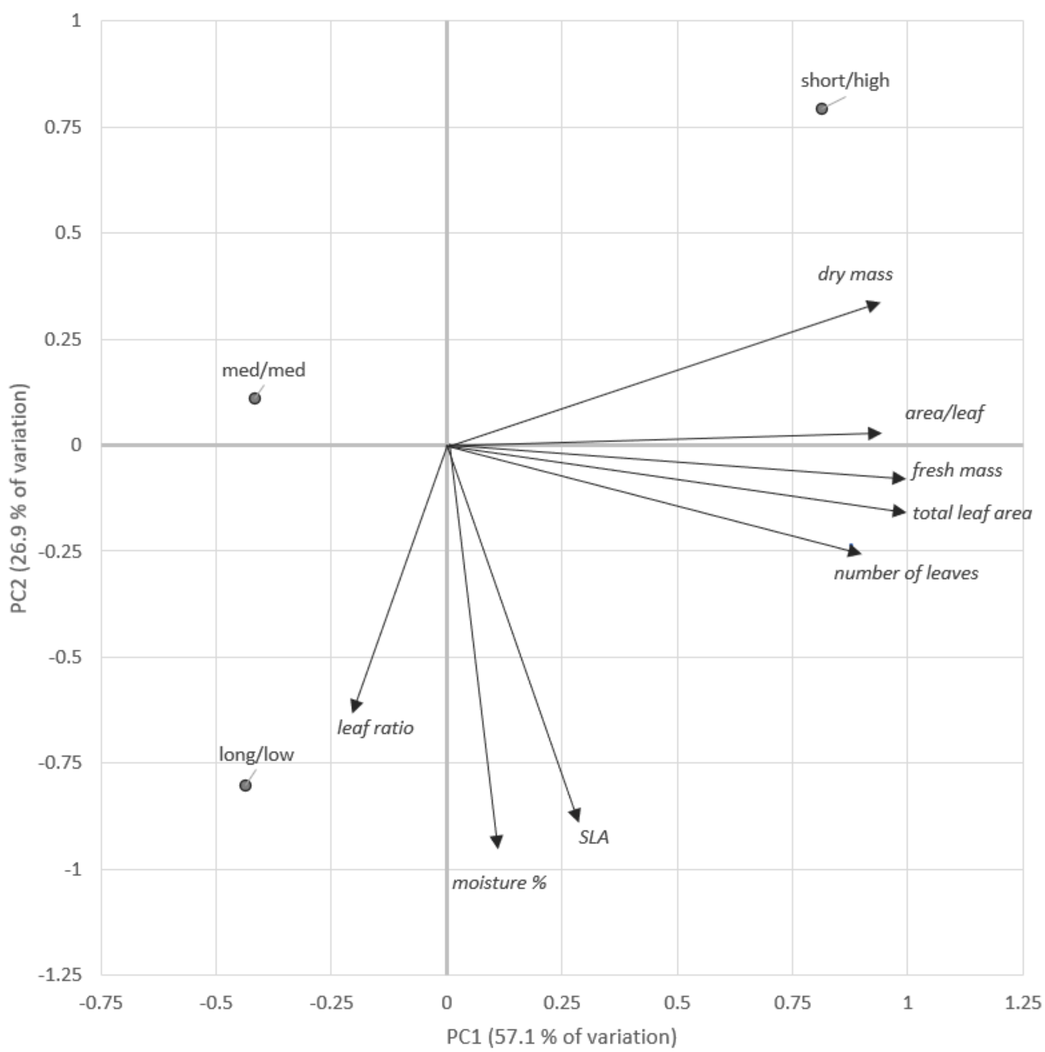

Figure 3-4 | Score and loading plot of principal component analysis (PCA) measuring growth and morphological characteristics of lettuce grown under three lighting treatments. The average scores from each treatment are plotted.

3.4 Conclusion

The current study results show that a low DLI delivered over varying photoperiods and light intensity influences the leaf composition and morphology in romaine lettuce. The goal of reduced DLI is to reduce overall growth while keeping all other factors as close to the control values as possible in maintaining crop quality. Of the treatments tested, the shortest photoperiod and highest PPFD provided the highest qualities of the characteristics measured. Results are primarily inconsistent with similar tests under growth promoting DLI, so more trials are required to reaffirm the results. Further research should investigate the periodic effect of low DLI values to determine growth trends at low light levels.

Connecting statement to Chapter 4

The previous chapter investigated the impact of photoperiod and light intensity under constant low DLI, to determine which would provide reduced growth while maintaining various aspects of quality. The results determined that high intensity delivered over shorter photoperiod was the best option of those tested. This chapter will investigate is delivering the same total light with the same intensity and photoperiod over different lighting schedules will further influence the growth and morphology of lettuce plants while improving various aspects of quality.

Chapter 4 The impact of reduced light intermittent lighting schedules on the growth of lettuce in a vertical in-home hydroponic system

Abstract

In closed plant production, there is ample research into how light influences plant growth to optimize production and produce high-quality products. In this research, plants grown under a reduced light environment were treated with light schedules to examine the impact on growth and morphology. The goal was to reduce growth while maintaining a high-quality crop, essentially 'pausing' the growth. Six reduced light treatments with light/dark intervals (daily light integral (DLI) = 4.3 $mol \cdot m^{-2} \cdot d^{-1}$) were tested: (1) 5 h continuous, (2) 60 min/60 min over 10 h, (3) 60 min/20 min over 6.7 h, (4) 20 min/60 min over 20 h, (5) 20 min/20 min over 10 h and, (6) 10 min/20 min over 15 h. The control trial was 16 h/8 h with a DLI of 13.75 $mol \cdot m^{-2} \cdot d^{-1}$. Plants were transplanted to the systems 14 and 28 days after seeding (DAS). For both ages of plants, the 10/20 treatment resulted in the lowest fresh and dry mass and largest specific leaf area (SLA). The 20/60 and 60/20 maximized biomass accumulation of 14 and 28 DAS samples but were proportionally much larger for 28 DAS and resulted in the same yield as the control. Between the 14 and 28 DAS, results are significantly different. The reduced DLI impacted the 14 DAS as expected, but the biomass accumulation in 28 DAS was much larger and comparable to the control.

4.1 Introduction

Light is a primary driver of plant growth and morphology, with the main influencing factors being quality (spectrum), quantity (intensity/total photons) and photoperiod (length of lighting). Daily light integral (DLI) measures the total photons delivered under a given photosynthetic photon flux density (PPFD) and photoperiod. Total DLI is strongly correlated with biomass accumulation and aesthetic qualities (compact leaves with high pigment content), and a minimum DLI of 6.5 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ is suggested to produce quality lettuce in the home (Paz et al., 2019). Total DLI alone does not impact the growth; photoperiod, light intensity and schedule all play a role (Liu et al., 2022). Intermittent lighting regimes vary from milliseconds (often called pulsed lighting) to seconds, minutes, and hours (Liu et al., 2022). This research omitted pulsed lighting and focused on intermittent and fluctuating lighting regimes with lighting fluctuations on the scale of minutes to hours. Closed plant production systems operate independently of natural light, so growers can employ novel lighting regimes to increase control over plant growth.

This research investigates the control and reduction of growth of lettuce (*Lactuca sativa*) using intermittent lighting schedules in a vertical in-home hydroponic system. This research uses lettuce as a reference crop is it is fast-growing and requires a low energy input (Lin et al., 2013; Silva et al., 2022). Our goal was to apply the results of an earlier study showing lettuce grown under a constant DLI of 4.3 $mol{\cdot}m^{-2}{\cdot}d^{-1}$, which maintained higher quality under short photoperiod and high PPFD compared to longer photoperiod and lower PPFD. Quality is defined as minimal etiolated growth (measured through leaf density and L:W ratio), while the fresh mass and leaf area will determine crop quantity. These trials deliver a DLI of 4.3 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ with constant or intermittent lighting schedules, outlined in detail below. We hypothesize that reducing the overall total dark period and minimizing the number of cycles will maintain higher crop quality.

4.2 Materials and methods

4.2.1 Plant culture

The experiment was conducted in a home environment (temperature 21 ± 2 °C; relative humidity 45 ± 5 %). Three Gardyn Home systems were housed in an office in Montreal, Canada. Each system had two layers of 80 % light-blocking shade cloth to reduce the impact of light pollution. Small fans were mounted to blow air across the base of the systems to maintain circulation. Two vertically mounted LED light bars (Gardyn Home Inc., Bethesda, MD) supplied light to each growing area. The Gardyn Home central processing unit (CPU) automatically controlled light intensity and photoperiod and was controlled from the app.

