From Lab to Greenhouse: Shedding Light on the Role of Spectral Quality and CO₂ Concentration in Tomato (*Solanum lycopersicum* L.) Production

By

Jason Lanoue

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ABSTRACT

FROM LAB TO GREENHOUSE: SHEDDING LIGHT ON THE ROLE OF SPECTRAL QUALITY AND CO\textsubscript{2} CONCENTRATION IN TOMATO (*Solanum lycopersicum* L.) PRODUCTION

Jason Lanoue
University of Guelph, 2020

Advisors:
Dr. Bernard Grodzinski
Dr. Xiuming Hao

During the light limiting winter months in Canada and other northern countries, supplementary lighting is needed during greenhouse production to meet consumer demand for fresh vegetables. The advancements in light-emitting diodes (LEDs) have made them a viable source for supplementary lighting, with many advantages over traditionally high pressure sodium (HPS) luminaries. Due to their low heat emittance, compact design, and ability to supply wavelength specific light, LEDs provide an unparalleled flexibility in the development and implementation of lighting strategies for crop production. This thesis is an investigation of spectral quality and CO\textsubscript{2} concentration and how these factors can affect tomato (*Solanum lycopersicum* L.) growth specifically tailored towards the implementation of LED lighting fixtures in commercial production. Fundamental pathways such as CO\textsubscript{2} and H\textsubscript{2}O gas exchange at both the leaf and whole plant level as well as the process of carbon export were examined under a variety of spectral qualities and CO\textsubscript{2} concentrations. Results from experiments involving whole plant gas exchange and carbon export were then used during a production style greenhouse experiment where the effects of continuous lighting on tomato physiology and yield were assessed. Results from this thesis aim to not only increase our understanding of how fundamental pathways respond to abiotic...
stress, but also how utilizing laboratory data can aid in the design and implementation of greenhouse lighting strategies to increase yield and sustainability of tomato production during light limiting periods.
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List of Abbreviations

AB: Alberta

AC: Ambient CO₂ (400 µL L⁻¹)

ADP: Adenosine diphosphate

ANOVA: Analysis of variance

ATP: Adenosine triphosphate

Ba¹⁴CO₃: radio-labelled barium carbonate

Bq: Becquerel

CAB13: Type III light harvesting chlorophyll a/b binding protein 13

CFL: Compact fluorescence light

Cₜ: Internal CO₂ concentration

CL: Continuous light

CLCT: Continuous light and constant temperature

CLVT: Continuous lighting and variable temperature

CO₂: Carbon dioxide

CRY: cryptochrome

DIT: Days into the treatment

DLI: Daily light integral

DNA: Deoxyribonucleic acid

EC: Elevated CO₂ (1000 µL L⁻¹)

FACE: Free-air concentration enrichment

Fₖ: Minimum fluorescence in a dark-adapted state (10 min of dark adaptation)

Fₘ: Maximum fluorescence in a dark-adapted state (10 min of dark adaptation)
\( F_v \): Variable fluorescence in a dark-adapted state \((F_m - F_o)\)

\( F_v/F_m \): Maximum quantum efficiency of PSII

FL: Florida

\( H^+ \): Hydrogen ion

\( H_2O \): Water (dihydrogen oxide)

GM: Geiger-Müller

GUT: Glucose transporter

G6P: Glucose 6-phosphate

HCl: Hydrochloric acid

HID: High intensity discharge

HPS: High pressure sodium

\( H_2SO_4 \): Sulfuric acid

IRGA: Infrared gas analyzer

KOH: Potassium hydroxide

\( K^+ \): Potassium ion

LCP: Light compensation point

LED: Light emitting diode

LSGC: Lighting Science Group Company

MA: Massachusetts

MB: Manitoba

MC: Mesophyll cell

MEXI: maltose excess I transporter

MH: Metal Halide
Min: Minute

mRNA: Messenger ribonucleic acid

n: Number of replicates

NADPH: Nicotinamide adenine dinucleotide phosphate

NaH¹⁴CO₃: Radio-labelled sodium bicarbonate

NCER: Net carbon exchange rate

NE: Nebraska

NY: New York

OH: Ohio

ON: Ontario

PAR: Photosynthetically active radiation

PCA: Principle component analysis

PHY: Phytochrome

Pᵢ: Inorganic phosphate

Pₙmax: Maximum light saturated photosynthetic rate as calculated using the equation \( y = y_o + a(1-e^{-b*x}) \)

Pₙ₅₀₀: Photosynthetic rate at a light level of 500 \( \mu \)mol m⁻² s⁻¹ PAR

PPC: Phloem parenchyma cell

PPE: Photosynthetic photon efficacy

ppm: Part per million

PSI: Photosystem I

PSII: Photosystem II

QY: Quantum yield (increase in the photosynthetic rate per additional photon)
r: Correlation coefficient
RPM: Revolutions per minute
RH: Relative humidity
RI: Rhode Island
RuBisCO: Ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP: Ribulose-1,5-bisphosphate
SAC: Short-term ambient CO₂ (Plants grown at EC but analyzed at AC)
SEC: Short-term elevated CO₂ (Plants grown at AC but analyzed at EC)
SPS: Sucrose phosphate synthase
SUT: Sucrose transporter
SWEET: Sugar will eventually be exported transporter
TC: Transfer cell
TPT: Triose phosphate translocator
USA: United States of America
UV: Ultraviolet
WUE: Water-use-efficiency
WUE₅₀₀: Water-use-efficiency at a light level of 500 µmol m⁻² s⁻¹ PAR
¹⁴C: Radio-labelled carbon
¹⁴CO₂: Radio-labelled carbon dioxide
Chapter 1: An Introduction

1.1 History of Tomato Production

Tomatoes (*Solanum lycopersicum* L.) originated from South America, specifically in the mountainous region of Peru and Ecuador (Encyclopaedia Britannica, 2018). Although generally observed to be red in colour, tomatoes can also be purple, green, orange or yellow and weigh anywhere from 20 grams to well over a kilogram depending on cultivar. Characterized as a fruit, the production of tomatoes can now be found around the world and is a staple cuisine of many cultures (Knapp, 2002).

In 2018, Ontario produced 6021 hectares of tomato, the vast majority of which (92.8%) being produced in conventional field agriculture (Ontario Ministry of Agriculture, Food and Rural Affairs, 2018). While controlled environment production (greenhouse) represented less than 8% of the total tomato production in Ontario, greenhouse tomato production is almost exclusively reserved for high value cultivars. For example, of the $444+ million tomato industry in Ontario, greenhouse production accounts for an astonishing 79% of the total tomato market value while representing less than 8% of total production (Ontario Ministry of Agriculture, Food and Rural Affairs, 2018). While the total farm gate value of field grown tomatoes has nearly doubled from 1979-2018, the farm gate value of greenhouse grown tomato has ballooned from $15.5 million to $355.2 million during the same period, representing nearly a 23-fold increase in value (Ontario Ministry of Agriculture, Food and Rural Affairs, 2018).

The vast majority of the increased value in greenhouse tomato production is due to the production of high value cultivars. While high value cultivars, which are normally sold as fresh fruit, can be grown in field production, the tendency is to produce them using greenhouse management. Greenhouse production offers many advantages over conventional field production for tomatoes. In Canada and many other northern latitude countries, field production seasons are relatively short and do not provide optimal conditions. Greenhouse crop production practices provide protection from cold temperatures while allowing for conditions, such as CO₂, temperature, light intensity, and light quality to be manipulated for optimal crop production.
One of the major obstacles during tomato production in the winter months is adequate light availability. Seasonal changes in the natural photoperiod can be sensed by plants and is an important regulator for many processes (photoperiodism) including the flowering process. Plants can be categorized into three main groups: short-day plants such as chrysanthemums, soybean, and cannabis; long-day plants such as spinach, petunia, and poppy; and day-neutral plants such as tomatoes, cucumbers, and rose. While these categories name the plants based on short or long days, it is in fact the length of the night which determines flower initiation. In short-day plants, flower initiation occurs when the night period length exceeds the critical photoperiod. Conversely, long-day plants flower when the night length falls below the critical photoperiod. For plants which are categorized as day-neutral, such as tomatoes, flower initiation occurs as a certain developmental stage, or due to some environmental stimuli and is not controlled by day length. Thus, understanding the effect of photoperiod length on morphological and physiological processes of tomato is important.

For tomato, a photoperiod up to 18h in length is associated with normal growth characteristics with 16-18h being the commercial standard (Demers & Gosselin, 2002). During much of the Canadian winter, natural solar photoperiods can be drastically shorter (<12h) and thus not allow for adequate growth rates for timely and profitable production. The addition of supplemental lighting during the light limiting months has been shown to increase vegetative biomass and yield within tomatoes (McAvoy & Janes, 1984). Traditionally, the use of high intensity discharge (HID) lights, specifically high pressure sodium (HPS) lamps has been employed in commercial tomato production. While HPS lights provide adequate light intensities to support production, the luminaries also have a large amount of energy converted to heat which, in some cases, can damage the plants (Cathey & Campbell, 1977).

More recently, the advent of light emitting diodes (LEDs) and the subsequent increase in their technological advancements compared to traditional lighting systems, has shown promise for the greenhouse industry. LED fixtures hold many advantages over traditional light sources. Unlike traditional lighting systems, LEDs are capable of emitting high intensity light while having a low heat emitting face. This characteristic allows for LED fixtures to be placed very close to plant tissue without harm, allowing for use as inter-canopy lights. LED lights are also now generally
more electrically efficient than traditional lighting systems which offset the high initial cost of purchase, allowing for more economical implementation for growers (Singh et al., 2015).

Furthermore, potentially the most advantageous aspect of LED lighting fixtures is the ability to emit wavelength specific light. Simply by altering the semi-conducting material composition, LEDs can produce various wavelengths of light, something which is difficult to do with traditional lighting systems. Having the capability to provide very narrow wavelengths to plants is one of the most useful properties of LED lights as it is known photosynthesis is driven by certain colours better than others (Mackinney, 1941; Sæbø et al., 1995). Furthermore, wavelength specific lighting can also alter plants function through non-photosynthetic processes such as changes in morphology, physiology, genetic responses, and chemical compound formation (Lanoue et al., 2019a). The following section will briefly detail the current literature related to individual wavelengths as it pertains to plant biology.

### 1.2 Effects of Spectral Quality on Plant Biology

#### 1.2.1 Red Light

Red light is a region of the visible light spectrum between approximately 625-740nm and thus has the lowest energy associated with any colour of visible light. Red light is absorbed highly by both chlorophyll \(a\) and \(b\) and thus is generally thought to drive high rates of photosynthesis (Mackinney, 1941; Hogewoning et al., 2012). However, when plants are exposed or grown under sole red illumination, they generally have a lower photosynthetic rate than when red is used in combination with blue light (Hogewoning et al., 2010). It has been proposed that under monochromatic red light, there is an imbalance between photosystem I and photosystem II which produces the lower photosynthetic rate (Tennessen et al., 1994). Both tomato and cucumber plants grown under sole red light have been shown to have a reduced stomatal conductance rate compared to plants grown under either a white light control or red light with additional blue light (Hogewoning et al., 2010; Liu et al., 2012). Thus, although red light is known to have an effect on the photosynthetic process directly, it can also affect stomatal regulation down stream, indirectly affecting photosynthesis.

Red light is also absorbed by the plant photoreceptor phytochrome. Absorption of red light by phytochrome is able to have effects on gene regulation which control functions such as transcription factors, cell metabolism, cell signaling, hormone-pathways, stress response, and
overall plant morphology (Tepperman et al., 2004). For example, in tomato, growth under sole red light increased stem elongation but decreased overall plant biomass compared to plants grown under white light (Liu et al., 2012). Such subtle morphological differences, such as increases in stem elongation due to red light, can have a large affect on growth when in a greenhouse production setting. Alterations in plant morphology can alter the canopy architecture and thus the light environment. In that way, subtle changes can have a large indirect effect on whole plant photosynthesis and over the course of production can have a substantial impact of overall biomass accumulation and yield (Lanoue et al., 2018b).

1.2.2 Orange Light

Orange light (560-625nm) is also described in the literature as amber or yellow light. Although orange light is a large component of the natural sun light spectrum and the HPS spectrum, it is grossly under researched as a sole light source. Liu et al., (2012) grew tomato plants under orange light and observed an increase in plant height compared to plants grown under a white light treatment. Physiologically, a recent study in which chrysanthemums were grown under white light but analyzed under different spectra, plants analyzed under an orange LED produced a high photosynthetic maximum (Leonardos et al., 2019). In fact, chrysanthemum plants analyzed under orange light produced a higher photosynthetic max than did plants analyzed under blue light, a colour normally associated with being highly absorbed by chlorophyll $a$ and $b$ (Leonardos et al., 2019).

Some research has suggested that the addition of orange light to a light treatment may affect the concentration of secondary metabolites. Radish sprouts grown under HPS lighting with a superimposed flashing orange light showed an increase in phenolic content compared to sprouts grown solely under HPS lighting (Urbinavičiūte et al., 2009). Similarly, baby leaf lettuce has also been shown to have increased phenolic concentration when grown under a light spectrum which had an enriched orange light component (Samuolienė et al., 2013). However, when examining other secondary metabolites, namely antioxidant concentrations, wheatgrass, barleygrass, and baby leaf lettuce showed similar concentrations when grown under spectra with or without orange light (Urbinavičiūte et al., 2009; Samuolienė et al., 2013). Thus, the effect of orange light may vary between species and even between secondary metabolites.
Research pertaining to the role that orange light could potentially play in a plant production setting has been poorly studied. One potential reason for this could be the energy conversion factor of orange LEDs. While blue, red, white, and, to some extent, green LEDs can be commonly found in everyday life (i.e., street lights, house hold light bulbs, business signs, etc.), narrow bandwidth orange LEDs are not as abundant. Thus, while LEDs can have high energy conversion factors upwards of 2 µmol J⁻¹, the technology used to manufacture orange LEDs has not been pushed forward by industrial demand like other wavelengths. While some research has been done using orange light, much experimentation is still needed as orange lights could potentially play a key role in light recipes in order to maximize biomass gain and ultimately yield.

1.2.3 Green Light

Green light is often thought of as the least useful of all wavelengths when it comes to plant growth. However, this is not the case. While green light is the least absorbed of all wavelengths, the reason why leaves appear green, it plays a vital role in regulating growth and photomorphogenic processes. Only around 10-50% of green light is reflected by the plant with the remainder being absorbed or transmitted through the leaf (Smith et al., 2017). The light which is absorbed is capable of exciting an electron and initiating photosynthesis similar to all other wavelengths (Lanoue et al., 2017). Indeed, cucumber leaves exposed to green light were observed to produce a higher quantum yield of CO₂ fixation (CO₂ fixed per photon) than exposure to blue light (Hogewoning et al., 2012). Furthermore, green light plays a vital role in many other physiological and morphological processes (Folta et al., 2007). One role of green light seems to be as an antagonist to the effects of blue light. For example, blue light is generally observed to decrease stem elongation in plants whereas green light drastically increased the rate of stem elongation (Folta et al., 2007). In fact, the rate of stem elongation of plants exposed to green light is even faster than from plants which are kept in darkness (Hernández & Kubota, 2012). Furthermore, the addition of green light to a red and blue light treatment showed an increase in stem elongation rates compared to plants without green light (Folta et al., 2007).

Similar to stem elongation, lettuce plants exposed to a red, blue, and green light treatment had an increased leaf area compared to plants which lacked the green light component (Kim et al., 2004). Taken together, increased stem elongation and enhanced leaf expansion are typical of a shade avoidance response (Zhang et al., 2011). Combining leaf optics and with the phenotype of plants
under green light, the ecological response makes intuitive sense. In a large vining greenhouse crop, leaves within the inner canopy region receive less ambient light than do leaves in the upper regions of the canopy. However, the light which does penetrate through the canopy is highly enriched in the green component. The elevated green component is due to leaf reflecting green light, bouncing it through the canopy and green light transmitting through the leaf (Terashima et al., 2009). This elevated green component will induce a shade avoidance response, causing greater leaf expansion in an attempt to capture more light which in turn will increase the plants overall photosynthesis. Thus, the implementation of green light into light recipes may increase overall plant growth (Kaiser et al., 2019a).

Green light not only plays a direct role in photosynthesis, but also an indirect role via the regulation of stomatal opening. In lettuce plants grown under sole green LEDs, leaves were observed to have a decrease number of stomata compared to leaves grown under either red or blue LEDs (Muneer et al., 2014). These intrinsic changes in leaf morphology led to a reduction in stomatal conductance values and transpiration rates when leaves are grown under green light (Muneer et al., 2014). While changes in leaf morphology are due to long-term exposure to green light, green light can also immediately affect stomatal behaviour. In a clever ‘light switch’ experiment, Frechilla et al. (2000), demonstrated that exposing *Vicia faba* leaves to blue light, followed by a pulse of green light, followed again by exposure to blue light showed immediate, light induced stomatal opening, closure, and re-opening in response to the respective light changes (Frechilla et al., 2000). A similar decrease in transpiration rate was observed by tomato plants grown under white light when leaves were exposure to green light compared to blue light (Lanoue et al., 2017; Lanoue et al., 2018b).

### 1.2.4 Blue Light

Blue light (400-495nm) has the highest photon energy out of any wavelength of light within the photosynthetically active radiation (PAR) region. Similar to red light, blue light is highly absorbed by both chlorophyll *a* and *b* which corresponds with the photosynthetic action spectrum from isolated chloroplast and absorption spectrum from intact leaf systems (McCree, 1972; Kasperbauer, 1987). The photosynthetic response to the addition of blue light is highly variable among species and can even change within a species depending on the amount of blue light present (Snowden et al., 2016). Compared to a sole red light spectrum, cucumber, radish, pepper, and
lettuce have shown increased photosynthetic rates when grown under a mix of red and blue light (Snowden et al., 2016). However, in tomato, no increase in the photosynthetic rate was observed when 16% blue light was added to a red light spectrum compared to a sole red light spectrum (Hernández & Kubota, 2012). However, a species-specific response to blue light has been observed. In cucumbers, the addition of 50% blue light to a red light spectrum increased the photosynthetic maximum of leaves, which in turn translated to increased growth (Hogewonning et al., 2010).

The addition of blue light to a red light spectrum has generally been shown to increase biomass under controlled environment conditions which lack natural solar radiation (Goins et al., 1997). Under greenhouse conditions (i.e. solar radiation present), tomato plants were observed to have a larger plant biomass when some blue light (6-12%) was used for supplemental lighting compared to sole red lighting (Kaiser et al., 2019b). Despite, increased stem elongation and leaf expansion under red supplementation which should increase overall light absorption, the addition of some blue light increased overall plant photosynthetic rates likely due to increases in chlorophyll concentrations and a more balanced photochemistry (Liu et al., 2012). However, increasing the supplemental blue light component to 26% of the total light intensity caused a reduction in total biomass accumulation (Kaiser et al., 2019b). Thus, the implementation of blue light to a supplemental light regime needs to be done cautiously.

Indirectly, blue light can influence CO$_2$ and H$_2$O gas exchange via control of stomatal function. The effect of blue light on stomatal opening is one of the most well studied phenomena in plant science (Assmann & Shimazaki, 1999; Kinoshita et al., 2001; Kinoshita et al., 2003; Shimazaki et al., 2007; Inoue & Kinoshita 2017). Even low levels (5 £mol m$^{-2}$ s$^{-1}$) of blue light has been shown to induce stomatal opening (Kinoshita et al., 2001). Many theories have been postulated as to the mechanistic explanation for blue induced stomatal opening including mediation via blue light sensitive photoreceptors (Kinoshita et al., 2001; Mao et al., 2005) and degradation of starch within the stomatal guard cell (Horrer et al., 2016). However, all of these mechanisms tend to have a common starting point which is the stimulation of the blue light absorbing photoreceptors, phototropin 1 and 2 (Kinoshita et al., 2001). Upon the absorption of blue light, a signal cascade is started with the end result being H$^+$ efflux from the guard cell, causing hyperpolarization leading to an influx of K$^+$ ions and ultimately stomatal opening (Kinoshita et al., 2001). The physiological
response to stomatal opening results in a higher water loss via transpiration from the leaf (Hogewoning et al., 2010; Lanoue et al., 2019a), and a lower water-use-efficiency than plants grown under light treatments with low components of blue light.