Lettuce (*Lactuca sativa* cv. Buttercrunch) was grown from seed. Seeds were germinated in 1-inch Rockwool (Grodan (ROXUL inc); Milton, ON) cubes in black 10" x 20" trays and irrigated every 3 days and fertilized every 7 days. During germination, the lighting schedule was light/dark 16 h/8 h at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seedlings were transplanted to the gardyn system 14 and 28 days after seeding (DAS) (Table 4-1). Each system has three towers, divided into 10 growing pods to allow up to 30 plants per system. All transplants were put onto the middle tower, where the lighting is most uniform. Salt nutrients were dissolved in tap water to an EC of 1.5 $\mu mS \cdot cm^{-1}$, which required about 1.25 g/L (MaxiGrow 10N-5P-14K, General Hydroponics, Santa Rosa, Ca) in the system 40 L reservoir. During the experiment, the system was irrigated for 5 minutes daily before the beginning of the light cycle. Lettuce was harvested after 14 days under all light treatments and replicates. Plants were weighed (DH100G, NEWCALOX, CHN) and measured immediately upon harvest. Samples were dried (80 °C; 32100C, Hamilton Beach, VA, USA) until changes in mass were no longer detectable day over day. Leaf measurements were taken with a vernier calliper (Digital Caliper; Mastercraft, TN, USA), and leaf area was measured using ImageJ from photos (12 MP wide-angle, Samsung S10) taken immediately after harvest.

4.2.2 Experimental design and data analysis

Plants were transplanted to the Gardyn systems 14 and 28 DAS. Plants were exposed to 6 lighting treatments with various light recipes (Table 4-1). Each treatment had the same PPFD of

238 µmol·m^{-2}·s^{-1} and total photoperiod of 5 hours with light delivered intermittently in 5 of 6 treatments (Table 4-1). The total DLI was 4.29 mol·m^{-2}·d^{-1}. A control crop was grown at the same PPFD over a photoperiod of 14 hours, resulting in a DLI of 13.75 mol·m^{-2}·d^{-1}. PPFD was measured 10 cm above the grow pods under each light fixture using a handheld quantum sensor (LI-250 with an LI-193 sensor; LI-COR Environmental, Lincoln, NE).

The experiment layout was randomized, with each Gardyn system acting as a block running 3 treatments simultaneously. Each treatment was repeated thrice in time. Treatments were randomized across Gardyn Home systems for each trial. Variations in results between treatments were evaluated with a two-way analysis of variance (ANOVA) with Duncan's post hoc test at a significance level of 0.05 using SPSS (IBM, IL, USA). A principal component analysis (PCA) was performed in Minitab (Minitab LLC, PA, USA) to determine how physical parameters were impacted by various qualities of the lighting treatments (length of continuous darkness, number of cycles, ratio of light to dark cycle length).

Table 4-1 **|** Description of light treatments trialled in intermittent lighting experiment. Interval on and off time indicates the length of the periods for the intermittent portion of the light treatment only. The ratio of light (L) to dark (D) indicates the ratio of these light intermittent light treatments.

	control	**continuous**	**60/60**	**60/20**	**20/60**	**20/20**	**10/20**
DLI (mol·m^{-2}·d^{-1})	13.75	4.29	4.29	4.29	4.29	4.29	4.29
Interval on time	16 h	5 h	60 min	60 min	20 min	20 min	10 min
Interval off time	8 h	19 h	60 min	20 min	60 min	20 min	20 min
Ratio (L:D)	2.00	0.26	1.00	3.00	0.33	1.00	0.50
# cycles/24 h	1	1	5	5	15	15	30
Continuous dark (h)	8.0	19.0	14.0	17.3	4.0	14.0	9.0

4.3 Results

Lettuce crops were grown on the Gardyn Home system under various lighting schedules for two weeks. All lighting trials had a DLI of about 4.4 mol·m^{-2}d^{-1} except for the control, which was 13 mol·m^{-2}d^{-1}. Lighting trials began simultaneously and ran until the total light desired was delivered. The goal is to reduce the overall mass accumulation while keeping the morphology comparable to the control.

4.3.1 Impact of total DLI

The growth of lettuce plants was impacted by reduced DLI lighting trials and the total reduction in DLI. The light treatments did not equally impact 14 DAS and 28 DAS plants. The 14 DAS fresh and dry yield was significantly lower than the control, while some treatments on 28 DAS were not significantly reduced (Figure 4-1 & Figure 4-2). The leaf length:width ratio was lower for 28 DAS compared to 14 DAS. Elongation under the light treatments was much more significant for the 14 DAS lettuce plants than for the 28 DAS (Figure 4-4). The fresh mass per area and SLA were not significantly different for the 14 and 28 DAS control plants. The SLA for 14 DAS was 237–355 % of the control, while 28 DAS was 134–186 %. Similarly, the fresh mass/area of 14 DAS was 54–67 % of the control compared to 28 DAS 75–84 % of the control. The growth/DLI (Figure 4-3) show more biomass accumulation of the 28 DAS plants under reduced light compared to the control, while the 14 DAS the control accumulated for mass per unit DLI higher.

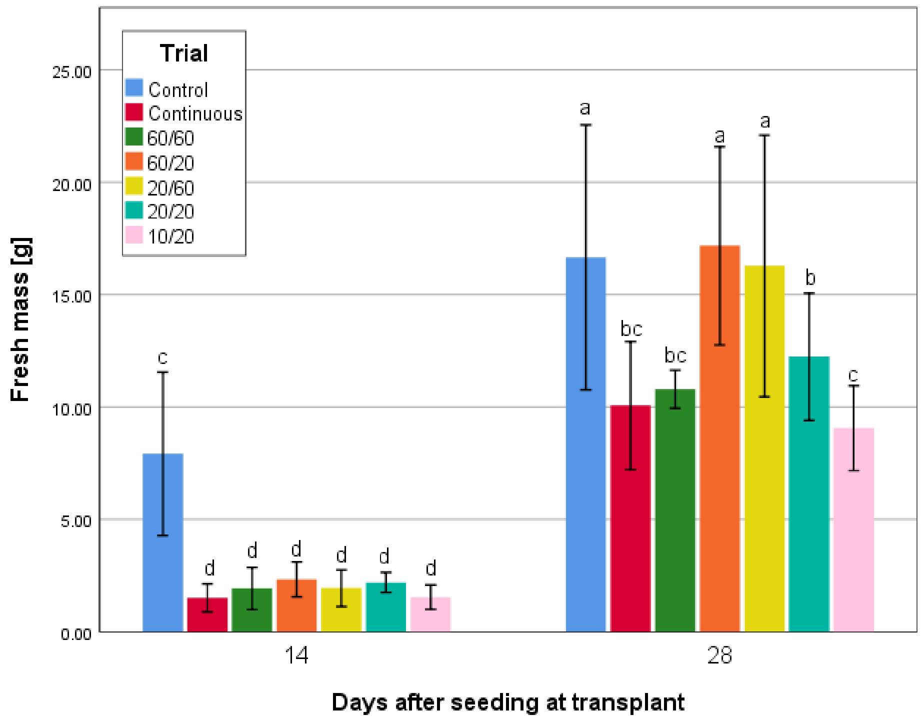

Figure 4-1 | Fresh mass of lettuce, in grams, at harvest under control (daily light integral (DLI) = 13.75 mol·m^{-2}·d^{-1}) and reduced light (DLI=4.29 mol·m^{-2}·d^{-1}) treatment for 14 and 28 days after seeding (DAS). Plants were grown at 200 µmol·m^{-2}·s^{-1} (DLI=11.5 mol·m^{-2}·d^{-1}) before transplant, for 14 or 28 days. Both 14 and 28 DAS were harvested after 14 days of light treatment. Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

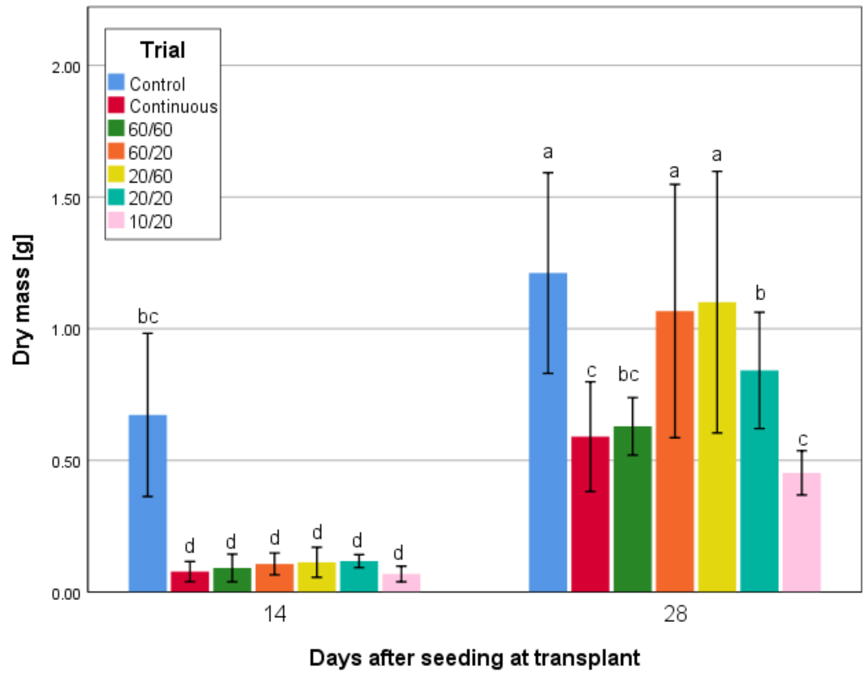

Figure 4-2 | Dry mass of lettuce, in grams, at harvest under control (daily light integral (DLI) = 13.75 mol·m^{-2}·d^{-1}) and reduced light (DLI=4.29 mol·m^{-2}·d^{-1}) treatment for 14 and 28 days after seeding (DAS). Plants were grown at 200 µmol·m^{-2}·s^{-1} (DLI=11.5 mol·m^{-2}·d^{-1}) before transplant. Both 14 and 28 DAS were harvested after 14 days of light treatment.Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

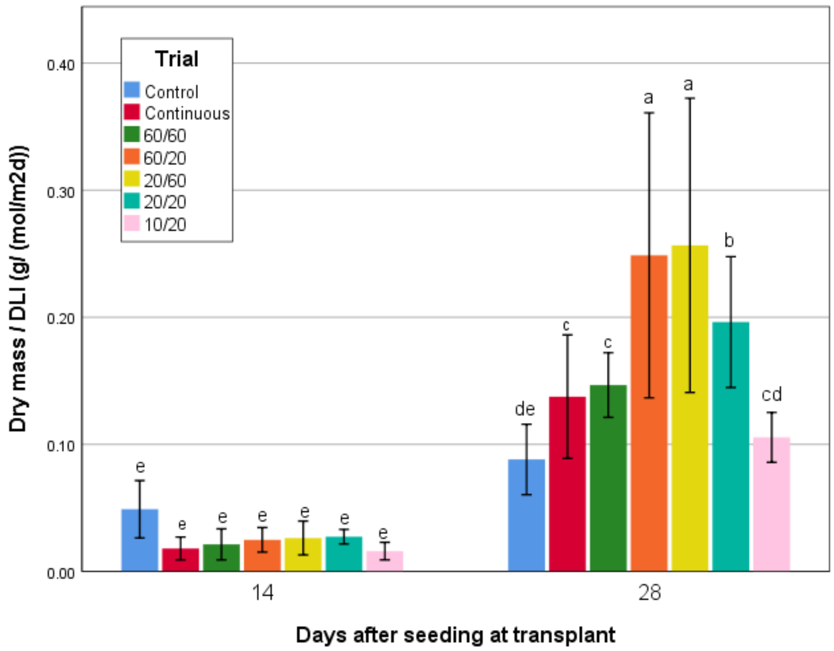

Figure 4-3 | Dry mass per unit daily light integral (DLI) ($g/mol·m^{-2}·d^{-1}$) of lettuce, at harvest under control (DLI = 13.75 $mol·m^{-2}·d^{-1}$) and reduced light (DLI=4.29 $mol·m^{-2}·d^{-1}$) treatment for 14 and 28 days after seeding (DAS). Plants were grown at 200 $\mu mol·m^{-2}·s^{-1}$ (DLI=11.5 $mol·m^{-2}·d^{-1}$) before transplant, for 15 or 28 days. Both 14 and 28 DAS were harvested after 14 days of light treatment. Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

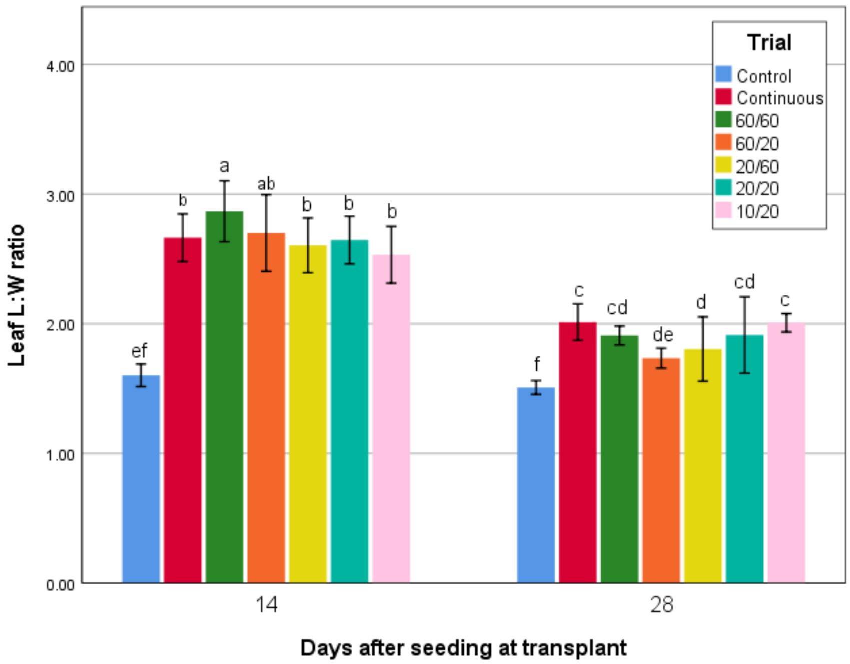

Figure 4-4 | Leaf length (L) to width (W) ratio of lettuce leaves at harvest under control (daily light integral (DLI) = 13.75 mol·m^{-2}·d^{-1}) and reduced light (DLI=4.29 mol·m^{-2}·d^{-1}) treatment for 14 and 28 days after seeding (DAS). Plants were grown at 200 µmol·m^{-2}·s^{-1} (DLI=11.5 mol·m^{-2}·d^{-1}) before transplant. Both 14 and 28 DAS were harvested after 14 days of light treatment. Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

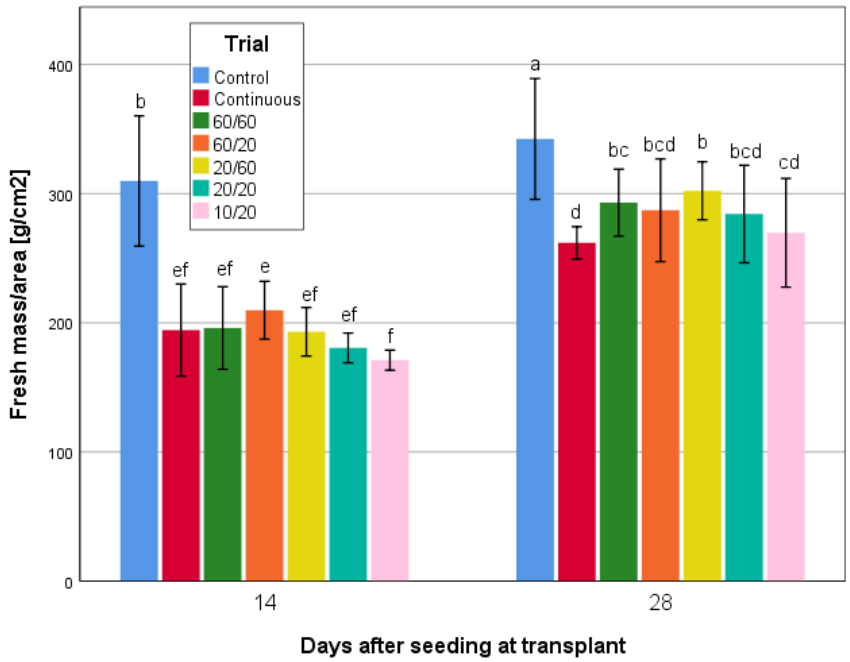

Figure 4-5 | Fresh leaf mass per leaf area of lettuce at harvest under control (daily light integral (DLI) = 13.75 mol·m^{-2}·d^{-1}) and reduced light (DLI=4.29 mol·m^{-2}·d^{-1}) treatment for 14 and 28 days after seeding (DAS). Plants were grown at 200 µmol·m^{-2}·s^{-1} (DLI=11.5 mol·m^{-2}·d^{-1}) before transplant for 14 or 28 days. Both 14 and 28 DAS were harvested after 14 days of light treatment. Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

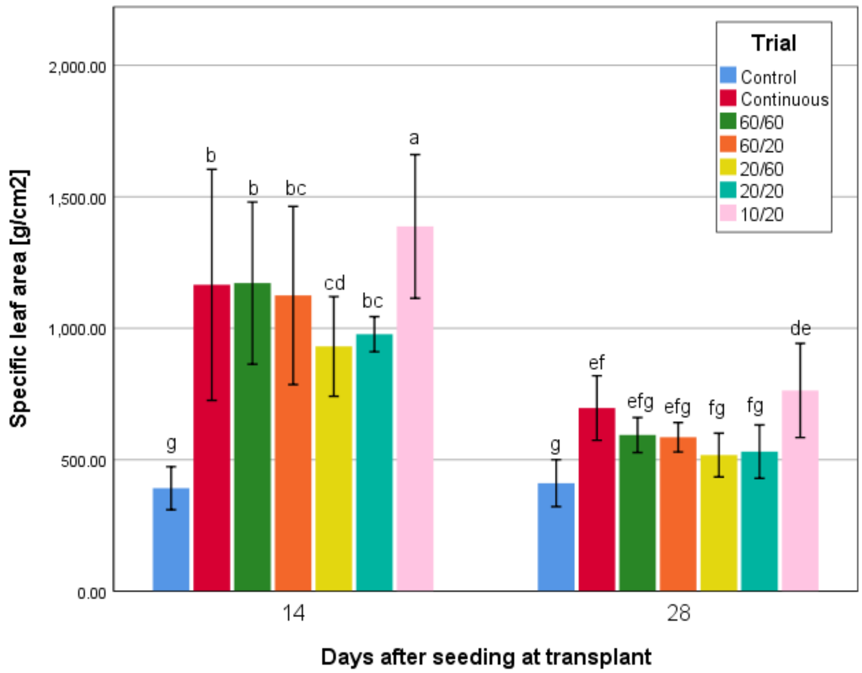

Figure 4-6 | Specific leaf area (SLA) (g/cm^2) of lettuce leaves at harvest under control (daily light integral (DLI) = 13.75 $mol \cdot m^{-2} \cdot d^{-1}$) and reduced light (DLI=4.29 $mol \cdot m^{-2} \cdot d^{-1}$) treatment for 14 and 28 DAS. Plants were grown at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI=11.5 $mol \cdot m^{-2} \cdot d^{-1}$) before transplant, for 14 or 28 days. Both 14 and 28 DAS were harvested after 14 days of light treatment. Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