While blue light can have a large role in controlling CO$_2$ and H$_2$O gas exchange and thus photosynthesis, it also plays a large role in phenotypic responses of plants. With the addition of blue light to a light spectrum, radish, soybean, tomatoes, and peppers were observed to be shorter than plants grown under sole red light (Liu et al., 2012; Snowden et al., 2016). This reduction in plant height has been linked to the role of cryptochrome as plants lacking either cryptochrome 1 or 2 were observed to have increased relative growth rates compared to wild-type plants under blue light (Parks et al., 2001). However, too much blue light can cause an unexpected result. In cucumber seedlings grown under sole blue LEDs, plant height was observed to increase compared to plants grown under sole red light (Hernández & Kubota, 2016). The increased plant height from plants grown under sole blue light is inconsistent with the general trend: as blue light increases, plant height decrease. While an exact mechanism for this phenomenon has yet to be produced, some hypotheses have been proposed. One hypothesis states that the lack of far-red light within an all blue light spectrum could contribute to the increased stem elongation (Hernández & Kubota, 2016). Another suggests a cooperative role between cryptochrome and phytochrome in suppressing stem elongation which would not occur in the sole blue light treatment (Folta & Spalding, 2001).

**1.2.5 Spectral Quality and Primary Light Reactions**

Photosynthesis is broken down into two main reactions: light-dependent reaction and light-independent reactions. As the name suggests the light-dependent reactions require light energy in order to take place. Light is absorbed from a source via the light harvesting complex in photosystem II. The light harvesting complex is made up of many protein subunits which ultimately move an electron from the water splitting reaction to the primary electron acceptor, chlorophyll $a$. Upon excitation of photosystem II, the electron is moved through the plastoquinone pool, Cyt $b_6/f$ complex, and plastocyanin, creating a proton gradient and ultimately ATP, to photosystem I where it is again excited by light absorption (i.e., classical “z” scheme; Yamori & Shikanai, 2016). The electron is then transferred to ferredoxin to ultimately reduce NADP$^+$ to NADPH which is used in the Calvin cycle to fix CO$_2$ during the light-independent reactions.
This traditional linear electron flow is also accompanied with cyclic electron flow around photosystem I (PSI) which helps balance the production of ATP and NADPH. In PSI cyclic electron flow, the electron passed to ferredoxin is transferred back to the plastoquinone pool. In this process, a proton gradient is still produced as is ATP without the production of NADPH. It is thought that the transition to cyclic electron flow aids in the control of reduced species (Munekage et al., 2004).

With the introduction of LEDs, the processes surround the primary light reactions can be examined under narrow waveband light. The state transition between state 1 (linear electron flow) and state 2 (cyclic electron flow) has been associated with the association of light harvesting complex II (LHCII) with either PSII or PSI (Shapiguzov et al., 2010). These state transitions are regulated by phosphorylation of a LCHII component. When dephosphorylated, LCHII is in state 1 and associated with PSII causing linear electron flow (Shapiguzov et al., 2010). When phosphorylated, state 2 is favoured and LCHII transition to PSI causing cyclic electron flow (Shapiguzov et al., 2010). Interestingly, these state transitions are linked to light quality. Under exposure to far-red light, state 1 was preferred and linear electron flow was favoured (Shapiguzov et al., 2010). However, under blue light, phosphorylation occurs and state 2 is initiated, preferentially driving cyclic electron flow (Shapiguzov et al., 2010; Miao et al., 2016).

State transitions are also important during fluctuating light environments, such as those in the forest understory and within the canopy of hire-wire greenhouse crop productions. Under short period of high intensity light, the transition from state 1 to state 2 helps to maintain ATP/NADPH balance and prevents the over-reduction of the plastoquinone pool (Grieco et al., 2012). Notably, there are also alterations in the blue:far-red which occurs during fluctuating light conditions, known to affect state transitions (Shapiguzov et al., 2010; Miao et al., 2016). Thus, via spectral manipulation, the balance of ATP/NADPH production can be optimized to maintain high levels of photosynthesis by avoiding over-reduced plastoquinone pools (Greieco et al., 2012; Miao et al., 2016).

1.3 Role of Elevated CO₂ in Plant Production

Fixation of atmospheric CO₂ makes up the backbone of plant growth from DNA to cell walls to secondary metabolites such as antioxidants. Ribulose-1,5-bisphosphate carboxylase/oxygenase
(RuBisCO) catalyzes the carboxylation of ribulose-1,5-bisphosphate (RuBP) in the first step of the Calvin Cycle eventually ending with the production of sugar molecules needed for plant growth. With the atmospheric level of CO2 on the rise globally (Booth et al., 2017), the effect elevated levels of CO2 can have on plant production is of grave importance. The increase in CO2 levels has positive theoretical consequences for plant growth. While RuBisCO is mostly thought of as catalyzing a carboxylation reaction, it can also catalyze an oxygenation reaction. Under elevated CO2 conditions the carboxylation reaction becomes favoured (compared to ambient CO2 conditions), and thus increases photosynthetic rates and produces higher amounts of sugars and building blocks for plant growth.

Across a broad range of agricultural plant species, a yield increase of approximately 33% has been observed with a doubling of the current atmospheric CO2 concentration (Kimball, 1983). In controlled environment agricultural production systems, the artificial increases in CO2 concentrations has led to increased production in C3 species such as tomato and pepper (Fierro et al., 1994). However, this increase seems to only hold true when plants are not sink limited. For instance, under long-term elevated CO2 concentrations carbon fixation rates are observed to reduce due to end product feedback inhibition (Harley et al., 1992). Indeed, production of tomato plants was reduced when CO2 levels were drastically increased (i.e., 2000 µL L⁻¹ or above; Madsen, 1974).

While elevated CO2 levels can affect plant growth directly via altered photosynthetic levels, it can also affect plant growth indirectly through stomatal control and changes in plant morphology. In general, stomatal conductance levels decrease by approximately 23% with a doubling of atmospheric CO2 levels (Field et al., 1995). While the exact mechanism responsible for stomatal closure due to elevated CO2 levels are unknown, ultimately, the increased efflux of K⁺ from guard cells facilitates the closure (Ainsworth & Rogers, 2007). Morphologically, elevated CO2 is observed to increase biomass accumulation in the leaves, stem, and roots of four-week-old cotton plants (Delucia et al., 1985). Furthermore, leaf area was also observed to increased under elevated CO2 (Delucia et al., 1985). However, the mechanism of increased biomass accumulation is somewhat unknown due to the vast array of physiological and morphological processes which can be affected by an increase CO2 concentration (Mott, 1990). For example, an increased CO2 concentration can lead to taller plants which can reduce mutual shading and place plants closer to
supplementary lighting fixtures allowing for increased light absorption. These interactions between different abiotic factors make it essential to not only examine plants under isolated stressors but also the relationship between many.

While both alterations in light quality and CO₂ concentrations can affect plant morphology and physiology separately, the integration of both is needed to understand how two common environmental parameters interact. In strawberry grown under ambient CO₂ (400 µL L⁻¹) and elevated CO₂ (1200 µL L⁻¹) under three different light qualities did not show an interaction between CO₂ concentration and light quality (Wu et al., 2012). Indeed, differences in shoot fresh weight between the three light qualities at ambient CO₂ were similar when analyzed at elevated CO₂ (Wu et al., 2012). However, due to the affects of both CO₂ and spectral quality can have on virtually all aspects of plant biology, research pertaining to the interaction between these factors needs much research.

1.4 Thesis Overview

How does spectral quality affect plant growth? Since discovering the array of light wavelengths that exist when sunlight was passed through a prism, this simple question has been asked by many, and many have attempted to answer it. As technology has improved, gone are the times of using prisms or filters to produce semi-selective wavelengths for research purposes. We now live in an age where LEDs allow us to make minute nanometer changes in wavelength while being more energy efficient, which has prompted scientist and growers to rethink the way plants are grown. However, despite advanced technological improvements in LEDs and light quality control, the question remains the same: How does spectral quality effect plant growth?

This thesis is a compilation of published research papers which examine how spectral quality and changes in CO₂ concentration can affect plant growth in multiple different physiological ways. Here, the effect of spectral quality and CO₂ concentration on the process of carbon export (Chapter 2) and whole plant gas exchange (Chapter 3) are examined. The knowledge acquired during these experiments was then implemented in a production style greenhouse setting where the effect of continuous lighting was tested (Chapter 4). Many research questions were posed along the way:

1) Does spectral quality have an effect on the rate at which photo-assimilates (sugars) are exported from a tomato leaf? (Chapter 2).
2) Is there an interaction between spectral quality and CO$_2$ concentration with respect to carbon export? (Chapter 2).

3) Does spectral quality alter primary whole plant gas exchange of a tomato plant? (Chapter 3).

4) Does short-term exposure to elevated CO$_2$ or growth under elevated CO$_2$ interact with spectral quality to affect whole plant CO$_2$ or H$_2$O gas exchange? (Chapter 3).

5) Can tomato plants be grown under continuous lighting using alternating red and blue spectrum supplemental lighting? (Chapter 4).

The objective of the thesis was two-fold: to improve our understanding of how plants are affected by spectral quality, and investigate a fundamental process believed to link photosynthesis to overall production. While photo-assimilates are produced primarily within the leaves of a tomato plant, the fruit is the marketable product. Thus, the process of moving sugars from the source (leaf) to the sink (fruit) is of fundamental importance. In tomatoes, this is a complicated multi-cellular process which is controlled by enzymes and transporters. It is this complexity and regulation which was also hypothesized to be controlled by spectral quality. Essentially, by exposing tomato leaves to various spectral qualities, the aim was to determine if the rate of carbon export could be increased, and thus have the potential for increased yield.

Leaves are the main photosynthetic organ of a tomato plant. However, simply extrapolating data acquired during leaf level experimentation does not allow for a detailed picture of what happens at a whole plant level. Thus, whole plant gas analysis is an essential part of understanding the effects of spectral quality on plant growth. By examining whole plants under different spectral qualities and CO$_2$ concentrations, the nuances of different plant architecture, leaf age, and microclimates can be explored. Using this multi-faceted approach involving leaf and whole plant measurements, a better understanding of the plant as a living organism can be determined. This knowledge can then aid growers and scientists in determining how tomato plants may react to changes in environmental parameters.

The final step was to use the knowledge acquired on carbon export, whole plant gas exchange and other relevant literature, and perform a production-style greenhouse experiment in order to see if laboratory theory would lead to a real-world production increase. For this, we chose to examine if
continuous lighting (24h) using an alternating red and blue LED light treatment was conducive for plant growth. Essentially, the question being asked was: Do plants need sleep? While it has been hypothesized that growth under continuous lighting would lead to increase in growth and production, traditionally it was observed that tomato plants grown under continuous lighting become chlorotic and performed poorly. However, we believed that alternating the spectrum of light given to the plants (red during the day (12h) and blue at night (12h)) could alleviate some of the typical injury associated with continuous lighting and potentially increase yield.

Thus, this thesis represents the evolution of the relationship between spectral quality, CO₂ concentration, and plant growth. It begins by trying to bring novel understand to the inner workings of a fundamental biochemical pathway and culminates with the use of a novel technique which could provide increased yield and energy savings simply by altering the spectral quality over a 24h period.
Chapter 2: Effects of Light Quality and Intensity on Diurnal Patterns and Rates of Photo-assimilate Translocation and Transpiration in Tomato Leaves

The work was described in a manuscript accepted in Frontiers in Plant Science: Crop and Product Physiology section and can be found at:


The manuscript has been altered to adhere to the University of Guelph thesis format. Additional information has also been added.

**Contributions:**

Jason Lanoue and Dr. Bernard Grodzinski designed the study. Jason Lanoue performed the experiments, collected the data, and analysed all data. Jason Lanoue prepared the manuscript. Jason Lanoue, Dr. Evangelos D. Leonardos, and Dr. Bernard Grodzinski reviewed and edited the manuscript.
2.1 Abstract

Translocation of assimilates is a fundamental process involving carbon and water balance affecting source/sink relationships. Diurnal patterns of CO\textsubscript{2} exchange, translocation (carbon export), and transpiration of an intact tomato source leaf were determined during \textsuperscript{14}CO\textsubscript{2} steady-state labelling under different wavelengths at pre-set photosynthetic rates. Daily patterns showed that photosynthesis and export were supported by all wavelengths of light tested including orange and green. Export in the light, under all wavelengths was always higher than that at night. Export in the light varied from 65-83\% of the total daily carbon fixed, depending on light intensity. Photosynthesis and export were highly correlated under all wavelengths (r=0.90-0.96). Export as a percentage of photosynthesis (relative export) decreased as photosynthesis increased by increasing light intensity under all wavelengths. These data indicate an upper limit for export under all spectral conditions. Interestingly, only at the medium photosynthetic rate, relative export under the blue and the orange light-emitting diodes (LEDs) were higher than under white and red-white LEDs. Stomatal conductance, transpiration rates, and water-use-efficiency showed similar daily patterns under all wavelengths. Illuminating tomato leaves with different spectral quality resulted in similar carbon export rates, but stomatal conductance and transpiration rates varied due to wavelength specific control of stomatal function. Thus, we caution that the link between transpiration and carbon export may be more complex than previously thought. In summary, these data indicate that orange and green LEDs, not simply the traditionally used red and blue LEDs, should be considered and tested when designing lighting systems for optimizing source leaf strength during plant production in controlled environment systems. In addition, knowledge related to the interplay between water and carbon movement within a plant and how they are affected by environmental stimuli, is needed to develop a better understanding of source/sink relationships.

2.2 Introduction

Different light quality, as well as intensity, provided by wavelength specific light-emitting diodes (LEDs) have been shown to affect both leaf CO\textsubscript{2} fixation and transpiration in tomato (Lanoue et al., 2017). In addition to photosynthesis, export is also a key process of a source leaf defining its strength. Export involves both the movement of carbon and water through the translocation conduit. For growth of a plant to actually occur, the photo-assimilates synthesized in leaves must be exported to growing sink tissues. Up to 80\% of fixed carbon is exported via phloem
translocation in the day and night periods (Grange, 1985; Geiger & Servaites, 1994; Lemoine et al., 2013). Importantly in studies using attached leaves, it is clear that most export occurs in the day-time rather than during subsequent night periods (Kalt-Torres et al., 1986; Leonardos & Grodzinski, 2000; Leonardos et al., 2003). Translocation at night can involve the breakdown of starch as well as mobilization of sugars (Geiger & Servaites, 1994; Lemoine et al., 2013). An important consideration regarding source/sink flow of assimilates is that the key processes which regulate translocation are not merely the classical enzymatic pathways of sucrose and starch metabolism (Geiger & Servaites, 1994; Lemoine et al., 2013), but also steps involving many transporters and temporary storage sites (Farrar & Farrar, 1986; Lemoine et al., 2013; Osorio et al., 2014).

Notably, at ambient CO₂ conditions and high light intensity, a high correlation is observed between photosynthesis and export in many C₃ and C₄ species (Grodzinski et al., 1998; Leonardos & Grodzinski, 2000). In tomato, a sucrose exporter, sucrose concentration and carbon export rates are highly correlated across a wide range of photosynthetic rates (Ho, 1976). An increase of carbon fixation of 1 mg C dm⁻² h⁻¹ results in an increase in export rate of 0.59 mg C dm⁻² h⁻¹ quantified using differential leaf weight analysis (Ho, 1976). Although much is known about sugar synthesis during photosynthesis, little is known about how light quality alters translocation patterns of attached source leaves.

An early study of translocation using detached sugarcane leaves at low light levels indicated that green light caused a decrease in translocation compared to red and blue (Hartt 1966). Furthermore, in a similarly designed ¹⁴CO₂ pulse-chase experiment performed with intact parsnip leaflets, no differences in translocation rates under different light spectra were observed (Hoddinott & Gorham, 1975). In order to deploy new LED technology for controlled environments the role of spectral quality on gas exchanges and export functions of the source leaf are required.

The inter-play between long-distance assimilate movement via the phloem (Münch, 1930) and the movement of H₂O within the xylem via transpiration (Dixon & Joy, 1894) is still poorly understood (Windt et al., 2006; Nikinmaa et al., 2013). Although studies with woody species using MRI or theoretical modelling have suggested that transpiration rate affects export (Windt et al., 2006; Nikinmaa et al., 2013), there has been very little experimental data linking H₂O and CO₂ exchanges with the mobility of H₂O and assimilates via translocation using intact herbaceous
plants (Johnson *et al.*, 1992). Furthermore, our understanding of carbon fixation and translocation suffers from the fact that very little data exists where researchers have used intact, attached leaves so that tissue turgor and metabolism have both not been jeopardised.

Regulation of plant growth and development is fine-tuned by photoreceptors (i.e. cryptochrome, phytochrome, etc.) responding to light of different spectral quality throughout the day (Chen *et al.*, 2004). There is a long-distance interplay between source and sink interactions that occurs throughout the day. It is well known that growth patterns of sink organs change in response to environmental stimuli including light intensity and quality (Heuvelink, 1989; Bertam & Karlsen, 1994; Lin, 2000a; Nozue & Maloof, 2006; Liu *et al.*, 2012). For example, long term acclimation to blue light causes tomato plants to be shorter (Lin, 2000a; Liu *et al.*, 2012). Thus, the problem with studying export from source leaves of plants which have been acclimated to wavelength specific lighting, is that one is comparing source/sink relationships in plants with different morphology and anatomy. Therefore, information about the effect of spectral quality on export is required before moving to plants acclimated to different abiotic stresses such as those occurring in greenhouses during the implementation of inter-canopy lighting (Hao *et al.*, 2012; Gomez & Mitchell, 2014).

With such a large gap existing in our understanding of source-to-sink metabolism with regards to light quality, we set out to challenge intact, attached source leaves with wavelength specific lighting and measure diurnal patterns of gas exchanges and carbon export. We hypothesize, that, due to the complexity of the carbon export pathway within the source leaf, the effects of wavelength specific lighting can be manifested independently of the effects of light quality on the primary photosynthetic reactions in the chloroplast. We developed a novel methodology to study diurnal patterns of photosynthesis and export, using non-acclimated source leaves under maximized sink demand. We used a steady-state $^{14}$CO$_2$ labelling technique and exposed mature tomato source leaves to LEDs with differing spectral quality. To better compare the diurnal carbon export patterns, we purposely established different photosynthetic rates at the beginning of the photoperiod to drive similar initial CO$_2$ influx rates among all lights. We also examined if there was any link between intact source leaf transpiration and carbon export.
2.3 Materials and Methods

2.3.1 Plant Material and Growth Conditions

Seeds of *Solanum lycopersicum* L. cv. ‘Bonny Best’ from William Dam Seeds (Dundas, ON, Canada) or cv. ‘Foronti’ from De Ruiter (Leamington, Ontario, Canada) were sown into 60 cavity potting trays in Sungro professional growing mix #1 (Soba Beach, AB, Canada) under a clear plastic lid to maintain a high relative humidity (RH; ~85%) and placed in a growth chamber (GC-20 Bigfoot series, Biochambers, Winnipeg, MB, Canada) at 22/18°C (day/night) with a 16/8h photoperiod and 200±25 μmol m$^{-2}$ s$^{-1}$ of photosynthetically active radiation (PAR) from compact fluorescence lights (CFLs; Sylvania Pentron 841 HO Ecologic, Wilmington, MA, USA; Figure 2.1). These cultivars were chosen to compare the potential difference between a determinate field tomato (cv. ‘Bonny Best’) and a common indeterminate greenhouse tomato (cv. ‘Foronti’). After germination, cv. ‘Bonny Best’ and ‘Foronti’ lids were removed and plants were grown at 65±10% RH, ambient CO$_2$ (400 µL L$^{-1}$), and 300±25 μmol m$^{-2}$ s$^{-1}$ PAR at canopy level. In addition, cv. ‘Foronti’ were grown under a CO$_2$ concentration of 1000 µL L$^{-1}$ during the day (06:00-22:00) and 400 µL L$^{-1}$ during the night period (22:00-06:00). Plants were watered with fertilizer (20-8-20; pH=6, EC=2.3mS cm$^{-1}$).