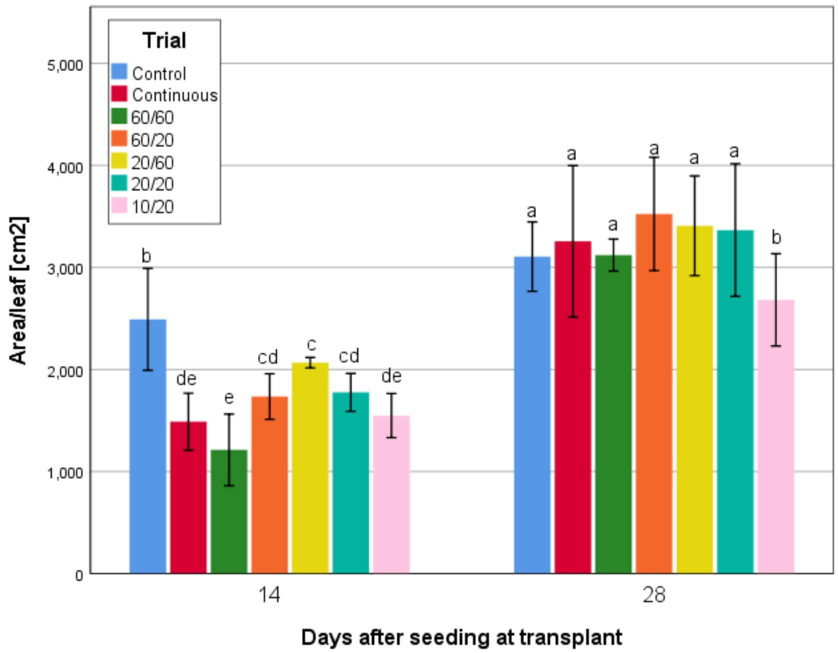

Figure 4-7 | Area per leaf (cm^2) of lettuce leaves at harvest under control (daily light integral (DLI) = 13.75 $mol \cdot m^{-2} \cdot d^{-1}$) and reduced light (DLI=4.29 $mol \cdot m^{-2} \cdot d^{-1}$) treatment for 14 and 28 days after seeding (DAS), for 14 or 28 days. Plants were grown at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI=11.5 $mol \cdot m^{-2} \cdot d^{-1}$) before transplant. Both 14 and 28 DAS were harvested after 14 days of light treatment. Different letters indicate a significant difference between means at $p < 0.05$ according to Duncan's Multiple Range post hoc analysis. Error bars show ± 1 standard deviation.

4.3.2 Impact of light trials on biomass accumulation and leaf morphology

Lighting treatments and age at transplant significantly impacted the growth of lettuce plants. The interaction effect between week and trial showed a much more significant impact of the trial on the 28 DAS plants, while there was no significant difference in yield between the 14 DAS plants (Table 4-2, Figure A0-4). The 28 DAS plants had the highest yield with 20/60 and 60/20 and the lowest with 10/20. The area/leaf was significantly impacted by the age of the plant and the lighting treatment; the trials had a more significant effect on 14 DAS plants than on 28 DAS (Figure A0-5). The 14 DAS plants were 48.6–82.9 % of the control, while 28 DAS were 86.3–104.8 % of the control. 28 DAS samples were unchanged from the control, except for the 10/20 trial, which had significantly reduced leaf area. Both 14 and 28 DAS plants had significantly larger ratios than the control, but the increase was more significant for 14 DAS crops. For 14 DAS, the ratios in 60/60 and 60/20 were the highest and 10/20 the lowest. Among both 14 and 28 DAS samples, 20/60 treatment reduced the ratio the most. There was a significant interaction effect for the 10/20 treatment, which resulted in the lower ratio for 14 DAS and the largest for 28 DAS (Figure A0-6, Table 4-3). SLA increased significantly in 14 DAS, 253–354 % of the control, while 28 DAS are 126–186 % of the control. SLA was maximized by 10/20 for both 14 and 28 DAS and minimized by 20/60 and 20/20.

Table 4-2 | Growth characteristics of lettuce grown under reduced daily light integral (DLI) lighting treatments. Mean ± 1 standard deviation (SD) followed by a letter, different letters indicate a significant difference between means (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) within the column according to Duncan's Multiple Range test.

A	B	Fresh mass (g)				Dry mass (g)				Moisture (%)			
14DAS	continuous	1.52	±	0.63	d	0.08	±	0.04	d	95.11	±	1.15	abc
	60/60	1.93	±	0.93	d	0.09	±	0.05	d	95.39	±	0.82	ab
	60/20	2.34	±	0.78	d	0.11	±	0.04	d	95.47	±	0.97	a
	20/60	1.95	±	0.81	d	0.11	±	0.06	d	94.22	±	1.11	cde
	20/20	2.20	±	0.45	d	0.12	±	0.02	d	94.54	±	0.59	bcd
	10/20	1.55	±	0.55	d	0.07	±	0.03	d	95.62	±	0.68	a
28DAS	cont	10.07	±	2.84	bc	0.59	±	0.21	c	94.23	±	0.87	cde
	60/60	10.79	±	0.85	bc	0.63	±	0.11	c	94.14	±	0.83	de
	60/20	17.18	±	4.41	a	1.07	±	0.48	a	94.31	±	1.06	cd
	20/60	16.28	±	5.81	a	1.10	±	0.50	a	93.36	±	0.94	ef
	20/20	12.24	±	2.82	b	0.84	±	0.22	b	93.18	±	0.77	f
	10/20	9.06	±	1.89	c	0.45	±	0.08	c	94.91	±	0.87	abcd
	A	***				***				***			
	B	***				***				***			
	A x B	***				***				NS			

A	B	Number of leaves				Fresh mass/area (g/cm^2)				SLA (cm^2/g)			
14DAS	continuous	5.50	±	0.80	b	194.32	±	35.68	de	1165.0	±	439.5	b
	60/60	6.43	±	1.72	b	195.96	±	31.96	de	1171.7	±	308.4	b
	60/20	6.00	±	1.35	b	209.71	±	22.34	d	1124.6	±	338.8	bc
	20/60	5.86	±	1.21	b	193.05	±	18.79	de	930.8	±	189.4	cd
	20/20	5.91	±	1.14	b	180.52	±	11.55	e	994.9	±	93.3	bc
	10/20	6.31	±	1.44	b	171.03	±	7.80	e	1387.3	±	273.1	a
28DAS	cont	10.00	±	1.13	a	261.88	±	12.53	c	696.6	±	122.7	ef
	60/60	9.89	±	1.27	a	292.96	±	25.93	ab	593.8	±	66.6	ef
	60/20	9.50	±	1.84	a	287.07	±	39.81	abc	585.3	±	55.9	ef
	20/60	10.08	±	1.88	a	302.11	±	22.46	a	517.6	±	83.3	f
	20/20	9.42	±	1.56	a	284.20	±	37.74	abc	530.9	±	101.6	f
	10/20	9.63	±	1.92	a	269.65	±	42.06	bc	763.4	±	179.1	de
	A	***				***				***			
	B	NS				**				***			
	A x B	NS				NS				NS			

Table 4-3 | Morphological characteristics of lettuce grown under reduced DLI lighting treatments. Mean ± SD followed by a letter, different letters indicate a significant difference between means (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) within the column according to Duncan's Multiple Range test.