2.3.1 Leaf Gas Exchange and $^{14}$C-Export

A steady-state $^{14}$CO$_2$ labelling technique was employed (Geiger & Fondy, 1979) using a custom-made leaf gas exchange/$^{14}$C labelling system previously as described in Leonardos *et al.*, 1996 & 2003. ‘Bonny Best’ and ‘Foronti’ plants 30-35 days after germination grown under ambient CO$_2$ (400 µL L$^{-1}$) were illuminated with white CFLs for 30 minutes at 300±25 μmol m$^{-2}$ s$^{-1}$ PAR at canopy level in order to prime all photosystems (Supplementary Figure 1). A similar photosystem priming was done with cv. ‘Foronti’ plants grown under elevated CO$_2$ 25-30 days after germination prior to leaves be placed in the chambers. Four plants for each experimental run were then transferred to the $^{14}$C system where the most distal leaflet on the 5$^{th}$ highest leaf was placed in a leaf chamber and sealed. Each of the 4 leaf chambers included a circulating water jacket for temperature control, a glass window on the top to allow light to illuminate the leaf, and a Geiger-Müller (GM) detector (model 7231, LND Inc., Oceanside, NY, USA) underneath the entire leaf area enclosed in the chamber (16 cm$^2$) for radioactivity monitoring.
The leaf was illuminated with one of seven spectra from custom LEDs provided by Lighting Science Group Company (LSGC; Warwick, RI, USA) including white, red-blue, red-white, red, blue, orange, and green (Figure 2.1). Photosynthetic rates achieved in the $^{14}$C system were set to either ~12 (high), 8 (medium), or 4 (low) μmol m$^{-2}$ s$^{-1}$, for experiments using cv. ‘Bonny Best’ at the start of the experiment before $^{14}$CO$_2$ was added, by adjusting light levels for each leaf chamber/light treatment using previous data (Lanoue et al., 2017). For cv. ‘Foronti’ analyzed at ambient CO$_2$ the photosynthetic rate was set to ~8 (medium) μmol m$^{-2}$ s$^{-1}$. For cv. ‘Foronti’ grown and analyzed under elevated CO$_2$ the photosynthetic rates were set at either ~20 (very high), 14 (high), 8 (medium), or 4 (low) μmol m$^{-2}$ s$^{-1}$. These photosynthetic rates were chosen, by design, to represent rates which were near saturation, in the linear phase of a light response curve, or a low, light limiting condition for each respective CO$_2$ condition. Each lighting treatment and photosynthetic rate was randomized daily to ensure there was no chamber bias. The experimental design was specific to our primary objective to compare daily export patterns under the different spectra, but at very similar CO$_2$ influx rates.
Figure 2.1: PAR spectrum of compact fluorescent lights within the Biochambers used to grow the plants specifically for the $^{14}\text{CO}_2$ leaf studies in which different PAR-38 LED floodlights from LSGC were tested. The PAR spectra of each of the PAR-38 LED floodlights show the wavelength composition generated by white, red-blue, red-white, red, blue, orange, or green LEDs. Each light spectra was determined using a spectroradiometer (Flame Spectrometer, Ocean Optics, Dunedin, FL, USA).
Only the source $^{14}$C-fed leaf was illuminated. The remainder of the plant was left in the dark to maximize sink demand and therefore maximize source activity and carbon export from the illuminated leaf. During the period of illumination, for both cv. ‘Bonny Best’ and ‘Foronti’ analyzed under ambient CO$_2$, chambers were set to 22°C, 50-60% RH and 405±10 μL L$^{-1}$ of CO$_2$ at an air flow rate of 500 cm$^3$ min$^{-1}$ per chamber. For cv. ‘Foronti’ analyzed under elevated CO$_2$ all parameters were identical besides a CO$_2$ concentration of 1000±10 μL L$^{-1}$ was used during the illumination period.

Radiolabelled CO$_2$ ($^{14}$CO$_2$) was generated in a large gas tight syringe by reacting either NaH$^{14}$CO$_3$ with 30% H$_2$SO$_4$ or Ba$^{14}$CO$_3$ with 30% HCl. $^{14}$CO$_2$ was drawn into a 60mL syringe and loaded onto a syringe pump (PHD 2000 Infusion, Harvard Apparatus, MA, USA). Once gas exchange was deemed to be steady (approximately 30 min after putting the leaf into a chamber), $^{14}$CO$_2$ was injected into the total air stream (2250 cm$^3$ min$^{-1}$) at 4 mL h$^{-1}$.

Leaves were illuminated for 15h during steady-state $^{14}$CO$_2$ labelling (7:00:00-22:00:00). Net carbon exchange rate (NCER) and transpiration rates were obtained by an infrared gas analyzer (IRGA; Li-COR CO$_2$/H$_2$O Gas analyzer 7000, Lincoln, NE, USA). Day-time carbon export was calculated as the difference between NCER measured by the IRGA and the $^{14}$C retention measured by the GM detector. The $^{14}$C retention measured by the GM detector was corrected for the specific activity in the air steam (54.75-159.33 Bq μmol$^{-1}$ C) and the GM detector efficiency (0.005326-0.0278) both of which remained steady throughout the day (i.e., each experimental run), but changed between runs.

After 15h, leaves were either removed from the system for analysis of the products made during the feed period under the LEDs, or subject to an 8h chase period in the dark while $^{14}$CO$_2$ was not injected. During this night-time chase period, temperature was lowered to 18°C and the flow rate was reduced to 150 cm$^3$ min$^{-1}$ per leaf chamber. For cv. ‘Foronti’ analyzed under elevated CO$_2$, the CO$_2$ concentration was also lowered to 400±10 μL L$^{-1}$ during the night period. The respired air was collected in chamber specific gas traps containing 40mL 20% KOH and the respired $^{14}$C was determined via liquid scintillation counting. NCER during the dark period (respiration rate) was again obtained by the IRGA as were transpiration rates. Night-time carbon export was calculated as the difference between $^{14}$C-retention measured by the GM detector and the respiration rate determined from the radioactivity in the KOH traps.
In order to determine the fate of all $^{14}$C assimilated, a carbon budget analysis was performed by integrating the day-time rates of carbon fixation and export as well as the night-time export and respiration values. Each of these values were then expressed as a percentage of total fixed carbon to allow for analysis of relative day-time and night-time export.

### 2.3.2 Carbon partitioning

Immediately after each experimental run, leaves were taken out of the chambers in order to determine $^{14}$C amounts in various forms (i.e., sucrose, starch). The area of the leaf enclosed by the chamber was imaged to determine leaf area then extracted three times using 80% boiling ethanol for 20-30 min, leaving an ethanol soluble fraction and ethanol insoluble fraction. Ethanol soluble fractions were then dried and suspended in a mixture of water and 99% chloroform (3:2 v/v), agitated, and centrifuged at 11,000 RPM to separate a water soluble fraction (primarily sugars) from chloroform soluble leaf components (chlorophyll, lipids, etc.). Ethanol insoluble fractions (primarily starch) were oven dried at 70°C for 48h, dry ground and suspended in 80% ethanol. $^{14}$C content of each fraction was determined using liquid scintillation counting.

### 2.3.3 Statistical Analysis

#### 2.3.4.1 Diurnal Patterns of Gas Exchange and Export for cv. ‘Bonny Best’ Grown and Analyzed Under Ambient CO$_2$

For each point/bar on a graph presented, an average ± the standard error is represented for the following number of replicates (n). During the day-time of high photosynthetic rate experiments starting at ~12 µmol m$^{-2}$ s$^{-1}$ for white n=14, for red-blue n=11, for red-white n=10, for red n=14, for blue n=11, for orange n=8, and for green n=8. During the subsequent 8h dark period of the same experiment, for white n=7, for red-blue n=5, for red-white n=5, for red n=7, for blue n=6, for orange n=2, and for green n=2.

During the day-time of medium photosynthetic rate experiments starting at ~8 µmol m$^{-2}$ s$^{-1}$ for white n=19, for red-blue n=15, for red-white n=17, for red n=21, for blue n=23, for orange n=11, and for green n=12. During the subsequent 8h dark period of the same experiment for white n=8, for red-blue n=7, for red-white n=4, for red n=13, for blue n=13, for orange n=6, and for green n=5.
During the day-time of low photosynthetic rate experiments starting at ~4 µmol m\(^{-2}\) s\(^{-1}\) for white n=15, for red-blue n=11, for red-white n=9, for red n=17, for blue n=15, for orange n=11, and for green n=9. For the subsequent 8h dark period of the same experiment for white n=7, for red-blue n=4, for red-white n=4, for red n=5, for blue n=8, for orange n=5, and for green n=5.

All light treatments were compared to the white light treatment (control) within a photosynthetic range using a Students t-test with a p<0.05 indicating a significant difference with SAS Studio 3.5.

2.3.4.2 Diurnal Patterns of Gas Exchange and Export for cv. ‘Foronti’ Grown and Analyzed Under Ambient CO\(_2\)

For each point/bar on a graph presented, an average ± the standard error is representative of n=8 during the day-time and n=4 during the night-time.

2.3.4.3 Diurnal Patterns of Gas Exchange and Export for cv. ‘Foronti’ Grown and Analyzed Under Elevated CO\(_2\)

For each point/bar on a graph presented, an average ± the standard error is represented for the following number of replicates (n). During the day-time of high photosynthetic rate experiments starting at ~20 µmol m\(^{-2}\) s\(^{-1}\) for white n=6, for red-blue n=7, for red-white n=8, for red n=7, for blue n=10, for orange n=9, and for green n=6. During the subsequent 8h dark period of the same experiment, for white n=3, for red-blue n=5, for red-white n=3, for red n=3, for blue n=4, for orange n=5, and for green n=4.

During the day-time of high photosynthetic rate experiments starting at ~14 µmol m\(^{-2}\) s\(^{-1}\) for white n=8, for red-blue n=7, for red-white n=6, for red n=9, for blue n=10, for orange n=6, and for green n=7. During the subsequent 8h dark period of the same experiment, for white n=4, for red-blue n=3, for red-white n=3, for red n=4, for blue n=5, for orange n=3, and for green n=3.

During the day-time of medium photosynthetic rate experiments starting at ~8 µmol m\(^{-2}\) s\(^{-1}\) for white n=6, for red-blue n=7, for red-white n=7, for red n=8, for blue n=6, for orange n=6, and for green n=6. During the subsequent 8h dark period of the same experiment for white n=4, for red-blue n=4, for red-white n=3, for red n=4, for blue n=3, for orange n=3, and for green n=4.

During the day-time of low photosynthetic rate experiments starting at ~4 µmol m\(^{-2}\) s\(^{-1}\) for white n=6, for red-blue n=8, for red-white n=9, for red n=8, for blue n=6, for orange n=10, and for green
n=6. For the subsequent 8h dark period of the same experiment for white n=3, for red-blue n=3, for red-white n=4, for red n=4, for blue n=3, for orange n=5, and for green n=3.

All light treatments were compared to the white light treatment (control) within a photosynthetic range using a Students t-test with a p<0.05 indicating a significant difference.

2.3.4.4 Correlation Analysis

For correlation analyses of cv. ‘Bonny Best’ displayed in Figure 2.7, hourly data from all export experimentation was pooled (n=3653). For correlation analyses of cv. ‘Foronti’ under elevated CO₂, hourly data from all export experimentation was pooled (n=2652). The first two hours of every experiment were excluded from the correlation analysis due to the fact that isotopic equilibrium was not met during this time period. The Pearson’s correlation coefficient was classified using guidelines specified by Mukaka (2012).

All statistics were performed using SAS studio 3.5.

2.4 Results – ‘Bonny Best’: Ambient CO₂

2.4.1 Diurnal Patterns and Correlation of NCER, C-Export, and Soluble Sugars

An initial high photosynthetic rate of ~12 µmol m⁻² s⁻¹ was established at the start of the photoperiod under all LEDs during an export experiment at high photosynthesis (white, red-blue, red-white; Figure 2.2A; red & blue Figure 2.2B; orange & green Figure 2.2C). The photosynthetic rate decreased in all treatments at about 14:00:00 (Figure 2.2A, 2.2B, & 2.2C) with the decrease being greater under the monochromatic red and blue LEDs (Figure 2.2B). Dark respiration rates following the period of illumination were similar (Figure 2.2A, 2.2B, & 2.2C).

Day-time export rates rose during the beginning of the day under all LEDs (Figure 2.2D, 2.2E, & 2.2F). During the rest of the illumination period with white, red-blue, red-white, orange, and green export remained steady (Figure 2.2D & 2.2F), whereas under red and blue, export dropped slightly following the photosynthetic pattern (Figure 2.2E). Night-time export rates were similar following all LED treatments (Figure 2.2D, 2.2E, & 2.2F).

When day-time export was expressed as a percentage of photosynthesis, similar patterns were observed under all light treatments (Figure 2.2G, 2.2H, & 2.2I). Relative day-time export increased throughout the day under all LED treatments (Figure 2.2G, 2.2H, & 2.2I).
**Figure 2.2:** Daily patterns of leaf NCER and export at an initial high photosynthetic rate of ~12 µmol m\(^{-2}\) s\(^{-1}\) as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from monochromatic LEDs (red & blue; orange & green) are shown in panels B, E, H and C, F, I respectively.
An initial medium photosynthetic rate of ~8 µmol m$^{-2}$ s$^{-1}$ was established at the start of the photoperiod under all LEDs in a following export experiment at medium photosynthesis (white, red-blue, & red-white Figure 2.3A; red & blue Figure 2.3B; orange & green Figure 2.3C). Similar to the high photosynthetic export experiment (Figure 2.2), photosynthetic rates trended downwards in all treatments around 16:00:00, more so in the red and blue treatments (Figure 2.3A, 2.3B, & 2.3C). Dark respiration rates were similar following all LED treatments (Figure 2.3A, 2.3B, & 2.3C).

Export rates from leaves illuminated with white, red-blue, and red-white increased to a maximum between 16:00:00-18:00:00 and remained steady thereafter until the end of the photoperiod (22:00:00; Figure 2.3D). Leaves illuminated with blue light reached a maximum export rate at ~10:00:00 then declined slightly thereafter (Figure 2.3E). Leaves illuminated with red light reached a maximum export rate around 12:00:00 then remained steady until the end of the photoperiod (Figure 2.3E). Export under orange and green increased until 14:00:00 and was sustained at a high rate throughout the remainder of the photoperiod (Figure 2.3F).

Under all lights, relative day-time export was seen to increase throughout the photoperiod (Figure 2.3G, 2.3H, & 2.3I). Interestingly, from 9:00:00 to 12:00:00, leaves illuminated with red-blue, blue, and orange LEDs showed a higher relative export rate than did leaves illuminated with white, red-white, red, and green LEDs (p<0.05; Figure 2.3G, 2.3H, & 2.3I). Illumination with orange and green light produced a similar, high relative export throughout the photoperiod (Figure 2.3I).
**Figure 2.3:** Daily patterns of leaf NCER and export at an initial medium photosynthetic rate of ~8 µmol m$^{-2}$ s$^{-1}$ as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs system, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from red & blue are shown in panels B, E, & H while orange & green are shown in C, F, & I respectively.
An initial low photosynthetic rate of \( \sim 4 \, \mu\text{mol m}^{-2} \, \text{s}^{-1} \) was established at the start of the photoperiod under all LEDs in a final export experiment at low photosynthesis (white, red-blue, & red-white Figure 2.4A; red & blue Figure 2.4B; orange & green Figure 2.4C). NCER remained stable throughout the day/night period under LEDs (Figure 2.4A, 2.4B, & 2.4C). Similar to NCER, both day/night export rates were similar under all LEDs (Figure 2.4D, 2.4E, & 2.4F). It is noteworthy for all photosynthetic rate experiments, day-time export rates were significantly higher than night-time export rates (Figure 2.2D, 2.2E, 2.2F, 2.3D, 2.3E, 2.3F, 2.4D, 2.4E, & 2.4F).

Relative export under all LEDs increased during the morning hours and became steady until 16:00:00 (Figure 2.4G, 2.4H, & 2.4I). At 16:00:00, under all LEDs, a noticeable increase in relative export was observed which persisted until the end of the photoperiod (Figure 2.4G, 2.4H, & 2.4I).

The average relative amount of newly fixed carbon from all photosynthetic rate experiments (Figure 2.2-2.4) which had been either exported, respired, or remained in the leaf after 15h or 23h are displayed in Figure 2.5. During the experiments at both high and low photosynthetic rates, export during the day and night, as well as the percentage of carbon remaining in the leaf after a 13h illumination period and subsequent 8h dark period was similar under all LED treatments (Figure 2.5A & 2.5C).

Interestingly, leaves illuminated with blue and orange LEDs produced a higher percentage of day-time export than leaves exposed to the control white LED (\( p<0.05 \); Figure 2.5B). Furthermore, leaves illuminated with blue and orange LEDs also produced a higher percentage of day-time export than leaves exposed to red-white LEDs (\( p<0.05 \); Figure 2.5B). Subsequently, these results were inversely related to the amount of newly fixed carbon which remained in the leaf at the end of the 15h illumination period (Figure 2.5B).
Figure 2.4: Daily patterns of leaf NCER and export at an initial low photosynthetic rate of ~4 µmol m\(^{-2}\) s\(^{-1}\) as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs system, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from red & blue are shown in panels B, E, & H while orange & green are shown in C, F, & I respectively.
Figure 2.5: A summary of carbon allocation at high (12 \text{ \mu mol m}^{-2} \text{s}^{-1}; \text{panel A}), medium (8 \text{ \mu mol m}^{-2} \text{s}^{-1}; \text{panel B}), and low (4 \text{ \mu mol m}^{-2} \text{s}^{-1}; \text{panel C}) photosynthetic rates to day-time export, storage in the leaf at the end of the photoperiod, night-time export, night-time respiration, and storage in the leaf after 23h pulse and chase experiment. Carbon allocation is expressed as a percentage of total carbon fixed during the photoperiod. A statistical difference (p<0.05) in the percentage of day-time export between an LED treatment and the white LED control is indicated with an asterisk (*).
Under all spectral conditions tested, including the orange and green, as the photosynthetic rate was raised by increasing light intensity, the amount of day-time carbon export also increased (Figure 2.6A). However, the slope of day-time export did not show the same extent of increase as did the amount of total fixed carbon under all wavelengths (Figure 2.6A). The relationship between total fixed carbon and day-time export is evident in Figure 2.6B, indicating a higher percentage of relative day-time export during the low photosynthetic experiments compared to both medium and high photosynthetic rates. The differences in day-time export under the different spectra at the middle photosynthetic rate noted in Figure 2.5B above are highlighted in Figure 2.6B that shows an interesting separation due to light quality.

At all light intensities, a very strong correlation between photosynthesis and export was observed (r=0.91; Figure 2.7A) and independent of the spectral quality the leaf was illuminated with (Table 2.1). A strong correlation (r=0.66) was also observed between the last hour day-time export and end of photoperiod soluble sugar content in the leaf (Figure 2.7B). A moderate to strong correlation between average day-time export and end of photoperiod soluble sugars was observed under each of the lights tested (r=0.52-0.87; Table 2.1). Notably, the green LED generated the strongest correlation between export and soluble sugar content (r=0.87; Table 2.1).
Figure 2.6: Total carbon fixed and exported during the photoperiod under low (4 µmol m$^{-2}$ s$^{-1}$), medium (12 µmol m$^{-2}$ s$^{-1}$), and high (12 µmol m$^{-2}$ s$^{-1}$) photosynthetic rates from leaves that were illuminated by various LED systems (Panel A). Panel B illustrates the relationship between export and photosynthesis during the day-time under different LED treatments. Relative export during the day-time was expressed as the percentage of the total fixed carbon.
**Figure 2.7:** Correlation analysis between hourly averages of carbon fixation and export under illumination with LEDs of different spectral qualities and intensities (Panel A). Panel B displays the correlation between the average export during the last hour of illumination and the soluble sugar in the leaf at the end of the illumination period. The solid black line (—) indicates the linear regression line within each dataset.
Table 2.1: Summary of wavelength specific correlation coefficients (r) for photosynthesis vs. export (Figure 2.7A), and export vs. soluble sugars (Figure 2.7B).

<table>
<thead>
<tr>
<th>LED Treatment</th>
<th>White</th>
<th>Red-Blue</th>
<th>Red-White</th>
<th>Red</th>
<th>Blue</th>
<th>Orange</th>
<th>Green</th>
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</thead>
<tbody>
<tr>
<td>Photosynthesis vs. Export</td>
<td>0.91</td>
<td>0.92</td>
<td>0.90</td>
<td>0.91</td>
<td>0.93</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>Soluble Sugar vs. Export</td>
<td>0.61</td>
<td>0.52</td>
<td>0.75</td>
<td>0.74</td>
<td>0.72</td>
<td>0.77</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Our results show the diurnal patterns of carbon fixation and export at three pre-established photosynthetic rates and different LED treatments. Of note, within each experimentation, only the intact source leaf was illuminated by an LED while the rest of the plant was kept in darkness to maximize sink demand. Also, plants used were all of similar size and sink demand. Thus, results presented are likely the result of the source leaf environment, specifically light quality and intensity.