A	B	Leaf length:width ratio				Area/leaf (mm^2)			
14DAS	continuous	2.66	±	0.18	b	1487.8	±	279.8	de
	60/60	2.87	±	0.24	a	1211.8	±	350.7	e
	60/20	2.70	±	0.30	ab	1734.8	±	223.8	cd
	20/60	2.60	±	0.21	b	2066.6	±	51.2	c
	20/20	2.65	±	0.18	b	1794.4	±	229.4	cd
	10/20	2.53	±	0.22	b	1561.2	±	249.1	de
28DAS	continuous	2.01	±	0.14	c	3255.9	±	741.4	a
	60/60	1.91	±	0.07	cd	3121.2	±	156.9	a
	60/20	1.73	±	0.08	d	3523.9	±	555.4	a
	20/60	1.81	±	0.25	d	3408.1	±	488.3	a
	20/20	1.91	±	0.29	cd	3365.8	±	649.4	a
	10/20	2.01	±	0.07	c	2682.3	±	451.9	b
	A	***				***			
	B	NS				***			
	A x B	**				*			

4.3.3 Principal component analysis of growth indices grown in 6 vacation mode light schedules

A principal component analysis (PCA) was performed on all treatment DAS for 14 and 28 DAS trials. The first 2 Eigenvalues accounted for 87.6 % of the variation in the DAS. PC1 explained 75.9 % and PC2 11.7 %. PC1 is positively correlated with fresh mass, dry mass, area/leaf, fresh mass/area, and the number of leaves and negatively to leaf ratio. PC2 was positively correlated with moisture and SLA. The loadings for the age of the plant were separated along the x-axis, and variation within the age groups spread along the y-axis (Figure A0-1). This indicated that most of the variation was driven by the age of the plants. The 28 DAS scores cluster with the loadings for fresh and dry mass, area per leaf and number of leaves, indicating the older plants drive more increase in these than 14 DAS. The 10/20 14 and 28 DAS treatments are the treatment pair clustered closest together, indicating the most consistent impact on growth regardless of plant age.

Another PCA was performed on the 14 and 28 DAS datasets separately to remove the variation caused by plant age. For the 14 DAS trial, the first 3 eigenvalues were greater than 1 and accounted for 74.7 % of the variation. PC1 explained 41.5 %, PC2 19.3 % and PC3 13.9 %.

In the 28D trial, the top 3 eigenvalues account for 74 % of the variation, with PC1 being 38.4 %, PC2 20.5 % and PC3 15.1 % (Figure A0-3). Loadings larger than 0.5 are related to the component. The 14 DAS PC1 is positively related to dry and fresh mass/area, while SLA and moisture content is negatively related. PC2 is positively related to fresh and dry mass and area per leaf and negatively related to leaf L:W ratio. PC3 is positively related to the number of leaves. For the 28 DAS trial, PC1 was positively related to moisture and SLA and negatively related to area/leaf and fresh and dry mass. PC2 is negatively related to the ratio and number of leaves. PC3 is related to fresh mass/area and SLA. The 14 DAS trials vary mostly across PC2, except for 10/20, which varies from the group along PC1 (Figure A0-2).

4.4 Discussion

4.4.1 Studies with an increase in uniform L/D cycles

The research focused on shortening the length and increasing the frequency of the light/dark (L/D) cycle is prevalent. Results show that as the number of L/D cycles increase and the light and dark periods in each cycle decrease, there is a significant, species-dependent (Garcia-Molina & Leister, 2020) impact on biomass accumulation (Ishii et al., 1995), distribution (Chen & Yang, 2018; Highkin & Hanson, 1954), plant morphology (Chen et al., 2022) and photosynthetic apparatus constitution (Garcia-Molina & Leister, 2020; Hang et al., 2019). Comparing an increasing number of L/D cycles with a 2/1 L/D ratio on lettuce biomass accumulation has increased (Chen & Yang, 2018), decreased (Zhou et al., 2020) and remained unchanged (Kang et al., 2013). Other trials showed a decrease in lettuce biomass with more L/D cycles of 6/6 and 4/4 (Hang et al., 2019) and a 1/2 ratio of 8/16, 4/8 and 2.67/5.33 (Ishii et al., 1995). The 28 DAS lettuce plants grown with 30 cycles (10/20 treatment) had a significantly lower dry mass. Samples with 15 light cycles (20/20 and 20/60) had the largest dry yields. Figure A0-2 shows these are the most substantial weighted scores for impact on dry mass positively and negatively. Lettuce leaf L:W ratio remained unchanged in L/D treatments of 8/16, 4/8 and 2.67/5.33 (Ishii et al., 1995) and 18/6, 9/3 and 6/2 under PPFD, ranging from 200-290 $\mu mol\cdot m^{-2}\cdot s^{-1}$ (Kang et al., 2013). The L:W ratio decreased from 6/6 to 4/4 (Hang et al., 2019). Our research found a significant reduction in ratio for 14 DAS treatments with 30 cycles, compared to 5 (60/60 and 60/20), which could contribute to leaf elongation by light treatments; there is no significant difference for 28 DAS (Figure 4-4). SLA significantly increased in 3/3 light cycles compared to 12/12 and 6/6 (Zhou et al., 2020). An increase in SLA indicates a thinner leaf. 10/20 was the largest SLA in both 14 and 28 DAS, while 20/20 and 20/60 maximized SLA for both.

4.4.2 Length of the continuous dark cycle

In trials with one cycle, growth impacts photoperiod, dark period, and light intensity. Lettuce is grown under constant photoperiod of 10 h (Maruo et al., 2000) and 16 h (Hiroki et al., 2014), with dark periods varying from 14–0 h, showing an increase in fresh and dry yield as the dark period decreased. Other studies have shown contrasting results; under a constant 16 h light period, as the dark period decreased from 8 h to 0 h, the yield drastically decreased, along with leaf length (Urairi et al., 2017). Our research showed that 4 h of continual darkness significantly increased the dry yield and area per leaf, which minimized SLA for 14 and 28 DAS treatments. However, the longest continuous dark period of 19 h (continuous light treatment) had the second-lowest yield of all treatments for both 14 and 28 DAS. Although no significant correlation was observed between the length of continuous darkness and dry yield, these results suggest there may be an impact on biomass accumulation.

During the light period, starch is stored in the plant to be used during the dark period. Leaf expansion occurs primarily during dark cycles when starch is not a limiting factor (Apelt et al., 2017; Pantin et al., 2011). Arabidopsis (*Arabidopsis thaliana*) grown under low light intensity of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over a 12 h photoperiod (DLI about 3 $mol \cdot m^{-2} \cdot d^{-1}$) had the lowest leaf expansion at the beginning of the dark period and a prominent peak at dawn (Pantin et al., 2011). The same paper reported that a starch mutant, which does not store starch while photosynthesizing, increased its expansion in the second half of the light cycle when it was minimized for the wild type, indicating plants in carbon starvation mode would expand more during the light cycle than in the dark. The largest leaf area under the reduced light treatments was 20/60, with the shortest continuous darkness, while the treatment with the most prolonged continuous darkness (continuous lighting treatment) had the second smallest leaves. This could be caused by a lack of photosynthate accumulation in the continuous lighting trial, leading to reduced leaf expansion in the dark period and minimal time for expansion over the 5 h photoperiod.

4.4.3 Intermittent and fluctuating light

Intermittent lighting regimes have very short L/D periods of minutes to about an hour. Most research focuses on extremely short lighting intervals (< 60 sec) or investigates fluctuating light (continuous light with variation in intensity), which mimics natural lighting conditions and sun flecks. In recent years, some research has been done focusing on intermittent lighting for applications in closed environments. Bhuiyan and van Iersel (2021) examined the impact of a 15 min/15 min L/D cycle over a 16 h photoperiod on lettuce compared to the same DLI delivered at a constant intensity over the same photoperiod. Their results show a drastic decrease in plant yield, leaf area, chlorophyll content and average photosynthetic rate while significantly larger SLA in fluctuating lighting conditions than constant.

In contrast, Chen and Yang (2018) reported larger fresh and dry mass, plant height and chlorophyll levels (not significant) for the lighting of 60 min/ 30 min throughout 24 h, compared to the control 16 h/8 h with the same light intensity. These studies suggest that a light period of larger than 15 minutes would promote the growth of lettuce. The 10/20 treatment in this study resulted in the lowest yield, which is in accordance with this trend. The intermittent light periods of 20 or 60 minutes resulted in yields higher than 10/20 and continuous lighting. SLA was the lowest for both 14 and 28 DAS for the 20 min light cycle, followed by the 60 min treatments, with 10/20 being the largest. Both treatments with 20 minute light intervals have the same number of light cycles, which could be a contributing factor to the results. Although, it is expected that increasing the number of cycles could decrease growth and increase SLA, and there is more time spent under photosynthetic induction, as discussed below.

4.4.4 Rate of photosynthesis, photosynthetic induction, lag time

Shifts in the light environment impact the rate of photosynthesis, although it is subject to a lag time after illumination, known as photosynthetic induction. The limiting processes are the activation of the ribose 1,5-bisphosphate (RuBP) regeneration pathway (2 minutes post illumination), the activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (5-10 minutes post illumination), initiation of enzymatic reactions and rates by opening stomata (up to 60 minutes post illumination (Jun & Hong, 2002; Kimura et al., 2020; Pearcy, 1990). This delay is in dark-adapted plants, but the stomatal aperture shifts continually as PPFD shifts. Short reductions in light intensity (3 minutes) do not impact the stomatal aperture but a large light reduction for 25 minutes causes a closure which limits the rate of photosynthesis, requiring 25 minutes to return to the pre-shade level (Lawson et al., 2012). Exposure to 15 minutes of reduced light will cause a 35 % restriction of the photosynthetic rate in the first few minutes after illumination (Lawson & Blatt, 2014). The rate of photosynthesis has not been directly tied to crop yield, although it is a significant contributing factor (Zelitch, 1982). Hang et al. (2019) reported the photosynthetic rate response to illumination for lettuce ‘Adriana’ and found a slow response in the first 3 minutes ending at about 65% of the max, and after 10 minutes, it reached about 80 % of the maximum rate. Bhuiyan and van Iersel (2021) investigated the impact of 15 minute fluctuations in light intensity on lettuce and found a reduction in net photosynthetic rate for L/D cycle of 360 $\mu mol \cdot m^{-2} \cdot s^{-1}$ /40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and especially 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ /0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Upon illumination, these treatments were unable to reach a steady-state photosynthetic rate and so were continually under a lower rate of photosynthesis. Zhou et al. (2020) reported that longer light periods induced a higher net light-saturated rate of photosynthesis, light saturation point and next photosynthetic rate at PPFD over 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when comparing 1 cycle (12 h/12 h) to 2 (6 h/6 h) and 4 (3 h/3 h) light/dark cycles. 10/20 yielded the lowest fresh and dry mass for all trials, and the increased L/D cycles with short photoperiods might not have allowed the plant to reach the maximum photosynthetic rate creating this result.

4.5 Conclusion

Overall, the most substantial impact of growth comes from the age of the crop. Beyond that, the light treatment growth rate appears to be driven most strongly by the number of cycles and length of continuous darkness. In the PCA analysis, 20/60 clusters with 20/20 and 60/20 along the x and y-axis in 14 DAS and the x-axis in 28 DAS, indicating a similar impact on plant growth and morphology among all 3 treatments. 60/20 the light period was dark enough and maybe the dark period short enough to minimize relaxation of some processes. 20/60 minimized the continuous dark period. The 10/20 treatment had the strongest impact on growth reduction but significantly increased SLA and ratio, indicating a reduction in the overall quality of the crops. The 60/60 treatment minimized yield while reducing leaf elongation and SLA indicating it provides good overall quality. Further research should compare similar trials under higher total DLI to investigate how the total overall reduction in light impacted the growth compared to the impact of the light recipes.

Chapter 5 Summary and general conclusion

5.1 General conclusion

This research aimed to examine the impact of various light schedules on the growth of lettuce under a low DLI. Current literature has focused on the impact of reduced light and of various lighting schedules, but not both. The experiments were carried out on Gardyn Home grow systems to provide feedback for the generation of a 'vacation mode' for their users, which 'pause' plant growth while they are away from home for an extended time.

Experiment one investigated the photoperiodic and intensity effect on the growth and morphology of lettuce under a reduced DLI. The results found that using a shorter photoperiod (5 h) and high intensity resulted in the smallest leaf elongation and thickest leaves while reducing the overall yield. Literature reported the opposite trends under growth promoting DLIs, leaving space for further investigation into the underlying metabolic impact on growth.

Experiment two applied the ideal light parameters collected in experiment one to develop various intermittent lighting schedules. The intermittent light and dark periods ranged from 10-60 minutes, and a continuous 5 h treatment was done, along with control at growth promoting DLI. It was found that the shortest light period of 10 minutes significantly reduced the growth. The treatment of 20 min light/60 min dark and 60 min light/20 min dark yielded the same fresh and dry mass as the control, notable as the DLI was about 1/3 of the control. The 60 min light/60 min dark treatment resulted in the best reduction in growth while maintaining the quality metrics. The literature around this research question is limited, as most have not been focused on applying intermittent lighting to closed plant production. The work that has been done (Avgoustaki et al., 2020; Bhuiyan & van Iersel, 2021; Chen & Yang, 2018) is unveiling interesting trends which indicate there could be a 'sweet spot' in interval length to promote growth with lower total light application.

In conclusion, we were able to show that the growth of lettuce under constant DLI can be influenced by both photoperiod and intensity as well as intermittent lighting schedules.

5.2 Contribution to knowledge

This research is, to our knowledge, the first research investigating the influence of various lighting schedules under low total light specifically with the aim to 'pause' plant growth. Results show under constant total low DLI, variations in intensity and photoperiod and intermittent lighting influence growth. Results show under low light intensity, trends in varying photoperiod and intensity are opposite compared to high light. In intermittent lighting, results show there is a light interval length threshold between 20 and 60 minutes above which growth is promoted and under which is reduced.

5.3 Suggested Work

The trends of photoperiodic impact under constant reduced DLI were opposite that of reported research under growth-promoting light levels. This should be further investigated and repeated to see if results replicate in another environment. If so, these trials should be performed under a greater range of DLI to determine if there is a light level where the trends in growth change.

These results and reviewed literature indicate that under intermittent lighting, there is a length of light and dark interval which can promote the growth of light under lower total DLI. To further elucidate this trend, research should be done into these the use of intermittent lighting schedules under different DLI with intervals ranging from 20-120 minutes. The dark interval in the same range should also be investigated. The necessity for continuous darkness under these lighting schemes should be investigated, and if so, the ideal length should be defined.

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Appendix

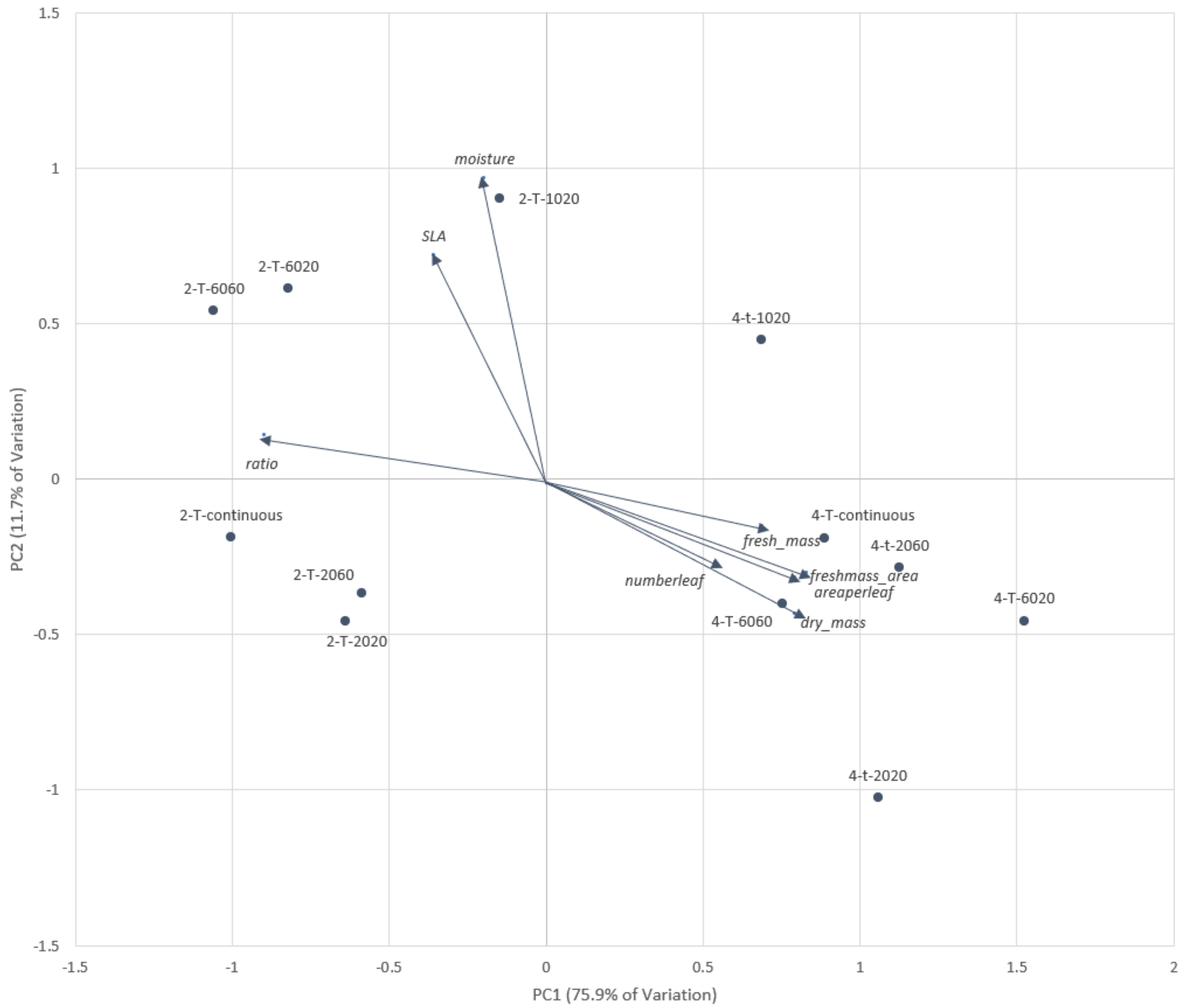

Figure A0-1 | Loading and score plot of principal component analysis (PCA) of data from 14 and 28 days after seeding (DAS) lettuce samples under low light treatments. 2 = 14 DAS samples, 4 = 28 DAS samples, T = treatment, NC = number of cycles, LD = ratio of light:dark of the intervals, CD = length of continual darkness, CL= length of one light cycle, ID = length of intermittent darkness.