2.4.2 Diurnal Patterns of Leaf Stomatal Conductance, Transpiration Rate, and Water-Use-Efficiency

During all photosynthetic rate experiments, day-time stomatal conductance and transpiration rates were higher than night-time rates (Figure 2.8A-2.8F). Stomatal conductance and transpiration rates increased until mid-day and decline thereafter (Figure 2.8A-2.8F). During all photosynthetic rate experiments, leaves illuminated with blue LEDs produced the highest stomatal conductance and transpiration rates (Figure 2.8A-2.8F). Day-time patterns of water-use-efficiency (WUE) reached a minimum in all experiments during the middle of the photoperiod then increased thereafter (Figure 2.8G, 2.8H, & 2.8I). Of note, during all photosynthetic rate experiments, leaves exposed to blue light produced the lowest WUE, while leaves exposed to orange and green produced among the highest (Figure 2.8G, 2.8H, & 2.8I).
Figure 2.8: Diurnal patterns of stomatal conductance (A, B, & C), transpiration rate (D, E, & F), and WUE (G, H, & I) of leaves during the light period under various wavelength specific LEDs. Panels A, D, & G show these values from export experiments done at an initial high photosynthetic level of \( \approx 12 \mu\text{mol m}^{-2}\text{s}^{-1} \). Panels B, E, & H show these values from export experiments done at an initial medium photosynthetic level of \( \approx 8 \mu\text{mol m}^{-2}\text{s}^{-1} \). Panels C, F, & I show these values from export experiments done at an initial low photosynthetic level of \( \approx 4 \mu\text{mol m}^{-2}\text{s}^{-1} \).
2.5 Results – ‘Foronti’: Ambient CO₂

2.5.1 Diurnal Patterns of NCER and Export

An initial photosynthetic rate of approximately 8 µmol m⁻² s⁻¹ was established and maintained throughout the day-time period when ‘Foronti’ tomato leaves were irradiated by all wavelengths of light (Figure 2.9A, B, C). When the lights were turned off (22:00), a steep drop in the NCER occurred which resulted in negative values (indicating respiration) which remained steady throughout the night period (22:00-06:00). The wavelength of light exposed to the leaf during the day-time had no influence of the respiration rate during the night period, resulting in similar respiration rates between all treatments (Figure 2.9A, B, C).

When leaves were exposed to the white, red-blue, red-white, red, and blue LEDs, export rates rose from the start of the day (06:00) to 12:00 (Figure 2.9D, E). After which, export rates under these LEDs, remained steady until the end of the day (22:00). Leaves exposed to either orange or green LEDs rose during the initial part of the day (06:00-12:00), remained steady from 12:00-17:00, then rose slightly until the end of the photoperiod (Figure 2.9F). Once the lights were turned off, the export rate under all light treatments decreased rapidly, reaching a minimum around 24:00 at which it remained during the night period.

Under all light treatments, the relative day-time export, an indication of the rate at which newly fixed carbon left the leaf steadily rose throughout the day reaching a maximum at 22:00 before the lights were turned off (Figure 2.9G, H, I).

The average percentage of newly fixed carbon which was exported during the day-time was higher when ‘Foronti’ leaves were exposed to a blue LED under short-term illumination compared to leaves exposed to the white light control (p<0.05; Figure 2.10). Furthermore, leaves exposed to a blue LED also had a higher percentage of newly fixed carbon exported during the day-time than did leaves exposed to a red-blue or a red-white LED (p<0.01; Figure 2.10). Subsequently, the percentage of newly fixed carbon which remained in the leaf after 24h was lowest in leaves exposed to a blue LED during the photoperiod (Figure 2.10). The percentage of newly fixed carbon which was either exported during the night-time or respired was similar between all LED treatments. However, the percentage of newly fixed carbon which remained in the leaf after a 23h
period was higher in leaves which had been exposed to red-white compared to those exposed to a blue LED (Figure 2.10).

**2.5.2 Diurnal Patterns of Leaf Stomatal Conductance, Transpiration Rate, and Water-Use-Efficiency**

Under all wavelengths of light, the stomatal conductance from ‘Foronti’ leaves increased to a maximum around 15:00 and then subsequently decreased until the end of the photoperiod (22:00; Figure 2.11A). Stomatal conductance values were at a minimum during the hour after the lights were turned off, and subsequently rose slightly during the night-time period (Figure 2.11A). Of note, leaves illuminated with the green LED produced the lowest stomatal conductance values.

Similar to stomatal conductance, the transpiration rates rose to a maximum around 15:00 than decreased until the end of the photoperiod (Figure 2.11B). Upon darkness (22:00), the transpiration rate dropped to a minimum than slowly rose throughout the night period (Figure 2.11B). Similar to the stomatal conductance values, leaves exposed to a green LED produced the lowest transpiration values.

Water-use-efficiency, a ratio of both the CO₂ and H₂O gas exchanges decreased during the morning hours until reaching a minimum around 15:00 (Figure 2.11C). Thereafter, the values of WUE under all spectral qualities rose until the end of the photoperiod (Figure 2.11C). Of note, leaves exposed to a green LED produced the highest WUE while leaves exposed to a blue LED produced the lowest throughout the photoperiod.
Figure 2.9: Daily patterns of leaf (cv. ‘Foronti’) NCER and export at an initial medium photosynthetic rate of ~8 µmol m$^{-2}$ s$^{-1}$ as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from monochromatic LEDs (red & blue; orange & green) are shown in panels B, E, H and C, F, I respectively.
**Figure 2.10:** A summary of carbon allocation with a ‘Foronti’ tomato leaf at a medium (8 $\mu$mol m$^{-2}$ s$^{-1}$) photosynthetic rate displaying the day-time export, storage in the leaf at the end of the photoperiod (15h), night-time export, night-time respiration, and storage in the leaf after 23h pulse and chase experiment. Carbon allocation is expressed as a percentage of total carbon fixed during the photoperiod. A statistical difference ($p<0.05$) in the percentage of day-time export between an LED treatment and the W LED control is indicated with an asterisk (*).
Figure 2.11: Diurnal patterns of stomatal conductance (A), transpiration rate (B), and WUE (C) from ‘Foronti’ leaves at a medium (8 µmol m⁻² s⁻¹) during the light period under various wavelength specific LEDs.
2.6 Results – ‘Foronti’: Elevated CO₂

2.6.1 Diurnal Patterns and Correlation of NCER and C-Export

An initial very high photosynthetic rate of approximately 20 µmol m⁻² s⁻¹ was established when leaves were exposed to all light treatments at the beginning of the very high light level elevated CO₂ (1000 µL L⁻¹) ‘Foronti’ export experiment (Figure 2.12A, B, C). Leaves which were exposed to either white, red-blue, red-white, red, or blue LEDs tended to have a decreasing photosynthetic rate throughout the day-time (06:00-22:00; Figure 2.12A, B). However, leaves which were exposed to either the orange or green light treatments maintained a photosynthetic rate around 20 µmol m⁻² s⁻¹ throughout the light period (Figure 2.12C). Upon the lights being turned off (22:00), the NCER of leaves under all light treatments dropped to a minimum at 23:00, then maintain a steady respiratory rate throughout the dark period (22:00-06:00; Figure 2.12A, B, C).

The export rate of leaves exposed to either white, red-blue, red-white, red, or blue LEDs rose from 06:00 to approximately 12:00 where it reached a daily maximum rate (Figure 2.12D, E). Thereafter, the export rate of leaves exposed to those light remained relatively steady until the end of the photoperiod (Figure 2.12D, E). When leaves were illuminated with either orange or green LEDs, the export rates rose steadily throughout the day, reaching a maximum rate around the end of the photoperiod (Figure 2.12F). Once the lights were turned off (22:00), the export rate from leaves under all light treatments dropped drastically (Figure 2.12D, E, F). The export rates during the night-time were similarly low for all light treatments which remained steady throughout the dark period (Figure 2.12D, E, F).

The relative day-time export rate (% export/photosynthesis) began around 50% during the first hour of the experiment and steadily rose throughout the day reaching a peak around 80% at 22:00 in all light treatments (Figure 2.12G, H, I).
Figure 2.12: Daily patterns of leaf NCER and export at an initial very high photosynthetic rate of ~20 µmol m⁻² s⁻¹ as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from monochromatic LEDs (red & blue; orange & green) are shown in panels B, E, H and C, F, I respectively.
An initial high rate of photosynthesis (~14 µmol m$^{-2}$ s$^{-1}$) was achieved from ‘Foronti’ leaves under high CO$_2$ when illuminated with all light treatments (Figure 2.13A, B, C). The established photosynthetic rate remained steady throughout the photoperiod until 22:00 when the lights were turned off. After a drastic drop in NCER related to the start of the dark period, respiratory rates were at a minimum in all light treatments at 23:00, rose until 24:00, then remained steady during the remainder of the night period (Figure 2.13A, B, C).

Under all light treatments, export rate from ‘Foronti’ leaves under high CO$_2$ at a high photosynthetic rate rose from the beginning of the experiment until approximately 14:00 after which they remained relatively steady (Figure 2.13D, E, F). At the end of the photoperiod, a drastic drop in the export rates occurred under all treatments. The export rates during the night period remained low and were similar between all light treatments (Figure 2.13D, E, F).

Under all light treatments, the relative export rate rose throughout the day, reaching a maximum of approximately 75-80% at 22:00 (Figure 2.13G, H, I).

A medium photosynthetic rate (~8 µmol m$^{-2}$ s$^{-1}$) was maintained under all light treatments throughout the photoperiod (Figure 2.14A, B, C). At the end of the photoperiod (22:00), a drastic drop in the NCER was observed and a negative NCER (indicating respiration) was observed throughout the night period.

Similar to export rates in the high photosynthetic rate experiment, rates of export increased under all light treatments until approximately 14:00, after which they remained steady until the end of the photoperiod (Figure 2.14D, E, F). During the subsequent night period, leaves which were exposed to all light treatments maintained similar rates and patterns of carbon export (Figure 2.14D, E, F).

The relative rates of carbon export increased throughout the day under all light treatments (Figure 2.14G, H, I). Interestingly, during the afternoon hours (13:00-22:00), leaves which were exposed to the blue LEDs produced a higher relative export rate than did leaves exposed to the red light treatment (Figure 2.14H).

A low photosynthetic rate (~4 µmol m$^{-2}$ s$^{-1}$) was supported by all wavelengths of light throughout the day (Figure 2.15A, B, C). At the end of the photoperiod (22:00) the lights were turned off and a drastic decrease in NCER occurred from leaves under all light treatments (Figure 2.15A, B, C).
During the dark period, respiration rates were similar between all light treatments the leaves were exposed to during the photoperiod.

When leaves were exposed to white, red-blue, red-white, red, or blue LEDs, the carbon export rate rose during the initial parts of the photoperiod until approximately 12:00 (Figure 2.15D, E). Thereafter, export rates achieved under these light treatments remained steady until the end of the photoperiod. When leaves were exposed to either orange or green LEDs, the export rose from the beginning of the photoperiod until approximately 17:00, after which it remained steady until the end of the photoperiod (Figure 2.15F).

The relative day time export of leaves exposed to white, red-blue, or red-white LEDs rose during the initial part of the photoperiod, plateaud between approximately 14:00-18:00, then rose again until the end of the photoperiod (Figure 2.15G). When leaves were exposed to red or blue LEDs, the relative export rate rose during the initial part of the photoperiod, plateaud from 12:00-18:00 then rose until the end of the photoperiod (Figure 2.15H). Under either orange or green illumination, the relative export rate from leaves rose steadily throughout the whole photoperiod (Figure 2.15I).

Carbon allocation budgets were produced from the very high (Figure 2.12), high (Figure 2.13), Medium (Figure 2.14), and low (Figure 2.15) photosynthetic rates showing the amount of newly fixed carbon which was exported during the day or night periods, the carbon which was respired during the night, or carbon which remained in the leaf. When ‘Foronti’ leaves which were grown and analyzed at elevated CO₂ (1000 µL L⁻¹) were analyzed under the very high, high, and low photosynthetic rates, carbon allocation budgets were similar between the light treatments within each photosynthetic level (Figure 2.16, 2.17, 2.19). Interestingly, similar to results with ‘Bonny Best’ and ‘Foronti’ under ambient CO₂, at the medium photosynthetic rate leaves which were exposed to blue light exported a higher percentage of newly fixed carbon during the 15h photoperiod compared to leaves exposed to white light (Figure 2.18). Furthermore, leaves illuminated with blue LEDs also exported more carbon during the 15h photoperiod than leaves exposed to red-white LEDs (Figure 2.18). Subsequently, the amount of carbon which remained at the end of the 15h photoperiod was less when leaves were exposed to blue LEDs compared to leaves illuminated with white or red-white light (Figure 2.18).
Figure 2.13: Daily patterns of leaf NCER and export at an initial high photosynthetic rate of ~14 µmol m$^{-2}$ s$^{-1}$ as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from monochromatic LEDs (red & blue; orange & green) are shown in panels B, E, H and C, F, I respectively.
**Figure 2.14:** Daily patterns of leaf NCER and export at an initial medium photosynthetic rate of \(~8 \mu\text{mol m}^{-2} \text{s}^{-1}\) as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from monochromatic LEDs (red & blue; orange & green) are shown in panels B, E, H and C, F, I respectively.
Figure 2.15: Daily patterns of leaf NCER and export at an initial low photosynthetic rate of ~4 µmol m⁻² s⁻¹ as affected by different spectral qualities. The NCER (A, B, & C), export (D, E, & F), and relative export (export as a percentage of photosynthesis; G, H, & I) exposed to mixed LEDs, white, red-blue, and red-white are shown in panels A, D, & G. The NCER, export, and relative export from monochromatic LEDs (red & blue; orange & green) are shown in panels B, E, H and C, F, I respectively.
Figure 2.16: A summary of carbon allocation with a ‘Foronti’ tomato leaf grown and analyzed under elevated CO$_2$ (1000 μL L$^{-1}$) at a very high (20 μmol m$^{-2}$ s$^{-1}$) photosynthetic rate displaying the day-time export, storage in the leaf at the end of the photoperiod (15h), night-time export, night-time respiration, and storage in the leaf after 23h pulse and chase experiment. Carbon allocation is expressed as a percentage of total carbon fixed during the photoperiod. A statistical difference ($p<0.05$) in the percentage of day-time export between an LED treatment and the white LED control is indicated with an asterisk (*).
Figure 2.17: A summary of carbon allocation with a ‘Foronti’ tomato leaf grown and analyzed under elevated CO₂ (1000 µL L⁻¹) at a high (14 μmol m⁻² s⁻¹) photosynthetic rate displaying the day-time export, storage in the leaf at the end of the photoperiod (15h), night-time export, night-time respiration, and storage in the leaf after 23h pulse and chase experiment. Carbon allocation is expressed as a percentage of total carbon fixed during the photoperiod. A statistical difference (p<0.05) in the percentage of day-time export between an LED treatment and the white LED control is indicated with an asterisk (*).
Figure 2.18: A summary of carbon allocation with a ‘Foronti’ tomato leaf grown and analyzed under elevated CO$_2$ (1000 µL L$^{-1}$) at a medium (8 µmol m$^{-2}$ s$^{-1}$) photosynthetic rate displaying the day-time export, storage in the leaf at the end of the photoperiod (15h), night-time export, night-time respiration, and storage in the leaf after 23h pulse and chase experiment. Carbon allocation is expressed as a percentage of total carbon fixed during the photoperiod. A statistical difference (p<0.05) in the percentage of day-time export between an LED treatment and the white LED control is indicated with an asterisk (*).
Figure 2.19: A summary of carbon allocation with a ‘Foronti’ tomato leaf grown and analyzed under elevated CO\textsubscript{2} (1000 µL L\textsuperscript{-1}) at a low (4 µmol m\textsuperscript{2} s\textsuperscript{-1}) photosynthetic rate displaying the day-time export, storage in the leaf at the end of the photoperiod (15h), night-time export, night-time respiration, and storage in the leaf after 23h pulse and chase experiment. Carbon allocation is expressed as a percentage of total carbon fixed during the photoperiod. A statistical difference (p<0.05) in the percentage of day-time export between an LED treatment and the white LED control is indicated with an asterisk (*).
To summarize all results related to spectral quality and photosynthetic rate of ‘Foronti’ leaves grown and analyzed at an elevated CO$_2$ concentration, the average relative day-time export has been plotted against the average day-time photosynthetic rate for all conditions (Figure 2.20). In general, similar to results from export experiments with ‘Bonny Best’, as the light intensity was increased, and subsequently the photosynthetic rate, the relative day-time export decreased under all light treatments (Figure 2.20). This trend indicates that as an increase in carbon influx occurred, more carbon was stored in the leaf at the end of the photoperiod instead of exported.

Similar to results observed during the ‘Bonny Best’ carbon export experiments, photosynthesis and carbon export were highly correlated under all wavelengths of light tested (Table 2.2). This result indicates that growing and exposing plants to elevated CO$_2$ levels did not affect the relationship between carbon fixation and carbon export.

**Table 2.2:** Summary of wavelength specific correlation coefficients (r) for photosynthesis vs. export.

<table>
<thead>
<tr>
<th>LED Treatment</th>
<th>White</th>
<th>Red-Blue</th>
<th>Red-White</th>
<th>Red</th>
<th>Blue</th>
<th>Orange</th>
<th>Green</th>
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<tbody>
<tr>
<td>Photosynthesis vs. Export</td>
<td>0.93</td>
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</table>
Figure 2.20: The relationship between export and photosynthesis during the day-time under different LED treatments from ‘Foronti’ leaves grown and analyzed under elevated CO$_2$. Relative export during the day-time was expressed as the percentage of the total fixed carbon.
2.6.2 Diurnal Patterns of Leaf Stomatal Conductance, Transpiration Rate, and Water-Use-Efficiency

At all photosynthetic levels, the stomatal conductance of leaves under all light treatments rose during the morning hours until reaching a maximum around 15:00 (Figure 2.21A, 2.22A, 2.23A, 2.24A). Thereafter, the stomatal conductance of all leaves decreased until reaching among the lowest day-time values at the end of the photoperiod. Once the lights were turned off, under all photosynthetic and light treatments, the stomatal conductance values dropped drastically, reaching the lowest levels during the initial hour of darkness (Figure 2.21A, 2.22A, 2.23A, 2.24A). During the subsequent night-time hours, the stomatal conductance rose slightly until the end of the night period under all conditions tested (Figure 2.21A, 2.22A, 2.23A, 2.24A). When leaves were exposed to the very high, high, and medium photosynthetic rates, those illuminated with blue light generally produced the highest day-time stomatal conductance values (Figure 2.21A, 2.22A, 2.23A). No difference regarding the night-time stomatal conductance values was observed.

Similar to the stomatal conductance day-time pattern, under all treatments tested, transpiration rates also rose during the initial hours, peaked around 15:00, then decreased until the end of the photoperiod (Figure 2.21B, 2.22B, 2.23B, 2.24B). During the day-time, under the very high, high, and medium photosynthetic treatments, leaves exposed to blue light generally produced the highest transpiration rates (Figure 2.21B, 2.22B, 2.23B). Similar to stomatal conductance, transpiration rates during the night period were lower than rates during the day, and no differences between the light treatments were observed (Figure 2.21B, 2.22B, 2.23B, 2.24B).