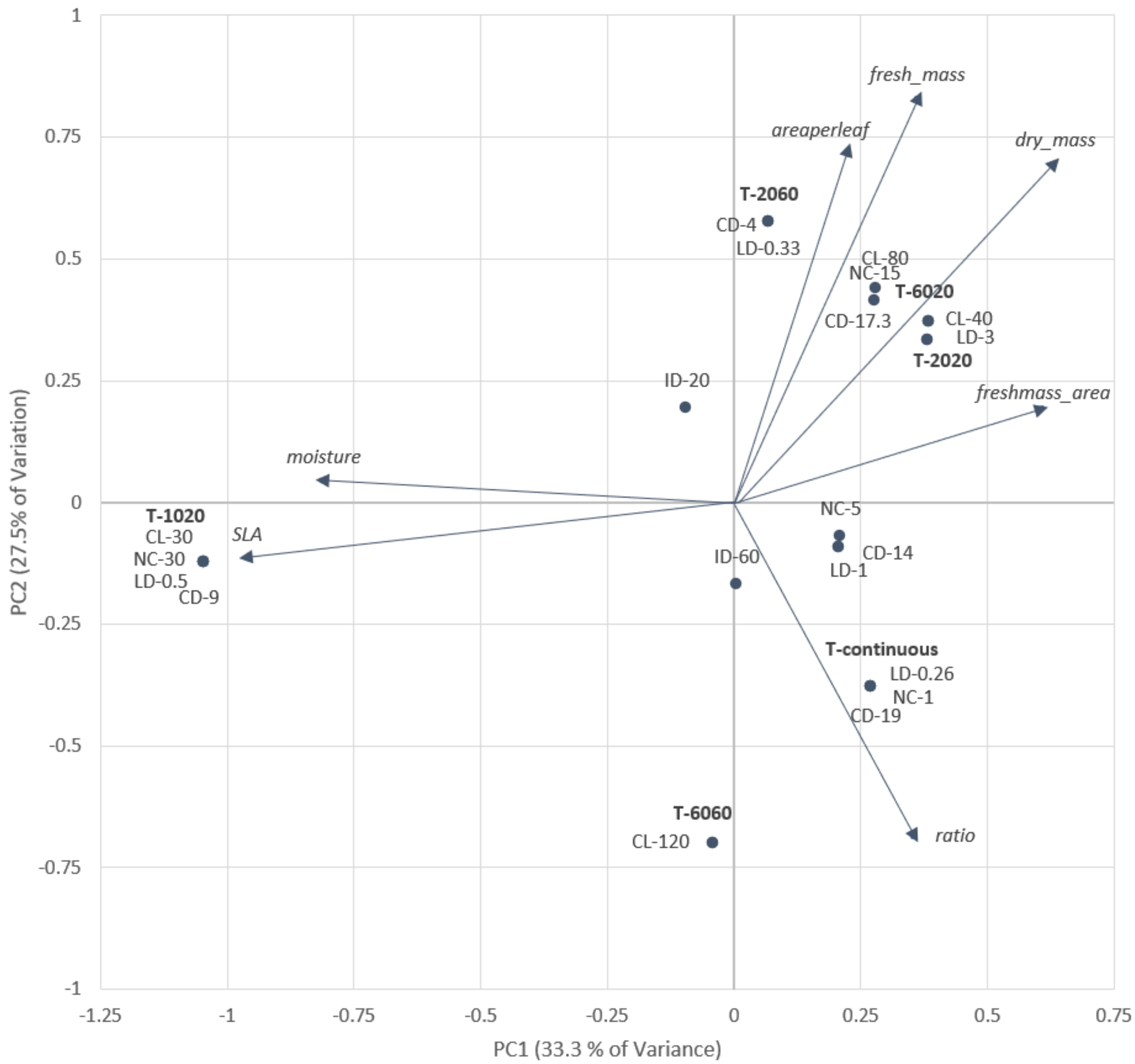

Figure A0-2 | Score and loading plot for 14 days after seeding (DAS) principal component analysis (PCA). 2 = 14 DAS samples, 4 = 28 DAS samples, T=treatment, NC= number of cycles, LD=ratio of light:dark of the intervals, CD= length of continual darkness, CL= length of one light cycle, ID = length of intermittent darkness

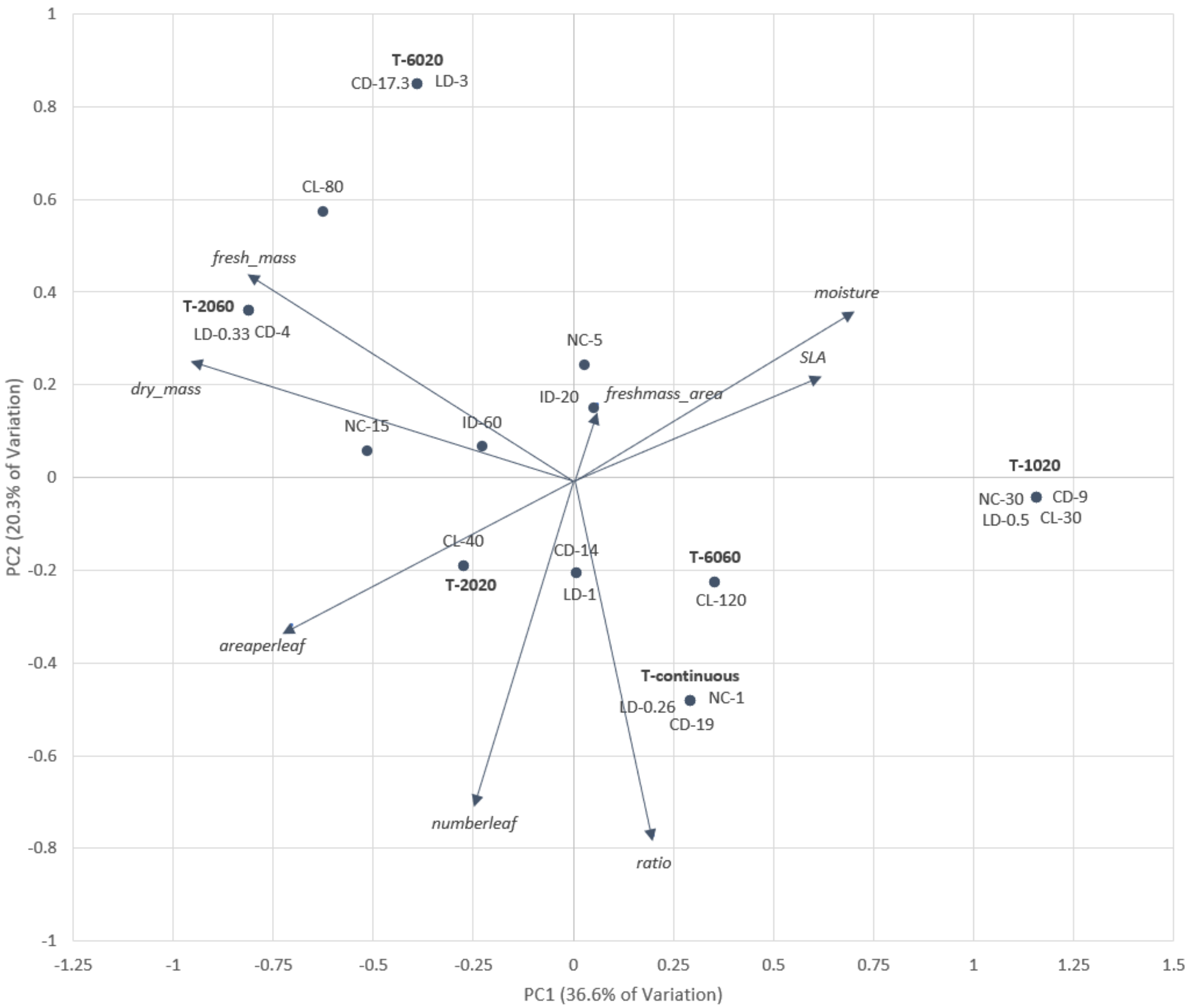

Figure A0-3 | Score and loading plot for 28 days after seeding (DAS) principal component analysis (PCA). 2 = 14 DAS samples, 4 = 28 DAS samples, T=treatment, NC= number of cycles, LD=ratio of light: dark of the intervals, CD= length of continual darkness, CL= length of one light cycle, ID = length of intermittent darkness