Inversely related to the transpiration rate, the WUE under all light treatments at all photosynthetic levels started at a high value during the first hour of the photoperiod, then decreased to a minimum around 15:00 (Figure 2.21C, 2.22C, 2.23C, 2.24C). The WUE then rose again until the end of the day period reaching values slightly lower than the first hour of the photoperiod (Figure 2.21C, 2.22C, 2.23C, 2.24C). During the very high, high, and medium photosynthetic rate experiments, leaves exposed to blue light generally had the lowest WUE during the day-time (Figure 2.21C, 2.22C, 2.23C, 2.24C).
Figure 2.21: Diurnal patterns of stomatal conductance (A), transpiration rate (B), and WUE (C) from ‘Foronti’ grown and analyzed under elevated CO₂ (1000 µL L⁻¹) leaves during the very high (20 µmol m⁻² s⁻¹) photosynthetic rate experiment under various wavelength specific LEDs.
Figure 2.22: Diurnal patterns of stomatal conductance (A), transpiration rate (B), and WUE (C) from ‘Foronti’ grown and analyzed under elevated CO₂ (1000 μL L⁻¹) leaves during the high (14 μmol m⁻² s⁻¹) photosynthetic rate experiment under various wavelength specific LEDs.
Figure 2.23: Diurnal patterns of stomatal conductance (A), transpiration rate (B), and WUE (C) from ‘Foronti’ grown and analyzed under elevated CO$_2$ (1000 µL L$^{-1}$) leaves during the medium (8 µmol m$^{-2}$ s$^{-1}$) photosynthetic rate experiment under various wavelength specific LEDs.
Figure 2.24: Diurnal patterns of stomatal conductance (A), transpiration rate (B), and WUE (C) from ‘Foronti’ grown and analyzed under elevated CO$_2$ (1000 µL L$^{-1}$) leaves during the low (4 µmol m$^{-2}$ s$^{-1}$) photosynthetic rate experiment under various wavelength specific LEDs.
2.7 Discussion

2.7.1 The Effect of Wavelength Specific LEDs on Photosynthesis and Export

Under all LED treatments at all photosynthetic levels, photosynthesis and export was sustained throughout the photoperiod during all experimentation (Figures 2.2-2.4, 2.9, 2.12-2.15). Consistent with previous studies, a high correlation between photosynthesis and export under both ambient CO$_2$ (r=0.91) and elevated CO$_2$ conditions (r=0.94) was observed (Grodzinski et al., 1998; Leonardos & Grodzinski, 2000). Furthermore, a high correlation between export and leaf soluble sugar content during ambient CO$_2$ experiments with ‘Bonny Best’ (r=0.66) was determined (Grodzinski et al., 1998; Leonardos & Grodzinski, 2000). Day-time export was always greater than night-time export under all conditions, consistent with previous studies using natural sunlight (Kalt-Torres et al., 1986) or artificial, multi-spectrum metal halide lights (Figures 2.2-2.4, 2.9, 2.12-2.15; Leonardos et al., 2003).

Importantly, leaves exposed to green produced similar photosynthetic and export rates when compared to other LED treatments (Figures 2.6, 2.9, 2.20). These results add to a growing consensus among literature indicating the ability of plants to function properly under green light (Sun et al., 1998; Terashima, et al., 2009; Wang & Folta, 2013). Furthermore, results displaying the diurnal patterns of photosynthesis and carbon export of leaves exposed to orange light introduce novel information pertaining to the function of the primary CO$_2$ gas exchange and carbon metabolism processes (Figures 2.2-2.4, 2.9, 2.12-2.15). The emphasis has traditionally been on red and blue LEDs because of their central role in the activation of the chlorophyll reaction centers. To our knowledge, the commercial application of orange and green LEDs for illumination in controlled environment systems has received little attention but, clearly warrant further examination.

Relative export increased throughout the photoperiod under all conditions (Figures 2.2-2.4, 2.9, 2.12-2.15). Tomatoes are known to store and re-mobilize sucrose in vacuoles throughout the day (Osorio et al., 2014). Similarly, in barley, vacuolar sucrose decreases in the afternoon in support of C-export (Farrar & Farrar, 1986). Starch synthesis and degradation occurs simultaneously during the light period (Stitt & Heldt, 1981; Zeeman et al., 2004; Osorio et al., 2014). Thus, the increase in relative export during the afternoon hours observed, specifically at the low
photosynthetic rates (Figures 2.4, 2.19), are consistent with the remobilization temporary stored assimilates.

During experimentation with ‘Bonny Best’ under ambient CO₂ at the medium photosynthetic rate, day-time export from blue and orange illuminated leaves were higher than leaves illuminated with white or red-white (Figure 2.3). Furthermore, during experimentation using ‘Foronti’ under both ambient and elevated CO₂ at the medium photosynthetic rate, blue light again was observed to increase the relative export rate during the day compared to leaves illuminated with either white or red-white light (Figures 2.9, 2.18). These results, for the first time, ubiquitously indicated a difference in total relative day-time export due solely to spectral quality at a very similar photosynthetic rate. It is important to note that the light level which was used during the medium photosynthetic rate experiment was during the exponential phase of the light response curve of different LED for the ‘Bonny Best’ tomato cultivar used in this study (Lanoue et al., 2017). During this phase, changes in light intensity and quality have the largest impact on a plant’s carbon status.

Furthermore, there was an increase in relative export during the morning hours of the medium photosynthetic rate experiment under blue, red-blue, and orange compared to white, red-white, red, and green (Figure 2.3). Interestingly, blue and red-blue had ~98% and 28% blue wavelength composition respectively, while white, red-white, red, and green had ~9%, 12%, 0.4%, and 2% blue wavelength composition respectively. Blue light activates cryptochrome (CRY) which is a known regulator of the circadian clock (Somers et al., 1998; Chen et al., 2004). The pathways and mechanisms controlling export, from sub-cellular to the tissue level, involve many photoreceptors and sites of regulation other than those affecting carbon fixation. The increased relative export during the morning hours of the medium photosynthetic rate experiment under blue and red-blue could be due to activation of CRY. This could induce cyclic electron flow (Figure 8-1; Finazzi et al., 2002; Munekage, et al., 2004; Shapiguzov et al., 2010) increasing ATP production needed in apoplastic phloem loading or expression levels of important enzymes and transporters facilitating export (Figures 2.25).

Interestingly, when cucumbers were grown under LEDs with varying red:blue ratios, plants grown under 100% blue light had the highest photosynthesis to specific leaf mass ratio (Hogewoning et al., 2010). Moreover, carbohydrate analysis determined that leaves grown under 100% blue had more sucrose and less starch (Hogewoning et al., 2010). Thus, having a high amount of sucrose at
the end of the photoperiod coupled with a high photosynthesis to specific leaf mass ratio indicates higher export under blue light consistent with findings presented in Figures 2.3, 2.9, and 2.18.

‘Bonny Best’ leaves under the orange LED, also had high relative export (Figure 2.3). The orange light contained only 0.67% of PAR within the blue region (Figure 2.1). To our knowledge, there is no evidence linking orange wavelengths to carbon export directly. However, orange light does bring about changes in tomato plant and leaf morphology during acclimation (Liu et al., 2011; Liu et al., 2012). Interestingly, orange wavelengths are major spectral components provided by high pressure sodium luminaries that have been utilized in research and commercial controlled environments. The high absorption of blue and orange light by pigments and molecules associated with the photosystems (e.g. carotenoids), are involved in energy trapping and therefore may increase cyclic energy transduction facilitating phloem loading and export in the light (Finazzi et al., 2002; Munekage, et al., 2004; Shapiguzov et al., 2010).

Our results indicated an increase rate of carbon export from leaves exposed to blue or orange LEDs only at the medium photosynthetic rate (Figures 2.6, 2.9, 2.20). Hartt (1966) indicated that the light saturation point for sugar export in a sugarcane leaf was much less than the saturation point of photosynthesis. Therefore, during high photosynthetic rate export experiments, the apoplastic phloem loading pathway of tomatoes may be at or close to the light saturation point and thus not be altered by different wavelengths of light. Furthermore, Windt et al., (2006) have hypothesized that there may be upper and lower limits to the rate of carbon export within the phloem. At low light levels, such as those during the low photosynthetic level export experiment, the rate of sucrose production may be slower than the rate of export not allowing for the discrimination between spectral qualities, thus indicating a lower boundary to carbon export. This is consistent with the decrease in relative export as the photosynthetic rates increase (Figures 2.6, 2.20).

2.7.2 Linking Transpiration and Carbon Export

The link among phloem and xylem osmotic pressures, stomatal conductance, transpiration rates and carbon transport is poorly understood (Smith & Milburn, 1980; Windt et al., 2006; De Swaef et al., 2013; Nikinmaa et al., 2013). As indicated by Johnson et al. (1992), an increase in leaf transpiration should increase the turgor potential gradient between the source and sink, leading to increased C-export. Results confirm that, in general, a higher leaf transpiration rate increased absolute C-export rates. However, as observed in Figures 2.8, 2.11, and 2.21-2.24, both stomatal
conductance and transpiration rates reached a maximum mid-day and declined thereafter following an inherent circadian rhythm unaffected by wavelength (Dodd et al., 2004), similar to whole plant patterns determined by Lanoue et al., (2017). Thus, the entrained circadian rhythm of stomatal function controlling daily transpiration rates is not observed in C-export patterns.

Exposing leaves to light of different spectral quality can greatly complicate attempts to relate phloem export and xylem water potential. For example, during the high photosynthetic rate experimentation, the carbon fixation rate was initially ~12 µmol m$^{-2}$ s$^{-1}$ (Figures 2.2, 2.22). While having similar photosynthetic rates, leaves illuminated with blue and red LEDs produced different stomatal conductance values and transpiration rates while producing similar export rates throughout the day (Figures 2.8, 2.22). Of note, transpiration under orange and green LEDs was lower than that under the blue and red while still producing high rates of photosynthesis and export.

Even though transpiration rates and export may be strongly linked in woody species, it has been noted that this relationship may not be as strong for herbaceous species such as tomato (Nikinmaa et al., 2013). Illuminating tomato leaves with different spectral quality resulted in similar carbon export rates, but stomatal conductance and transpiration rates varied due to wavelength specific control of stomatal function (Kinoshita et al., 2001; Lanoue et al., 2017). Thus, we caution that the link between transpiration and carbon export may be more complex than previously thought. Nevertheless, knowledge related to the interplay between water and carbon movement within a plant and how they are affected by environmental stimuli, is needed to develop a better understanding of source/sink relationships during crop production.

2.7.3 The Effects of CO$_2$ Concentration on Carbon Export

The use of elevated CO$_2$ concentration (800-1000 µL L$^{-1}$) is a common practice during greenhouse tomato production as it has been shown to increase growth and yield when light is not limited (Yelle et al., 1990; Fierro et al., 1994). Thus, one would expect that under elevated CO$_2$ conditions and higher carbohydrate production, a higher rate of carbon export would be needed to avoid end product feedback inhibition when comparing similar light levels (Lanoue et al., 2018b). However, during our experimentation, instead of setting the light levels, photosynthetic levels were set via adjusting the light level to normalize the amount of carbon influx allowing for a controlled comparison between light treatments. Thus, running a similar experiment in which, the light levels are similar between ambient and elevated CO$_2$ conditions, one could speculate that plants grown
and analyzed under elevated CO$_2$ may have a higher absolute rate of export as photosynthesis and export are highly correlation (Table 2.2). That being said, on a relative basis, the lower photosynthetic rates achieved from leaves exposed to ambient CO$_2$ at the same light level would cause a higher relative export as demonstrated in Figure 2.20 by a decreasing percentage at higher photosynthetic levels.

The carbon export pathway is a complicated multi-cellular process involving many enzymes and transporters (Lemoine et al., 2013), all of which can potentially be regulated by CO$_2$ levels. In monocot species such as rice, elevated CO$_2$ levels have been shown to increase the expression and activity of sucrose phosphate synthase (SPS) and sucrose transporter (SUT; Ono et al., 2003; Fukayama et al., 2011; Xu et al., 2018). The increase in activity and expression of SPS and SUT respectively have led to increased carbon export rates and thus diminished end product feedback inhibition of photosynthesis under elevated CO$_2$ (Ono et al., 2003; Xu et al., 2018). However, this observation may not hold true for dicot species such as tomato. In tomato and potato, another Solanaceae species, SUT expression was unaffected by an increase in CO$_2$ concentration (Xu et al., 2018). Thus, a difference in export rates when plants are grown under different CO$_2$ concentrations is not hypothesized simply by examining expression levels of key transporters. However, it should be noted that this seems to be species specific as two apoplastic loading species, rice and tomato, show different trends (Xu et al., 2018).

2.7.4 Implications

The pathway of carbon export is complex, involving numerous cells, enzymes, and transporters all of which are involved in water and carbon transport and leaf homeostasis (Figure 2.25; Stitt et al., 1987; Riesmeier et al., 1992; Sauer et al., 2004; Walters et al., 2004; Hackel et al., 2006; Sauer, 2007; Chen et al., 2012; Lemoine et al., 2013; Feng et al., 2015). Enzymes and transporters such as triose phosphate translocator (TPT; Figure 2.25; Stitt et al., 1987), SPS (Figure 2.25; Stitt et al., 1987; Huber & Huber, 1990a), sugar will eventually be exported transporter (SWEET; Figure 2.25; Chen et al., 2012; Feng et al., 2016), and SUT (Figure 2.25; Riesmeier et al., 1992) provide potential sites of regulation which are susceptible to environmental stimuli. For example, SPS, a crucial enzyme involved in sucrose production has been observed to be affected by light intensity and quality via phosphorylation (Huber & Huber, 1990a; Huber & Huber, 1990b; Tepperman et al., 2004; Suetsugu et al., 2014; Shi et al., 2016).
It is unlikely that differences in export at a medium photosynthetic rate are due solely to illumination with different spectral quality (Figures 2.3, 2.9, 2.23). If spectral quality were the sole reason for differences in export, one would expect that export would be affected by light quality at both all photosynthetic rates. It is more probable that an inter-play between spectral quality and intensity can explain the results seen. Under high light intensity, the process of light absorption via the antenna complex is saturated and not able to differentiate between spectral qualities. During low irradiance levels, plants struggle to capture enough light needed to sustain growth and thus will utilize whichever spectrum is available in a sufficient manner. Thus, it is in this middle light level in which sufficient light is available to sustain energy production but below saturation rate in which processes such as energy transduction can be influenced by light quality.
Figure 2.25: Potential sites of carbon and water regulation which can be affected by light intensity and quality within a tomato source leaf. ATP and NADPH are produced via the light reactions through the movement of electrons between photosystem II (yellow triangle) and photosystem I (red triangle) allows for the conversion of CO$_2$ to triose phosphate via the Calvin cycle (1). Triose phosphate is then moved out of the chloroplast into the mesophyll cell (MC) via the anti-port mechanism of the triose phosphate/phosphate translocator (TPT; blue oval) where it is converted to sucrose (2; Stitt et al., 1987; Huber & Huber, 1990a; Walters et al., 2004). Triose phosphate can also be made into starch in a multiple step process. Starch, which is a storage molecule in tomatoes, can be broken down into either maltose or glucose and transported into the MC via a maltose excess I transporter (MEXI; red circle) or glucose transporter (GUT; orange circle) respectively (3; Schleucher et al., 1998; Niittylä et al., 2004). Both maltose and glucose can then be converted to sucrose in the MC. Sucrose is then able to proceed via multiple pathways. Sucrose can enter the vacuole via an anti-port tonoplast membrane located H$^+$/sucrose transporter (black oval) and conversely leave the vacuole via a tonoplast membrane located H$^+$/sucrose symport (black rectangle) (4; Schulz et al., 2011; Etxeberria et al., 2012). Sucrose can also move into the apoplast via the sugars will eventually be exported transporter (SWEET; red rectangle) directly from the MC or by first symplastically diffusing into the phloem parenchyma cell (PPC) (5; Chen et al., 2012; Feng et al., 2015). The mechanism of sucrose efflux via SWEET is currently speculated to be bidirectional uniport, however lacks concrete evidence (Chen et al., 2015). Once in the apoplast, sucrose enters the phloem directly or the transfer cell (TC) then enters the phloem symplastically. Entering the phloem directly or the TC is catalyzed by a co-transport H$^+$/sucrose transporter (SUT; orange oval) (6; Riesmeier et al., 1992). ATPase enzymes (dark blue circle) are responsible for maintaining H$^+$ gradients across membranes and are usually found close to enzymes using H$^+$ symport or anti-port mechanism (7). The movement of water between the xylem and phloem has also been proposed to affect carbon export rates (8; Smith & Milburn, 1980; Windt et al., 2006; De Swaef et al., 2013; Nikinmaa et al., 2013).
2.7.5 Conclusion

In summary, the implications of quantifying day-time carbon export patterns show that an important, fundamental, pathway (carbon export) connecting source and sink tissue, can be sustained throughout the photoperiod by wavelength specific LEDs, including orange and green. Importantly, under all LED treatments, it was determined that day-time C-export is much greater than night-time export and similar patterns of export were observed. Significantly, at a medium photosynthetic rate, blue and orange LEDs produced an increase in day-time export rates compared to those of the white and red-white illuminated leaves. The correlation between photosynthesis and export under all wavelengths was high under both ambient and elevated CO\textsubscript{2} (r=0.91 and r=0.94 respectively), consistent with previous literature. There are many sites of regulation controlling carbon metabolism and H\textsubscript{2}O status within the leaf. Understanding the effect of light intensity and spectral quality on the fundamental carbon export pathway is central to the understanding of source leaf function in both natural and controlled environment production systems.
Chapter 3: Effect of Elevated CO₂ and Spectral Quality on Whole Plant Gas Exchange Patterns in Tomato

The work was described in a manuscript accepted in PLoS One and can be found at:


The manuscript has been altered to adhere to the University of Guelph thesis format.

Contributions:

Jason Lanoue and Dr. Bernard Grodzinski designed the study. Jason Lanoue performed the experiments, collected the data, and analysed all data. Jason Lanoue prepared the manuscript. Jason Lanoue, Dr. Evangelos D. Leonardos, Shalin Kholsa, Dr. Xiuming Hao, and Dr. Bernard Grodzinski reviewed and edited the manuscript.
3.1 Abstract

In controlled environment plant production facilities, elevating either light or CO\textsubscript{2} levels generally has led to increased biomass and yield due to enhanced canopy photosynthesis. Today, advancements in light-emitting diodes (LEDs) have made this technology a viable option for both supplementary lighting in greenhouses and a sole lighting source in controlled environment chambers. Our study used tomato plants grown under both ambient CO\textsubscript{2} (AC) and elevated CO\textsubscript{2} (EC) conditions then exposed them to various CO\textsubscript{2} and lighting treatments during both whole plant and leaf level measurements. Plants grown under EC reached the first flower developmental stage 8 days sooner and were approximately 15cm taller than those grown under AC. However, under AC plants had more leaf area while their dry weights were similar. Of note, under EC chlorophyll \textit{a} and \textit{b} were lower, as were carotenoids per unit leaf area. Whole plant analyses, under all CO\textsubscript{2} challenges, showed that plants exposed to high pressure sodium (HPS), red-blue LED, and red-white LED had similar photosynthesis, respiration, and daily carbon gain. Under different light qualities, day-time transpiration rates were similar among CO\textsubscript{2} conditions. Day-time water-use efficiency (WUE) was higher in plants grown and exposed to EC. Similarly, WUE of plants grown under AC but exposed to short-term elevated CO\textsubscript{2} conditions was higher than those grown and tested under AC during all light treatments. Under all CO\textsubscript{2} conditions, plants exposed to red-white and red-blue LEDs had lower WUE than those exposed to HPS lighting. Assessing alterations due to CO\textsubscript{2} and light quality on a whole plant basis, not merely on an individual leaf basis, furthers our understanding of the interactions between these two parameters during controlled environment production. Principle component analyses of both whole plant and leaf data indicates that increasing CO\textsubscript{2} supply has a more dramatic effect on photosynthesis and WUE than on transpiration.

3.2 Introduction

An increase in atmospheric CO\textsubscript{2} is now inevitable, with some predictions indicating concentrations in excess of 1100ppm by the end of the century (Booth et al., 2017). While plants in natural environments are just beginning to be exposed to increasing CO\textsubscript{2} concentrations, elevated CO\textsubscript{2} (EC) has been a staple fertilization method in controlled environment production with varied results (Grodzinski, 1992; Mortensen, 1987). Within high value greenhouse crops, such as
tomatoes, growth under EC has led to increases in both biomass production and yield (Hurd, 1968; Wittwer, 1966).

Light plays a critical role in production as it too increases biomass accumulation when intensity is increased (Evans & Mitchell, 1979). Recent advancements in light-emitting diode (LED) technology, including lower production cost and increased energy efficiency, have made them a viable alternative to high pressure sodium (HPS) lighting for both sole and supplemental lighting (Bugbee, 2017; Nakamura et al., 1994; Singh et al., 2015). A specific advantage of LEDs is their ability to generate wavelength specific lighting. Plants of all species have been observed to have different responses in morphology, primary gas exchanges, and gene expression to illumination with varying wavelengths (Gomez & Mitchell, 2015; Lanoue et al., 2017; Liu et al., 2012; Runkle & Heins, 2001; Tepperman et al., 2004).

Whole plant gas exchange analysis allows for a non-destructive estimation of daily growth patterns and water loss (Dutton et al., 1988; Lanoue et al., 2017; Leonardos et al., 2014). The use of whole plant gas exchange systems provides additional information regarding leaf age and light interception within a plant canopy which is often not apparent during leaf analysis (Davis & McCree, 1978; De Vries, 1982; Dutton et al., 1988; Lanoue et al., 2017; Leonardos et al., 2014). Accounting for differences in plant and leaf morphology is especially important when trying to compare plants which were grown under different conditions known to alter plant architecture, such as light quality and CO₂ concentration (Kramer, 1981; Liu et al., 2012; Woodward & Kelly, 1995; Yelle et al., 1989; Yelle et al., 1990). Furthermore, obtaining whole plant data allows for a greater understanding of the interactions between environmental stimuli. This, in turn, can allow for the translation of information to controlled environment production and climate regulation related to common inputs such as CO₂ and light (Kumar et al., 2016; Tremblay & Gosselin, 1998).

The use of whole plant analysis allows for the study of net carbon exchange rate (NCER), transpiration, and water-use efficiency (WUE) on plants at similar developmental stage and size with inherently different morphologies due to their growth conditions. Our study, by design, used plants grown under either ambient CO₂ (AC) or elevated CO₂ (EC). Whole plant and leaf CO₂ and H₂O gas exchanges were then analyzed at a similar developmental stage under lights of differing spectral quality and various CO₂ conditions. Our primary objective was to assess how primary gas
exchanges of CO₂ and H₂O of tomato plants with different canopy architecture are affected by light quality and CO₂ concentration and how whole plant response compare to leaf responses.

3.3 Materials and Methods

3.3.1 Plant Material and Growth Conditions

Seeds of Solanum lycopersicum L. cv. ‘Bonny Best’ (William Dam Seeds; Dundas, ON, Canada) were sown into 60 cavity potting trays in Sungro professional growing mix #1 (Soba Beach, AB, Canada) and placed in growth chambers (GC-20 Bigfoot series, Biochambers, Winnipeg, MB, Canada). Temperature was set to 22/18°C (d/n) with a 16/8h photoperiod. Plants were illuminated with 300±25 µmol m⁻² s⁻¹ of photosynthetically active radiation (PAR) from compact fluorescent lights (Sylvania Pentron 841 HO Ecologic, Wilmington, MA, USA). Relative humidity was maintained at 60±10%. Growth chambers contained either AC (400±10 µL L⁻¹) or EC (1000±20 µL L⁻¹). Growth chamber CO₂ conditions were rotated periodically to eliminate chamber bias. Plants were watered with fertilizer as needed (20-8-20; pH=6, electrical conductivity=2.3mS cm⁻¹). For both whole plant and leaf experiments, plants grown under AC were analyzed when they were 40-46 days after sowing and plants grown under EC were analyzed when they were 33-38 days after sowing. This was done in order to use plants which were at the first flower developmental stage. This stage was chosen as it represents the transition point for solely vegetative growth to a combination of vegetative and reproductive growth. Furthermore, this stage allowed for a defined point in which plants grown under different CO₂ conditions were at the same developmental stage. All experiments were performed in the Controlled Environment Systems Research Facility at the University of Guelph.

3.3.2 Growth Analysis

3.3.2.1 Diurnal Patterns of Whole Plant Gas Exchanges

The whole plant gas exchange system is identical to that used in Lanoue et al. (2017). Gas exchange measurements were made by sampling each chamber for 90s, cycling through all 6 chambers every 9-minutes throughout day/night periods. Two chambers were illuminated with high pressure sodium (HPS) lights (Agrolite XT; Phillips Lighting, Markham, ON, Canada), two chambers were illuminated with red-blue (LsPro VividGro V1 Grow Fixture; Lighting Science Group Company (LSGC) Warwick, RI, USA) LEDs, and two chambers were illuminated with
red-white (LSGC) LEDs (Figure 3.1). Light treatments were rotated between chambers to remove chamber bias.

Ambient CO$_2$ during analysis was 400±10µL L$^{-1}$ and EC during analysis was 1000±10µL L$^{-1}$. Plants grown under AC conditions were analyzed under either AC or short-term exposure to elevated CO$_2$ (SEC). Plants grown under EC conditions were analyzed only under EC. During EC and SEC analysis, night time CO$_2$ levels were 400±10µL L$^{-1}$. Plants which were grown under fluorescent lighting were placed in the chambers the day before around 15:00:00 and measurements used for the calculations of gas exchange were taken from the following day/night period. Lights were set to 1000±10 µmol m$^{-2}$ s$^{-1}$ at the top of the plant canopy as determined by a Li-COR quantum sensor with a photoperiod of 16/8h. Temperature and relative humidity were 22/18°C and 60±5% respectively.

3.3.2.2 Biomass Partitioning (Destructive Analysis)

Following each whole plant experiment, plants were removed from the chambers and leaf area was measured using a leaf area meter (Li-COR 3100, Li-COR Inc. Lincoln, NE, USA). The roots were washed free of dirt then plant material (leaves, stems, and roots) were partitioned and dried in an oven for 48h at 80°C then weighed.

3.3.2.3 Chlorophyll Content

Prior to entering the whole plant gas exchange system, 6 SPAD measurements were taken from each plant. Two SPAD measurements were taken from each of the upper, middle, and lower ranked leaves. SPAD measurements were similar between leaf ranks and thus, data was pooled. This protocol was repeated at the end of each experimental run. SPAD readings were not altered during whole plant analysis allowing for pooling of reading taken before and after the experiment run (12 measurements in total). SPAD measurements were then converted to chlorophyll content using correction equations generated by spectrophotometer pigment analysis in which chlorophyll concentrations were assigned to SPAD values. Chlorophyll correction curves were generated by extracting leaf punches in 100% dimethyl formamide for 28h at 4°C. Samples were than analyzed at 663.8nm, 646.8nm, and 480nm wavelengths using a spectrophotometer. Concentrations of chlorophyll $a$, $b$, and carotenoids were determined via equations from Porra et al. (1989) and Wellburn (1994). Correlation equations were determined for plants grown under both AC and EC.
**Figure 3.1**: The PAR spectra of the HPS, red-blue LED, and red-white LED floodlights show the wavelength composition. Each light spectrum was determined using a spectroradiometer (Flame Spectrometer, Ocean Optics, Dunedin, FL, USA).
3.3.3 Leaf Gas Exchange Measurements

The fifth most fully expanded leaf was placed in the chamber of a Li-COR 6400 (Li-COR Inc. Lincoln, NE, USA) which was fitted with a 2cm x 3cm clear top chamber. The leaf temperature within the chamber was held at 22°C with a relative humidity of 55-65%. CO₂ conditions included AC, EC, SEC, and short-term ambient CO₂ (SAC) in which plants were grown under elevated CO₂ than analyzed at ambient CO₂ levels. Lights used to generate the leaf gas exchange curves were specially designed LEDs (PAR 38, LSGC) as well as an HPS luminary. LEDs produced the following peak wavelengths: red (660nm), blue (440nm), orange (595nm), green (500nm), white, red-blue, or red-white (Figure 2.1). Three leaves, each from a different plant, were used for each light treatment. Light curves began at a high light intensity and decreased incrementally which follows the procedure from Evans & Santiago (2014). At each light level, the photosynthetic rate was allowed to reach steady-state then a 2-minute period was averaged to produce values for that light level. Of note, for plants under the CO₂ condition SAC, a light curve was not performed, but a measurement at 500 µmol m⁻² s⁻¹ light level was obtained.

3.3.4 Statistical Analysis

All statistics were performed using SAS studio 3.5. Means comparisons were done using a one-way ANOVA with a Tukey Kramer adjustment at p<0.05. Principle component analysis (PCA) (Abdi & Williams, 2010; Wold et al., 1987) was applied to determine the relationship between CO₂ and H₂O gas exchange under different light qualities and CO₂ concentrations for both whole plant and leaf data. For both whole plant and leaf PCA, the analysis was performed using daily averages from each individual experimental run.

3.4 Results

Plants grown under EC reached the flowering stage on average 8 days sooner than did plants grown under AC conditions. Upon destructive biomass harvest, plants grown under EC conditions produced a larger root dry mass than plants grown at AC (Figure 3.2). Plants grown under AC conditions produced a larger stem dry mass than plants grown under EC (Figure 3.2). However, under both conditions, plants had similar total dry matter at their respective first flower developmental stage (Figure 3.2).
Figure 3.2: Biomass partitioning of plants grown under AC or EC. Different letter groups (a,b) represent statistical differences within each plant section at p<0.05 with n=42.
Plants which were grown under EC conditions were taller at the time of whole plant analysis (Table 3.1). An increased leaf area was determined from plants grown under AC conditions (Table 3.1). However, plants grown under EC had a higher specific leaf mass (Table 3.1). Furthermore, plants grown under EC also produced a higher root:shoot (Table 3.1).

**Table 3.1:** Morphological parameters of plants grown under AC and EC. Statistical differences (a,b) within each parameter at p<0.05 with n=20 for plant height and n=42 for leaf area, specific leaf mass, and root:shoot.

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Plant Height (cm)</th>
<th>Leaf Area (cm²)</th>
<th>Specific Leaf Mass (g m⁻²)</th>
<th>Root:Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambient</strong> (400 µL L⁻¹)</td>
<td>26.87(0.55)ᵇ</td>
<td>1992.06(58.32)ᵃ</td>
<td>40.74(0.65)ᵇ</td>
<td>0.073(0.0002)ᵇ</td>
</tr>
<tr>
<td><strong>Elevated</strong> (1000 µL L⁻¹)</td>
<td>41.41(0.63)ᵃ</td>
<td>1512.73(44.47)ᵇ</td>
<td>50.49(0.82)ᵃ</td>
<td>0.097(0.0004)ᵃ</td>
</tr>
</tbody>
</table>

Pigment analysis determined that short-term illumination under different spectral quality did not alter pigment content and thus SPAD readings taken prior to and after each experiment were pooled. Plants grown under EC had lower levels of chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a:b, and carotenoids compare to plants grown under AC conditions (Table 3.2).

**Table 3.2:** Pigment analysis of plants grown under AC and EC. Statistical differences (a,b) within each parameter at p<0.05 with n=42.

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Chlorophyll a (µg cm⁻²)</th>
<th>Chlorophyll b (µg cm⁻²)</th>
<th>Chlorophyll a+b (µg cm⁻²)</th>
<th>Chlorophyll a:b</th>
<th>Carotenoids (µg cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambient</strong> (400 µL L⁻¹)</td>
<td>43.06(0.47)ᵃ</td>
<td>11.91(0.13)ᵃ</td>
<td>54.97(0.60)ᵃ</td>
<td>3.62(0.0014)ᵃ</td>
<td>10.38(0.095)ᵃ</td>
</tr>
<tr>
<td><strong>Elevated</strong> (1000 µL L⁻¹)</td>
<td>34.03(0.43)ᵇ</td>
<td>9.55(0.14)ᵇ</td>
<td>43.58(0.57)ᵇ</td>
<td>3.58(0.010)ᵇ</td>
<td>8.50(0.078)ᵇ</td>
</tr>
</tbody>
</table>
Figure 3.3 displays the primary diurnal whole plant NCER of tomatoes plants which were grown under either AC or EC conditions. Plants were then exposed to either AC, EC, or SEC conditions during whole plant analysis under either HPS (Figures 3.3A, 3.3D, 3.3G, and 3.3J), red-white LED (Figures 3.3B, 3.3E, 3.3H, and 3.3K), or red-blue LED (Figures 3.3C, 3.3F, 3.3I, and 3.3L). Due to vastly similar NCER between light treatments, Figure 3.3 was formatted to emphasize the differences between CO₂ conditions. Net carbon exchange rates were then expressed on a plant basis (Figures 3.3A, 3.3B, and 3.3C), a leaf area basis (Figures 3.3D, 3.3E, and 3.3F), a dry weight basis (Figures 3.3G, 3.3H, and 3.3I), and a chlorophyll basis (Figures 3.3J, 3.3K, and 3.3L). This was done in order to compare plants on the same metric while taking into account any intrinsic differences in morphologies brought about during growth under different CO₂ conditions.

Under all CO₂ conditions and light treatments, day-time (06:00:00-22:00:00) NCER followed similar patterns, being steady during the morning hours and then decreasing during the afternoon hours until the end of the photoperiod (Figure 3.3). Notably, under SEC, the drop in day-time NCER was observed to be more drastic than under EC conditions (Figure 3.3). Under all CO₂ and light conditions, night-time patterns of respiration were similar (Figure 3.3).

The integration of the data presented in Figure 3.3 is presented in Figure 3.4. This data represents the carbon gained by the plant during the light period via carbon assimilation and the subsequent carbon lost during the night period due to respiration (Figure 3.4). Due to vastly similar NCER between light treatments, Figure 3.4 was formatted to emphasize the differences between CO₂ conditions. On both a plant and dry weight basis, plants under the SEC condition accumulated the most carbon by the end of the photoperiod under all lighting conditions (Figures 3.4A-3.4C, 3.4G-3.4I). When expressed on a leaf area basis, plants under both EC and SEC conditions showed an increase in carbon accumulation under all light treatments compared to the AC condition (Figures 3.4D-3.4F). Although on a plant basis the carbon gained during the photoperiod is similar between EC and AC plants, the decrease in leaf area of EC plants compared to AC plants (Table 3.1) causes the increase in carbon gained under the EC condition (Figures 3.3D-3.3F). On a chlorophyll basis, similar to the NCER data in Figures 3.3J-L, plants under both EC and SEC conditions gained more carbon during the photoperiod under all light conditions than plants under AC conditions (Figures 3.4J-L). Similar to the decrease in leaf area, acclimation to EC caused a decrease in the amount of chlorophyll (Table 3.2) and thus, on a chlorophyll basis, an increase in carbon accumulation during
the photoperiod was observed under all light treatments from EC plants compared to plants under AC (Figures 3.4J-L). Consistent with the results obtained in Figures 3.3, under all CO2 and light treatments, the amount of carbon loss during the night period was similar (Figure 3.4).
Figure 3.3: Diurnal patterns of whole plant CO$_2$ gas exchange of tomatoes at the first flower developmental stage grown at either ambient or elevated CO$_2$. Plant grown under AC or EC conditions were analyzed under the same CO$_2$ conditions as well as AC plants analyzed under short-term elevated CO$_2$ (SEC) conditions. Plants were analyzed under either HPS (panels A, D, G, & J), red-white LED (panels B, E, H, & K), or red-blue LED (panels C, F, I, & L). Panels A-C are NCER normalized on a plant basis, panels D-F are NCER normalized on a leaf area basis, panels G-I are NCER normalized on a total dry weight basis, and panels J-L are NCER normalized on a chlorophyll basis. Each point and error bars represent the averages and standard error of n=14 respectively.
**Figure 3.4:** Diurnal patterns of whole plant carbon gain/loss of tomatoes at the first flower developmental stage grown at either ambient or elevated CO$_2$. Plant grown under AC or EC conditions were analyzed under the same CO$_2$ conditions as well as AC plants analyzed under short-term elevated CO$_2$ (SEC) conditions. Plants were analyzed under either HPS (panels A, D, G, & J), red-white LED (panels B, E, H, & K), or red-blue LED (panels C, F, I, & L). Panels A-C are NCER normalized on a plant basis, panels D-F are NCER normalized on a leaf area basis, panels G-I are NCER normalized on a total dry weight basis, and panels J-L are NCER normalized on a chlorophyll basis. Each point and error bars represent the averages and standard error of $n=14$ respectively.
Day-time average whole plant photosynthetic rate was highest when plants were exposed to SEC on a plant basis under all light conditions (Figure 3.5A). Average day-time photosynthetic rates were similar between lights within each CO₂ condition on a plant basis (Figure 3.5A). Under all light conditions, plants exposed to SEC produced higher night-time respiration rates than did plants grown and analyzed under EC (Figure 3.5B). When plants were analyzed under the red-blue LED, plants exposed to SEC produced higher respiration rates than plants grown and analyzed under AC (Figure 3.5B).

On a leaf area basis, day-time average whole plant photosynthetic rates were highest when plants were grown and analyzed under EC and when plants were exposed to SEC under all light conditions (Figure 3.5C). Within each CO₂ condition, lights produced similar average day-time photosynthetic rates (Figure 3.5C). Within each CO₂ condition, night-time respiration rates were similar between light treatments (Figure 3.5D). When analyzed under HPS and red-white LED, average respiration rates were similar regardless of CO₂ conditions (Figure 3.5D). When analyzed under red-blue LED, plants analyzed under EC produced higher average respiration rates than plants analyzed under AC (Figure 3.5D).

Plants exposed to SEC produced the highest day-time average photosynthetic rate compared to those grown and analyzed under either AC or EC under all light treatments when normalized or a dry weight basis (Figure 3.5E). Within each CO₂ conditions, all light treatments produced similar average day-time photosynthetic rates (Figure 3.5E). Similar night-time respiration rates were observed under all CO₂ and light treatments (Figure 3.5F).

When normalized on a total chlorophyll content basis, both plants grown under EC and analyzed under EC and SEC produced the highest average day-time photosynthetic rates under all light treatments (Figure 3.5G). When analyzed under red-blue LED, plants grown and analyzed under EC also produced a higher average photosynthetic rate than did plants exposed to SEC (Figure 3.5G).
Figure 3.5: Day and night time average NCER normalized on a plant (panel A – photosynthesis; B – respiration), leaf area (panel C – photosynthesis; D – respiration), dry weight (panel E – photosynthesis; F – respiration), and chlorophyll basis (panel G – photosynthesis). Different upper-case letter groups (A, B, C or X, Y) represent statistically different values between light treatments at the same CO$_2$ analysis conditions within a panel at p<0.05 where n=14 for day and night-time averages respectively. Different lower-case letter groups (a, b, c or x, y) represent statistically different values within the same light treatments at different CO$_2$ analysis conditions within a panel at p<0.05 where n=14 for day and night-time averages respectively.
Diurnal patterns of whole plant transpiration rate followed similar patterns under all CO\textsubscript{2} and light treatments (Figures 3.6A, 3.6B, and 3.6C). Day-time transpiration rates increased from the start of the photoperiod and reached a maximum around midday (12:00:00-14:00:00) than decreased until the end of the photoperiod (22:00:00) under all light and CO\textsubscript{2} treatments (Figures 3.6A, 3.6B, and 3.6C). Night-time transpiration rates were lower than day-time conditions under all treatments and remained steady during the night period (Figures 3.6A, 3.6B, and 3.6C).

Day-time patterns of WUE for plants grown under and analyzed under both AC and EC followed similar patterns throughout the photoperiod (Figures 3.6D and 3.6F). WUE were highest at the start of the photoperiod, decreased to a minimum around midday (12:00:00-14:00:00) than increased to levels comparable to the start of the day, at the end of the photoperiod (Figures 3.6D and 3.6F). Of note, under all CO\textsubscript{2} conditions, plants illuminated with red-blue and red-white LEDs had lower WUE during the beginning and middle of the day than plants illuminated with HPS lighting (Figures 3.6D and 3.6F). However, at the end of the day, all light treatments under AC and EC converged and similar efficiencies were observed (Figures 3.6D and 3.6F). Interestingly, patterns of day-time WUE were observed to be different under SEC than the other two CO\textsubscript{2} conditions (Figure 3.6E). WUE was still determined to be highest at the start of the day and decrease until midday (Figure 3.6E). However, instead of increasing back to efficiencies similar to the beginning of the photoperiod, only a slight increase, followed by a decrease until the end of the photoperiod was observed (Figure 3.6E). Unlike both AC and EC conditions, end of photoperiod WUE in SEC plants were among the lowest efficiencies observed during the photoperiod (Figure 3.6E).
Figure 3.6: Diurnal patterns of whole plant H$_2$O gas exchange of plants grown at either AC or EC. Plants were either exposed to the same CO$_2$ concentration as their growth condition (panels A, D – ambient; panels C, F – elevated) or SEC (panels B, E). Panels A-C are whole plant transpiration rates and panels D-F are whole day-time WUE. Each point and error bars represent the averages and standard error of n=14 respectively.
Average day-time transpiration rates were similar under all CO$_2$ treatments within a light treatment (Figure 3.7A). Within the AC treatment and the SEC, all light treatments produced similar average day-time transpiration rates (Figure 3.7A). Within the EC treatment, plants illuminated with the red-blue LED produced higher average day-time transpiration rates than plants illuminated with HPS (Figure 3.7A). Average day-time WUE was greater under illumination with HPS or RW light during EC and SEC conditions compared to AC experiments (Figure 3.7B). When illuminated with a red-blue LED, WUE was greater under SEC than other CO$_2$ conditions (Figure 3.7B). Of note, under illumination with a red-blue LED, plants grown and analyzed under EC produced higher day-time WUE than did plants grown and analyzed under AC (Figure 3.7B). Importantly, within each CO$_2$ condition, plants illuminated with either red-blue or red-white LED produced lower average WUE than did plants under HPS illumination (Figure 3.7B).