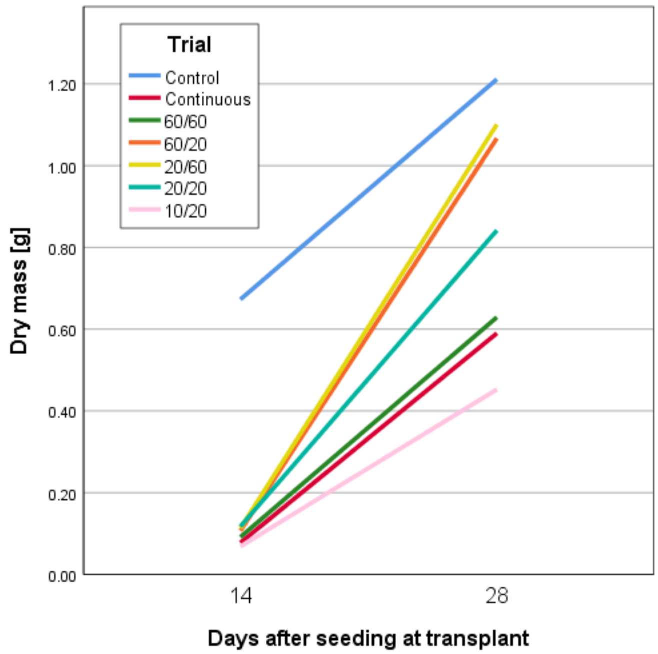

Figure A0-4 | Dry mass (g) of lettuce at harvest for 14 and 28 days after seeding DAS under control (daily light integral (DLI) = 13.75 mol·m^{-2}·d^{-1}) and reduced light (DLI=4.29 mol·m^{-2}·d^{-1}) treatment

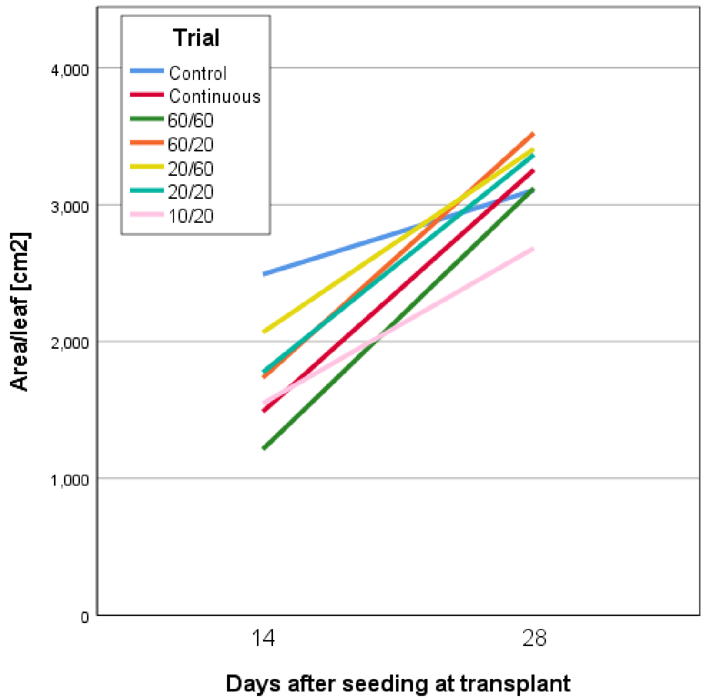

Figure A0-5 | Area per leaf (cm^2) of lettuce at harvest for 14 and 28 days after seeding (DAS) under control (daily light integral (DLI) = 13.75 $mol \cdot m^{-2} \cdot d^{-1}$) and reduced light (DLI=4.29 $mol \cdot m^{-2} \cdot d^{-1}$) treatment

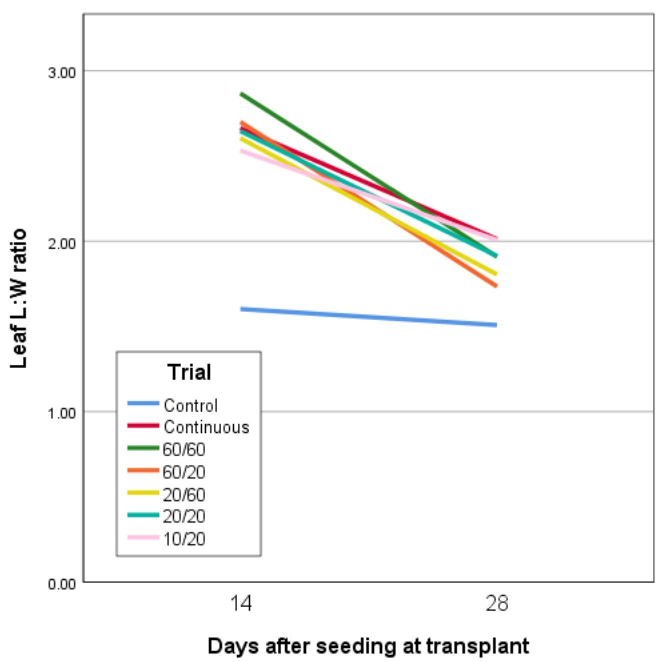

Figure A0-6 | Leaf length (L) to width (W) ratio of lettuce at harvest for 14 and 28 days after seeding (DAS) under control (daily light integral (DLI) = 13.75 mol·m^{-2}·d^{-1}) and reduced light (DLI=4.29 mol·m^{-2}·d^{-1}) treatment.

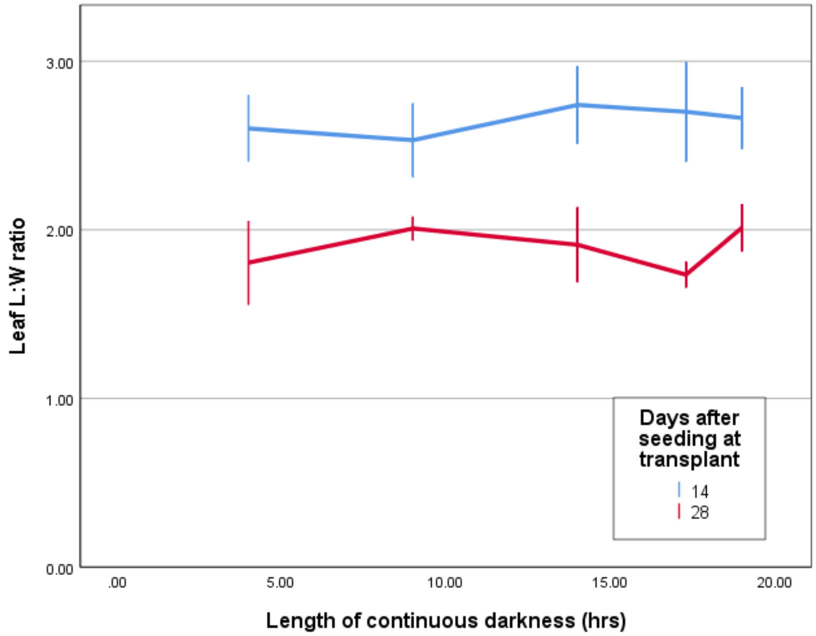

Figure A0-7 | Trend of leaf length (L) to width (W) ratio as the length of continuous darkness increases of lettuce 14 and 28 days after seeding (DAS) under low light treatment (daily light integral (DLI) =4.29 mol·m^{-2}·d^{-1}). Error bars show ± 1 standard deviation.

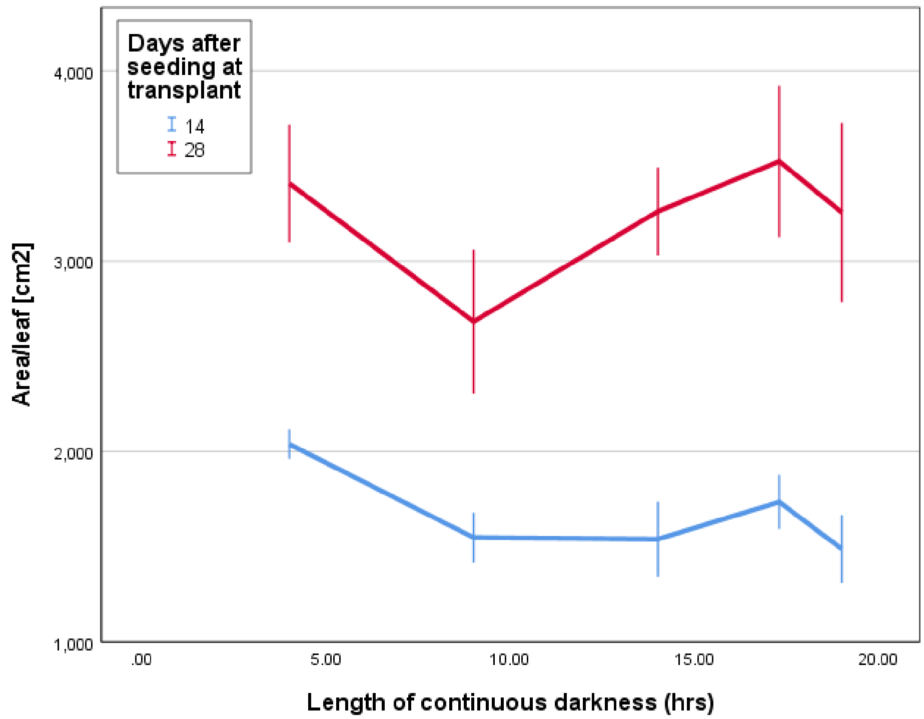

Figure A0-8 | Trend of area/leaf (cm^2) ratio as the length of continuous darkness increases of lettuce 14 and 28 days after seeding (DAS) under low light treatment (daily light integral (DLI)=4.29 $mol \cdot m^{-2} \cdot d^{-1}$). Error bars show ± 1 standard deviation.