Between the different light treatments, but within CO$_2$ conditions, leaf respiration rates were similar (Table 3.3; Appendix I). When examining within a light treatment, but between CO$_2$ treatments, the same is true except for the SEC condition under orange light which produces a lower respiration rate than leaves in the same light treatment under AC conditions (Table 3.3). Light quality was observed to have no affect on the light compensation point under AC conditions (Table 3.3). However, under EC treatments, leaves exposed to blue light had a higher light compensation point than all other light treatments except red-blue (Table 3.3). Similarly, leaves exposed to blue light produced a higher light compensation point under SEC conditions than leaves exposed to either orange or green LEDs (Table 3.3).

Quantum yield is a measure of how much carbon is being fixed based on the amount of light the leaf is exposed to. On a leaf area basis, blue light produced the lowest quantum yield among all light treatments under all CO$_2$ conditions (Table 3.3). Furthermore, under SEC conditions, leaves exposed to red LEDs also produced a lower quantum yield than all other light treatments (Table 3.3). Different CO$_2$ treatments showed no effect on quantum yield in most lights, however, leaves exposed to HPS, white, blue, orange, and green light produced increased quantum yields under the SEC treatment (Table 3.3). When examining the quantum yield on a chlorophyll basis and considering the intrinsic anatomical differences brought about by acclimation to EC, leaves exposed to EC had higher quantum yields than those under AC under all wavelengths (Table 3.3).
Expressing quantum yield taking on a chlorophyll basis shows that plants under EC conditions are more efficient at fixing carbon than plants under AC conditions.

Under all wavelengths, at each CO$_2$ conditions, a similar maximum photosynthetic level was produced on both an area and chlorophyll basis respectively (Table 3.3; Appendix II). Interestingly, on a chlorophyll basis, in all light treatments except HPS and the orange LED, leaves under the EC condition produced higher maximum photosynthetic levels than leaves under AC conditions (Table 3.3). These results, considering the change in chlorophyll content due to CO$_2$ growth conditions (Table 3.2), show that there is no photosynthetic acclimation, like that observed when normalizing on leaf area, from leaves grown under EC conditions compared to AC conditions.
Figure 3.7: Average day-time transpiration rates (panel A) and average day-time WUE (panel B). Different letter groups (A, B, C) represent statistically different values between light treatments at the same CO$_2$ analysis conditions within a panel at p<0.05 where n=14. Different lower-case letter groups represent statistically different values within the same light treatments at different CO$_2$ analysis conditions within a panel at p<0.05 where n=14.
Table 3.3: A summary of the major physiological traits determined by analysis of leaf gas exchange of plant exposed to various CO$_2$ and light treatments (Appendix I & II). Respiration values were calculated as the average of 3 replicates when the light level was 0 µmol m$^{-2}$ s$^{-1}$, the light compensation point, and quantum yield were calculated from a regression line (y=mx+b) fitted to the values between the light levels of 0-100 µmol m$^{-2}$ s$^{-1}$. The photosynthetic max (P$_{n_{\text{max}}}$) was calculated from $y=y_0+a(1-e^{bx})$. Note, quantum yield and P$_{n_{\text{max}}}$ are given on a leaf area basis and a chlorophyll basis to show the difference when expressing data on two different normalization factors. Letter groups (A,B,C,D) indicate significant differences (p<0.05) as per multiple means comparison with a Tukey-Kramer adjustment at the same CO$_2$ conditions between the different light treatments. An Asterisk (*) indicates a significant difference (p<0.05) under the same light treatment between AC (control) CO$_2$ condition and either EC or SEC.
<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>CO₂ Condition</th>
<th>Respiration (µmol CO₂ m⁻² s⁻¹)</th>
<th>Light Compensation Point (µmol m⁻² s⁻¹)</th>
<th>Quantum Yield (µmol CO₂ m⁻² s⁻¹/µmol m⁻² s⁻¹)</th>
<th>Pₙmax (µmol CO₂ m⁻² s⁻¹)</th>
<th>Quantum Yield (µmol CO₂ g Chl⁻¹ s⁻¹/µmol CO₂ m⁻² s⁻¹)</th>
<th>Pₙmax (µmol CO₂ g Chl⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>HPS</td>
<td>AC</td>
<td>-1.54(0.33)A</td>
<td>30.61(6.21)A</td>
<td>0.049(0.0006)A</td>
<td>19.52(3.40)A</td>
<td>0.090(0.001)A</td>
<td>35.52(6.18)A</td>
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<td>24.66(1.98)A</td>
<td>0.13(0.001)A</td>
<td>44.85(3.60)A</td>
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<td>20.10(0.96)A</td>
<td>0.15(0.001)A</td>
<td>46.13(2.21)A</td>
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<td>-1.67(0.32)A</td>
<td>20.88(1.95)A</td>
<td>0.056(0.002)A</td>
<td>20.88(1.95)A</td>
<td>0.10(0.004)A</td>
<td>37.98(3.55)A</td>
</tr>
<tr>
<td></td>
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<td>24.69(5.13)AB</td>
<td>0.063(0.002)A*</td>
<td>23.59(0.24)A</td>
<td>0.12(0.003)A</td>
<td>42.91(0.44)A</td>
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<td>EC</td>
<td>-1.73(0.09)A</td>
<td>27.81(1.77)B</td>
<td>0.061(0.002)A</td>
<td>23.39(1.98)A</td>
<td>0.14(0.004)A</td>
<td>53.67(4.55)A*</td>
</tr>
<tr>
<td>Red-Blue</td>
<td>AC</td>
<td>-2.07(0.20)A</td>
<td>31.28(1.39)A</td>
<td>0.056(0.003)A</td>
<td>20.83(1.19)A</td>
<td>0.096(0.005)A</td>
<td>37.90(2.17)A</td>
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<td>SEC</td>
<td>-1.69(0.06)A</td>
<td>26.59(1.10)AB*</td>
<td>0.059(0.002)A*</td>
<td>23.33(1.96)A</td>
<td>0.11(0.004)AB</td>
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<td>50.19(1.86)A*</td>
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<tr>
<td>Red-White</td>
<td>AC</td>
<td>-1.82(0.09)A</td>
<td>29.74(1.39)A</td>
<td>0.056(0.003)A</td>
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<td>25.07(4.32)B</td>
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<td>21.62(0.18)A*</td>
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<td>49.62(0.42)A*</td>
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</table>
Under AC, EC, and SEC CO₂ conditions, leaves exposed to light from a blue LED produced the lowest photosynthetic rate at a light level of 500 µmol m⁻² s⁻¹ on both a leaf area and chlorophyll basis (Table 3.4). This result is reflective of the low quantum yield values produced from leaves exposed to blue light, as seen in Table 3.3. Similar to the results in Table 3.3, when Pn₅₀₀ was expressed on a chlorophyll basis, leaves under EC conditions produced a higher photosynthetic rate than AC conditions under all light treatments except HPS and green (Table 3.4). Leaves exposed to white, red-white, red-blue, and green LEDs all showed a decrease in transpiration under SEC compared to AC conditions. Leaves exposed to blue light provided the lowest WUE under AC, SEC, and SAC conditions compared other light treatments (Table 3.4). This is reflective of leaves exposed to blue light producing both among the highest transpiration rates and the lowest Pn₅₀₀ rates. Consistent with whole plant data, leaves under the SEC condition produced a higher WUE than leaves under AC under all light treatments (Table 3.4). This result is in part due to the increase in Pn₅₀₀ under SEC compared to AC as well as the reduced transpiration rate brought about by increasing the CO₂ concentration.

Table 3.4: Leaf CO₂ and H₂O gas exchanges under various CO₂ and light qualities at a light level of 500 µmol m⁻² s⁻¹. Of note, photosynthesis is expressed on both a leaf area and chlorophyll basis. Letter groups (A,B,C,D) indicate significant differences (p<0.05) as per multiple means comparison with a Tukey-Kramer adjustment at the same CO₂ conditions between the different light treatments. An asterisk (*) indicates a significant difference (p<0.05) between AC (control) and SEC under the same light treatment but between different CO₂ conditions. A caret symbol (^) indicates a significant difference (p<0.05) between EC (control) and SAC under the same light treatment but between different CO₂ conditions.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>CO₂ Condition</th>
<th>P₅₀₀ (µmol CO₂ m⁻² s⁻¹)</th>
<th>P₅₀₀ (µmol CO₂ g Chl⁻¹ s⁻¹)</th>
<th>Transpiration₅₀₀ (mmol H₂O m⁻² s⁻¹)</th>
<th>WUE₅₀₀ (µmol CO₂ m⁻² s⁻¹/ mmol H₂O m⁻² s⁻¹)</th>
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<tr>
<td>HPS AC</td>
<td>12.66(1.16)ABC</td>
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</tr>
<tr>
<td>SEC AB*</td>
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<td>31.68(3.08)AB</td>
<td>1.34(0.07)AB</td>
<td>13.14(1.83)B*</td>
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</tr>
<tr>
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<td>15.35(0.93)AB</td>
<td>35.21(2.13)AB</td>
<td>1.79(0.31)A</td>
<td>8.95(1.20)A</td>
<td></td>
</tr>
<tr>
<td>SAC BC</td>
<td>12.94(1.62)A</td>
<td>29.69(3.22)A</td>
<td>2.00(0.34)AB</td>
<td>6.60(0.51)BC</td>
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<tr>
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<td>28.52(2.65)A</td>
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<tr>
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<td>---------------</td>
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<td>30.50(1.21)A^</td>
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<tr>
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<td>22.47(3.19)A^</td>
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<td><strong>Blue</strong></td>
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<td>22.47(2.92)A^</td>
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<td>SAC</td>
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<td>26.17(1.05)Aa^</td>
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<td>28.97(3.87)A</td>
<td>1.37(0.07)B</td>
<td>10.23(0.69)A</td>
</tr>
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</table>

Principle component analysis is a statistical analysis which allows for the assessment of how strongly a set parameter, in this case CO₂ condition and light quality, affect the response variables.
(Photosynthesis, transpiration, and WUE). Upon performing a PCA on whole plant data, Figure 3.8 was obtained. PCA was performed on each individual run (Appendix III), however, for simplicity, Figure 3.8 only shows the average values. Values associated with EC and SEC (i.e., Triangles and squares) experiments tend to associate more with the photosynthesis and WUE vectors, independent of light quality (Figure 3.8A). Of note, values associated with plants illuminated with red-blue and red-white LEDs under all CO\(_2\) conditions are more closely associated with the transpiration vector (Figure 3.8A). Taken together, these results indicate that transpiration rate is controlled more so by spectral quality while photosynthesis and WUE are more influenced by elevations in the CO\(_2\) concentration (Figure 3.8A). When expressing photosynthetic data on a chlorophyll basis, EC conditions produce a stronger influence on photosynthesis and WUE (Figure 3.8B) than when photosynthesis is expressed on a leaf area basis (Figure 3.8A). For example, note the rightward shift of the triangular symbols in Figure 3.8B. The shift to the right due to EC exposure indicates the importance of considering changes in plant anatomy and biochemistry when analyzing primary gas exchanges.

Similar to whole plant data, a PCA was performed on all experimental runs of leaf data at a light level of 500 µmol m\(^{-2}\) s\(^{-1}\) (Appendix IV), and for ease of interpretation, the averages are displayed in Figure 3.9. Results from the PCA involving leaf data are consistent with that in Figure 3.8 (Figure 3.9). On a leaf area basis, plants exposed to EC or SEC conditions tend to affect both photosynthesis and WUE more than transpiration (Figure 3.9A). Furthermore, consistent with Figure 3.8B, when leaf photosynthesis was expressed on a chlorophyll basis, plants under the EC conditions tend to affect the photosynthetic rate and WUE more than when expressed on a leaf area basis (Figure 3.9B). Again, the data presented in Figure 3.9B emphasizes the importance of recognizing key anatomical and biochemical differences brought about by acclimation to different CO\(_2\) treatments.
**Figure 3.8:** Principle component analysis of the impact of CO$_2$ condition and light quality on whole plant gas parameters such as the average photosynthesis, transpiration, and WUE of a tomato at the first flower developmental stage. Values identified by a circle (●) indicates plants grown and analyzed at AC, a triangle (▼) indicates plants grown and analyzed at EC, and a square (■) indicates plants grown at ambient CO$_2$ then analyzed under SEC. Panel A represents all values normalized on a leaf area basis. Panel B represents photosynthesis on a chlorophyll basis (µmol CO$_2$ g Chl$^{-1}$ s$^{-1}$), transpiration on an area basis (mmol H$_2$O m$^{-2}$ s$^{-1}$), and the resulting WUE (µmol CO$_2$ g Chl$^{-1}$/ mmol H$_2$O m$^{-2}$).
Figure 3.9: Principle component analysis of the impact of CO$_2$ condition and light quality on leaf gas parameters such as average photosynthesis, transpiration, and WUE of a tomato at the first flower developmental stage and a light level of 500 µmol m$^{-2}$ s$^{-1}$. Values identified by a circle (●) indicates plants grown and analyzed at AC, a triangle (▼) indicates plants grown and analyzed at EC, a square (■) indicates plants grown at ambient CO$_2$ then analyzed under SEC, a diamond indicates plants grown under elevated CO$_2$ then analyzed under SAC. Panel A represents all values normalized on a leaf area basis. Panel B represents photosynthesis on a chlorophyll basis (µmol CO$_2$ g Chl$^{-1}$ s$^{-1}$), transpiration on an area basis (mmol H$_2$O m$^{-2}$ s$^{-1}$), and the resulting WUE (µmol CO$_2$ g Chl$^{-1}$/ mmol H$_2$O m$^{-2}$).
3.5 Discussion

The effect of growing plants under EC on flowering time is highly variable among species (Mortensen, 1987; Springer & Ward, 2007). For tomato, growth under EC generally results in a decrease in the time to flowering (Hicklenton & Jolliffe, 1978; Micallef et al., 1995) which is confirmed by the 8-day decrease observed in our study. Furthermore, growth under EC increases biomass gain within tomatoes (Kimball & Mitchell, 1979). In this study, we have purposely compared plants at a similar developmental stage when grown under the different CO₂ levels. Doing so, an increase in total dry biomass was not apparent, likely due to the age difference (Figure 3.2). However, at the first flower developmental stage, an increase in root biomass was observed when plants were grown under EC which is consistent with results from many species including tomato (Fig 1; Pregitzer et al., 2008; Reimert et al., 1997; Rogers et al., 1992). An increase in root biomass could be associated with an increase in plant biomass gain at similar ages, generally observed due to the ability of a plant to uptake more water and nutrients needed for growth (NeSmith & Duval, 1998).

Consistent with the effects of long-term growth under EC, tomato plants exhibited an increase in stem elongation compared to AC grown plants (Table 3.1; Kramer, 1981). Plants grown under EC produced less leaf area than those grown under AC conditions which opposes the results from Ho (Table 3.1; Ho, 1977). However, plants in our study were analyzed at the same developmental stage and thus intrinsically different ages which could account for the differences in results observed. Furthermore, an increase in specific leaf weight was observed by plants grown under EC (Table 3.1). An increase in specific leaf weight is consistent with literature and due to an increase of non-structural carbohydrates, particularly starch, at EC levels which accumulate within the leaves (Poorter et al., 1997).

Chlorophyll a, chlorophyll b, and total chlorophyll, elements involved in light absorption, all decreased in plants grown under EC (Table 3.2). Similar results were observed in tomato and Trifolium subterraneum (Cave et al., 1981; Madsen, 1976). It has been proposed that an increase in CO₂ levels leads to a degradation of chloroplast as a result of the excess starch accumulation (Madsen, 1976). However, other literature in various species has shown no significant effect of CO₂ concentration on chlorophyll content (Kim & You, 2010; Mulholland et al., 1997). Thus, while
results presented in table 3.2 clearly indicate decreases in chlorophyll and carotenoid content, the literature surrounding this topic is still variable.

Whole plant NCER under all CO₂ conditions show no difference due to spectral quality which confirms results published in Lanoue et al. (Figure 3.3; Lanoue et al., 2017). This result indicated that when considering the complexity of a whole plant canopy, short-term exposure to wavelength specific lighting is unable to affect the primary photosynthetic and respiratory processes. Interestingly, day-time NCER decreases in the latter part of the day under all CO₂ conditions but is observed to decrease more when plants were exposed to SEC (Figure 3.3). A decrease in photosynthetic rate has also been observed in rice and was observed to be more dramatic with an increased CO₂ concentration (Winder et al., 1998). It has been suggested that under high carbohydrate production, such as the conditions used in EC and SEC experiments, an end product feedback causes an inhibition of the photosynthetic apparatus resulting in the drop off in NCER later in the photoperiod (Figure 3.3; Delucia et al., 1985; Goldschmidt & Huber, 1992; Stitt, 1986).

When plants were grown under AC than exposed to SEC an increase in average whole plant photosynthesis was observed compared to plants grown and analyzed under AC (Figure 3.5). Short-term exposure to elevated CO₂ leading to an increase in the photosynthetic rate is attributed to the increase of the carboxylation reaction of RuBisCO and subsequent decrease in photorespiration (Besford, 1993; Bowes, 1991; Stitt, 1991). Under long-term acclimation to EC, after an initial increase in photosynthesis, a decrease is reported in tomato when compared to plants grown under AC indicating acclimation of the photosynthetic machinery to EC (Yelle et al., 1989). However, it is important to note that plants grown under different CO₂ conditions have different anatomy and biochemical features, specifically, different chlorophyll content on a leaf area basis (Table 3.2). Therefore, when normalizing photosynthetic rates on a chlorophyll basis, plants grown under EC produced approximately a 52% increase in the photosynthetic rate under all light treatments compared to the ambient grown plants (Figure 3.5G).

Night-time respiration rates are associated with day-time photosynthetic rates and carbohydrate status (Azcon-Bieto & Osmond, 1983; Whitehead et al., 2004). This association is clearly indicated by results in Figure 3.5B where plants analyzed under SEC produced higher average photosynthetic rates and subsequent higher respiration rates than did plants with a lower photosynthetic rate. However, when normalizing on both leaf area and dry biomass, this
relationship is not observed (Figure 3.5D and 3.5F). Thus, the effect of CO$_2$ concentrations on night-time respiration is still debateable as both increases and reductions have been reported elsewhere (Xu et al., 2015). Importantly, whole plant night-time respiration rates are not observed to be affected by day-time light quality which confirm previous results for tomato reported by Lanoue et al. (2017).

While both CO$_2$ and H$_2$O gas exchange with the external environment are mediated via stomata, transpiration control is also linked to cryptochrome (CRY), a blue light receptor, which is known to follow an entrained circadian rhythm (Toth et al., 2001). Furthermore, the diurnal patterns of CRY follow closely to that of transpiration, indicating a strong link between CRY activation and transpiration rates (Dodd et al., 2004; Mao et al., 2005). Thus, the results in Figure 3.6 indicate that all light treatments provide adequate amounts of blue light to maintain normal circadian rhythms of stomatal behaviour.

Whole plant WUE describes the relationship between water loss via transpiration and carbon fixation via photosynthesis of a canopy (Figure 3.7B). Plants grown under AC were exposed to SEC, a decrease in WUE was observed in the latter part of the photoperiod (Figure 3.6E). This decrease is due to the drastic reduction in photosynthetic rate under this condition, something which is not as strongly apparent in AC or EC conditions, coupled with the entrained circadian rhythm of transpiration rate (Figures 3.3 and 3.6). As mentioned above, the reduction in the photosynthetic rate is consistent with feedback inhibition via plant carbon status and thus able to affect WUE more so during SEC challenge than AC or EC conditions (Delucia et al., 1985; Goldschmidt & Huber, 1992; Stitt, 1986).

Decreases in WUE were observed when plants were illuminated with either red-blue or red-white LEDs compare to illumination with HPS under all CO$_2$ condition (Figure 3.7B), confirming results first noted at AC (Lanoue et al., 2017). Water-use-efficiency results indicate that plants grown under EC and those grown under AC but analyzed under SEC fix more carbon per water loss via transpiration than plants under AC (Figure 3.7B). Due to the similarity of day-time transpiration rates among CO$_2$ conditions, it follows that the increase in WUE under EC and SEC are predominantly due to an increase in photosynthetic rates.

Average day-time transpiration rate under AC were unaffected by spectral quality, as were rates of plants analyzed under SEC (Figure 3.7A). Plants grown and analyzed under EC which were
illuminated with a red-blue LED produced higher average day-time transpiration rates than plants illuminated with HPS lighting (Figure 3.7A). This result is attributed to the increased amount of blue light emitted by the red-blue LED which is known to cause stomatal opening (Assmann et al., 1985; Kinoshita et al., 1999; Kinoshita et al., 2001). It is noteworthy that the whole plant chambers used during our experiments control air temperature more tightly than in a greenhouse. Thus, it is unlikely that excess heat generated by HPS lighting will affect our gas exchange measurements as it may in greenhouses equipped with HPS lamps.

It is well known that photosynthesis and transpiration are tightly linked as they both rely on stomatal opening (Daloso et al., 2016). In general, under all wavelengths of light tested, leaf transpiration rates were reduced by short-term exposure to elevated CO₂, consistent with literature (Ainsworth & Long, 2014). However, contrary to leaf results from free-air concentration enrichment (FACE) experimentation indicating a decrease in transpiration rates under EC (Ainsworth & Long, 2014; Leakey et al., 2009; Swann et al., 2016), no differences were observed during whole plant H₂O gas exchange analysis under increased CO₂ (Figure 3.7A). However, similar transpiration rates from plants exposed to different CO₂ conditions has been previously observed. Using lisianthus plants grown under AC conditions, whole plant transpiration rates were similar when analyzed under AC and SEC conditions (data unpublished). Similarly, wheat leaves which were exposed to elevated CO₂ produced similar stomatal conductance values to leaves exposed to ambient CO₂ over a wide range of light intensities (Tazoe et al., 2009). In Arabidopsis thaliana plants grown under both AC and EC conditions, whole plant transpiration rates were also observed to be similar (Leonardos et al., 2014). It is noteworthy to bring to the readers attention that when expressed on a plant basis, plants acclimated to EC would have a lower transpiration rate compared to plant grown under AC. However, due to differences brought about during growth under different CO₂ conditions (Table 3.1), when expression transpiration data on the traditional leaf area basis, this difference is negated due to the difference in leaf area.

The discrepancy between the response of leaf and whole plant transpiration resulting from exposure to SEC is likely due to the additional complexity involved in measurements of whole plant gas exchange. Measuring whole plant transpiration (Figure 3.6) shows the complicated nature of simple extrapolation of leaf data (Table 3.2) as it does not account for differences in leaf age, canopy architecture, micro-climate, and mutual shading (Medrano et al., 2015). During leaf
level measurements, controlling leaf temperature can be done accurately. However, controlling canopy temperature can be more difficult during whole plant measurements. During SEC, it is possible that the leaf temperature of the plants was increased compared to plants under AC (Morison & Gifford, 1984). The reaction will then be to increase transpiration in order to regulate the leaf temperature. It can be hypothesized that under SEC, the increase in leaf temperature is able to compensate for the affect of high CO\textsubscript{2} on stomatal function, leading to a higher whole plant transpiration rate than one would expect when modelling using solely leaf data. Furthermore, mature leaves under low light intensity, such as those deep within the canopy, naturally transpire at a lower rate. Thus, differences in transpiration rate between AC and SEC conditions will be minimal. The complexity brought about by measuring a whole plant instead of merely a leaf, illustrates the difference between CO\textsubscript{2} and H\textsubscript{2}O gas exchange via stomata of newly formed and mature leaves under different environmental parameters.

A principle component analysis was performed to assess the effects of changes in the light spectrum and/or CO\textsubscript{2} conditions on photosynthesis, transpiration, and WUE (Figure 3.8). The analysis determines that values associated with EC plants and those analyzed under SEC are more closely associated with changes in photosynthesis and WUE (Figure 3.8). In contrast, these same points are observed to have a relatively small influence on transpiration rates (Figure 3.8). Figure 3.8 indicates that both red-blue and red-white LEDs affect transpiration rates more than illuminating plants with HPS lighting. These results on whole plant CO\textsubscript{2} and H\textsubscript{2}O gas exchange confirm leaf studies of Xanthium strumarium L. which showed a lower stomatal sensitivity to increasing CO\textsubscript{2} concentrations (Sharkey & Raschke, 1981). Furthermore, PCA performed on leaf data shows similar results with plant exposed to elevated CO\textsubscript{2} levels tending to affect photosynthesis and WUE more than transpiration (Figure 3.9). The affects of both light quality and CO\textsubscript{2} concentration have on transpiration and WUE have implication in water and nutrient management during winter greenhouse production when humidity is generally low, inhibiting stomatal function (Alloway, 2008; Lange et al., 1971).

Taken together, results presented above show evidence that plants acclimated to EC and those exposed to SEC produce higher photosynthetic rates while having similar whole plant transpiration values compared with plants under AC during all light treatments. Furthermore, illumination with either red-blue or red-white LED produced lower WUE than illumination with HPS luminaries,
likely due to a higher blue light component. Results presented here allow for a greater understanding of the inter-play between a common greenhouse production method, CO$_2$ enrichment, and a new, fast-evolving technology, wavelength specific LED lighting. By doing such research on a whole plant, a better understanding of the physiological response can be obtained while considering leaf age and canopy architecture, factors which are not fully taken into account when conducting leaf level measurements. This information can lead to the optimization of lighting strategies as well as watering and fertilizing regimes in greenhouse production providing increased sustainability and yield.
Chapter 4: Alternating Red and Blue Light Emitting Diodes Allows for Injury-Free Tomato Production with Continuous Lighting

The work was described in a manuscript accepted in Frontiers in Plant Science: Crop and Product Physiology section and can be found at:


The manuscript has been altered to adhere to the University of Guelph thesis format.

**Contributions:**

Jason Lanoue and Dr. Xiuming Hao designed the study. JingMing Zheng, Celeste Little, and Alyssa Thibodeau handled crop maintenance. Jason Lanoue, JingMing Zheng, Celeste Little, and Alyssa Thibodeau took growth and morphological measurements. Jason Lanoue performed physiological measurement. Jason Lanoue performed the data analysis. Jason Lanoue prepared the manuscript. Jason Lanoue, Dr. Bernard Grodzinski, and Dr. Xiuming Hao reviewed and edited the manuscript.
4.1 Abstract

Plant biomass is largely dictated by the total amount of light intercepted by the plant (daily light integral (DLI) – intensity × photoperiod). Continuous light (CL, 24h lighting) has been hypothesized to increase plant biomass and yield if CL does not cause any injury. However, lighting longer than 18h causes leaf injury in tomato characterized by interveinal chlorosis and yield is no longer increased with further photoperiod extension in tomatoes. Our previous research indicated the response of cucumbers to long photoperiod of lighting varies with light spectrum. Therefore, we set out to examine greenhouse tomato production under supplemental CL using an alternating red (200 µmol m$^{-2}$ s$^{-1}$, 06:00-18:00) and blue (50 µmol m$^{-2}$ s$^{-1}$, 18:00-06:00) spectrum in comparison to a 12h supplemental lighting treatment with a red/blue mixture (200 µmol m$^{-2}$ s$^{-1}$ red + 50 µmol m$^{-2}$ s$^{-1}$ blue, 06:00-18:00) at the same DLI. Our results indicate that tomato plants grown under supplemental CL using the red and blue alternating spectrum were injury-free. Furthermore, parameters related to photosynthetic performance (i.e., P$_{\text{max}}$, quantum yield, and F$_{V}$/F$_{m}$) were similar between CL and 12h lighting treatments indicating no detrimental effect of growth under CL. Leaves under CL produced higher net carbon exchange rates (NCER) during the subjective night period (18:00-06:00) compared to plants grown under 12h lighting. Notably, 53 days into the treatment, leaves grown under CL produced positive NCER values (photosynthesis) during the subjective night period, a period typically associated with respiration. At 53 days into the growth cycle, it is estimated that leaves under CL will accumulate approximately 800 mg C m$^{-2}$ more than leaves under 12h lighting over a 24h period. Leaves grown under CL also displayed similar diurnal patterns in carbohydrates (glucose, fructose, sucrose, and starch) as leaves under 12h lighting indicating no adverse effects on carbohydrate metabolism under CL. Taken together, this study provides evidence that red and blue spectral alternations during CL allow for injury-free tomato production. We suggest that an alternating spectrum during CL may alleviate the injury typically associated with CL production in tomato.

4.2 Introduction

Continuous light (CL) means a constant flux of energy into photosynthesis, theoretically leading to increased growth and the potential for higher yield. In lettuce, low irradiance CL has already been shown to increase plant growth (Kitaya et al., 1998; Ohtake et al., 2018). However, in plants such as tomato, potato, and eggplant, CL has been associated with negative responses in growth
traits (Velez-Ramirez et al., 2011). In tomatoes, a down regulation in photosynthesis due to excess accumulation in carbohydrates, decreased maximum quantum efficiency of photosystem II (PSII), and early leaf senescence has been reported during extended photoperiods leading to CL-injury such as chlorosis and decreased production (Demers et al., 1998; Velez-Ramirez et al., 2017).

The underlying mechanism of CL-injury in tomato has yet to be determined. However, comparisons between CL-tolerant wild-type tomatoes and CL-sensitive domesticated tomatoes via RNA sequencing has shown that a downregulation of the gene *type III light harvesting chlorophyll a/b binding protein 13 (CAB13)* during CL confers injury (Velez-Ramirez et al., 2014). Furthermore, differences (mis-matching) between the external light/dark cycles and a plant’s internal circadian rhythms has been demonstrated to decrease the photosynthetic rate and show injuries related to CL (Velez-Ramirez et al., 2017b). The role of circadian asynchrony in CL-injury is strengthened by the circadian oscillations of light harvesting complex genes as determined by mRNA analysis (Kellman et al., 1993). Such oscillations may not allow the plant to fully utilize the CL during periods of low gene expression of proteins which make up the light harvesting complex.

The role of photoreceptors (i.e., phytochrome and cryptochrome) in CL-injury has also been postulated (Velez-Ramirez et al., 2011). Demers and Gosselin (2000) noted that tomato grown under CL with metal halide (MH) lamps had more severe leaf chlorosis and decreased photosynthetic rates compared to plants grown under CL with high pressure sodium (HPS). Of note, MH luminaries have a higher blue light component than HPS indicating that spectral quality may play a role in CL-injury. A recent study indicates that the overexpression of phytochrome A diminishes the injury associated with CL in tomato (Velez-Ramirez et al., 2019). This result indicates a potential for spectral modifications in alleviating CL-injury.

In addition, the role of temperature has been investigated with respect to CL-injury. The presence of a thermoperiod (oscillations in temperature during a 24h period) has been shown to negate the effect of CL-injury in many species (Hillman, 1956; Velez-Ramirez et al., 2011; Matsuda et al., 2014; Haque et al., 2017; Hao et al., 2017a; Hao et al., 2018b). In tomato, a temperature drop of 10°C during what would be the night period during CL increased maximum quantum efficiency of photosystem II (*Fv/Fm*) to levels similar to a control tomato plant grown under a conventional 12h day/12h night period (Haque et al., 2017). Using *Fv/Fm* as an indication of photoinhibition
(i.e., degradation of photosystem II antenna complex), an increase in this value indicates a reduction in CL-injury facilitated by a temperature drop. In addition, a temperature drop has been shown to alleviate the inhibition on photosynthesis due to excess carbohydrate production during CL (Demers & Gosselin, 1999; Matsuda et al., 2014; Haque et al., 2015; Haque et al., 2017). The carbohydrate status (glucose, fructose, sucrose, and starch) at the end of the light period from tomatoes grown under CL and a variable temperature (CLVT) was similar to plants grown under CL and a constant temperature (CLCT; Haque et al., 2017). Interestingly, at the end of what would have been the night period under CLVT, there was an increase in starch, sucrose, glucose, and fructose compared to the end of the day values (Haque et al., 2017). These results coupled with a high photosynthetic rate from plants under CLVT indicates that the accumulation of carbohydrates may not be solely responsible for CL-injury as it was not observed in CLVT treatment. Instead, it has been hypothesized that a temperature drop during CL may upregulate CAB13 allowing for normal energy balance and CL-injury free growth (Haque et al., 2017).

Most studies to date have used sole artificial CL with constant temperature and spectrum in controlled growth chambers and observed CL related injury in tomato (Hillman, 1956; Golbig et al., 1997; Matsuda et al., 2014; Haque et al., 2015; Haque et al., 2017; Velez-Ramirez et al., 2017b). The exceptions (Arthur et al., 1930; Demers et al., 1998) used high intensity discharge lights with fixed light spectral composition as supplemental lighting within a greenhouse. However, due to the intrinsic properties of the lighting fixtures, temperature control was an issue and thus thermoperiod effects are likely to play a factor in the results obtained. Also, our previous research on cucumbers shows that response to long photoperiods including CL varies with light spectrum (Hao et al., 2018c).

The introduction of light-emitting diodes (LEDs) as a supplemental lighting fixture not only allows for better temperature control within greenhouses, but also provides the ability to administer time-dependent wavelength specific light during the photoperiod. Furthermore, using LEDs allows for the economical implementation of lighting fixtures which can alter spectral quality without the need for added units. With this ability, we set out to assess the physiological and morphological effects of supplemental CL with alternating spectrum provided by LED fixtures.

The objective of the study was to assess the effects of supplemental CL with alternating light spectrum on tomato growth. We hypothesize that by alternating the light spectrum, CL-injury may
be alleviated during tomato production due to the drastic shift in spectral quality, decreased light intensity during the night period, and blue light alleviating carbohydrate accumulation (Lanoue et al., 2018a). The hypothesis was tested during the winter months in a Canadian greenhouse when supplemental lighting is most needed to achieve adequate tomato production.

4.3 Materials and Methods

4.3.1 Plant Material and Experimental Design

Tomato (*Solanum lycopersicum* L.) seedlings cv. ‘Endeavour’ were grafted onto cv. ‘Maxifort’ (a common scion and rootstock combination used in greenhouse production) in a double stemmed system. Transplants (5 weeks old – planted on October 14th, 2018) were placed into two adjacent double layers polyethylene (one year old) greenhouses (50 m² growing area) at the Harrow Research and Development Centre, Agriculture and Agri-Food Canada, Harrow, Ontario, Canada (42.03°N, 82.9°W) on November 9th, 2018 at a plant density of 1.75 plants m⁻² (3.5 stems m⁻²). The plants were drip-irrigated using a complete nutrient solution (Ontario Ministry of Agriculture, Food and Rural Affairs, 2010). The electrical conductivity and pH were set at 2.8 dS m⁻¹ and 5.8 respectively.

The greenhouses were divided into two sections via white curtains which were impenetrable to light. Each section was further divided into two blocks (i.e. 4 blocks per treatment). Two supplemental lighting treatments were used in the experiment: a conventional 12h (06:00h-18:00h) lighting system providing red and blue light from LED fixtures (Pro 650e, LumiGrow Inc., Emeryville, California, USA) at the same time and a CL system with 12h of red light during the day (06:00h-18:00h) and 12h of blue light during the night (18:00h-06:00h) from LEDs (LumiGrow Pro 650e, Table 4.1). The two lighting treatments provided the same DLI (Table 4.1). The lighting treatments were chosen for multiple reasons. Firstly, the prevalence of red + blue LED lighting fixtures is common (respective to total LED fixtures) during greenhouse production. Thus, the implementation of an alternating red and blue CL light treatment would be easily facilitated by growers if deemed useful. Secondly, as observed in Chapter 2, blue light has been determined to export sugars at a higher rate than other wavelengths. It is then thought that blue light can negate over-accumulation of carbohydrates and avoid feed-back inhibition of
photosynthesis which has previously been hypothesized to cause CL-injury (Velez-Ramirez et al., 2017a).

Application of both supplemental lighting treatments began on November 15th, 2018 and continued to May 16th, 2018 with harvest beginning on January 28th, 2019. Throughout the experiment, supplemental lighting remained on regardless of ambient DLI (Figure 4.1) to ensure both treatments received the same total DLI. The daytime temperature was held between 21-24°C depending on the ambient solar radiation while night temperature was maintained at 20±0.5°C. Relative humidity of 70±10% was maintained during both day and night periods. Greenhouses were CO₂ enriched to approximately 800 µL L⁻¹.

### Table 4.1: Photosynthetic photon flux density of supplemental lighting treatments during the day and night periods as measured above plant canopy (1m below the LED fixtures). Peak output measured by a spectrometer (Flame spectrometer, Ocean Optics, Dunedin, FL, USA) from the red and blue LEDs was 660nm and 447nm, respectively.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>06:00-18:00h</th>
<th>18:00h-06:00h</th>
<th>Daily Light Integral (mol m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red (µmol m⁻² s⁻¹)</td>
<td>Blue (µmol m⁻² s⁻¹)</td>
<td>Red (µmol m⁻² s⁻¹)</td>
</tr>
<tr>
<td>12h Lighting</td>
<td>200</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Continuous Lighting</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 4.1: Total natural solar radiation as measured outside of the greenhouse using a Li-COR LI-200R pyranometer. Measurements were taken every 15 min between the wavelengths of 400-1100nm throughout the course of the experiment. Measurements during the photoperiod were then averaged to provide average solar radiation readings for each individual day. The line plot represents average daily solar radiation while the bar graphs indicate average daily solar radiation throughout the month. Breaks in the line plot data indicate periods of time which were not documented due to technical malfunction.
4.3.2 Growth and Destructive Measurements

Growth measurements were performed 18 days into the treatment (DIT) and included plant height, stem diameter, leaf number, leaf length, leaf diameter, and chlorophyll content of the 5th leaf from 12 plants per treatment. At 55 DIT, growth measurements were again performed and included plant height, leaf number, cluster number, leaf length, width, and chlorophyll content of the 5th and 10th leaf from 12 plants in each treatment.

Destructive measurements were performed on eight plants per treatment 19 DIT and four plants per treatment 50 DIT. The leaf area was measured with a leaf area meter (Li-COR 3100, Li-COR Inc. Lincoln, NE, USA). The leaves, stems, and fruits, when applicable, were weighed (fresh weight) then placed in an oven at 65°C until each component was dry then weighed (dry weight).

Leaf chlorophyll was measured using a SPAD meter (model 502, Konica Minolta, Osaka, Japan) and values were converted to chlorophyll content using treatment specific correction equations generated by spectrophotometric pigment analysis. Chlorophyll correction curves were generated by extracting leaf punches in 95% ethanol at 78°C for approximately 3h until the tissue was cleared. Samples were then analyzed at 664.2nm, 648.6nm, and 470nm wavelengths using a spectrophotometer. Concentrations of chlorophyll a, b, and carotenoids were determined via equations from Lichtenthaler (1987).

4.3.3 Leaf Gas Exchange: Day and Night Measurements

The 5th leaf was placed in the chamber of a Li-COR 6400 (Li-COR Inc. Lincoln, NE, USA) which was fitted with a 2cm x 3cm clear top chamber. The leaf temperature was set to 24°C with a relative humidity of 55-65% and a CO2 level held at 800 µL L⁻¹, similar to the growth conditions. Four leaves from separate plants under each treatment were used at 20 DIT and 53 DIT for both day and night measurements. Measurements taken during the day were preformed on cloudy days to maximize the effect of supplemental lighting while minimizing the effect of natural light. Leaves were kept in the chamber until a steady-state photosynthesis rates were obtained then the average from a 2-minute period was taken.

4.3.4 Leaf Gas Exchange: Light Response Curves

The 5th leaf was placed in the chamber of a Li-COR 6400 which was fitted with a red/blue (88red/12blue) LED Li-COR standard light source (2cm x 3cm). The leaf temperature was set to
24°C with a relative humidity of 55-65% and a CO₂ level held at 800 µL L⁻¹, similar to growth conditions. Eight leaves from separate plants under each treatment were used at 18 DIT and four leaves were used at 43 DIT. Measurements were performed on cloudy days. Light curves began at a high light intensity and decreased gradually similar to the procedure from Evans & Santiago, (2014). The light level was set to 1500 µmol m⁻² s⁻¹ until steady-state then the light curve began with light intensity steps of: 1500, 1500, 1000, 750, 500, 250, 100, 75, 50, 25, and 0 µmol m⁻² s⁻¹. At each light level, the photosynthetic rate reached a steady-state (2-4 min) then a measurement was taken for that light level. Photosynthetic rates were plotted against light intensity and fitted to a regression line following the equation \( y = y_0 + a(1 - e^{bx}) \) using SigmaPlot 10.0 to determine the photosynthetic maximum. A linear regression (\( y = mx + b \)) using the photosynthetic rates between the light levels of 0-100 µmol m⁻² s⁻¹ was used to calculate both light compensation point (LCP) and quantum yield (QY).

4.3.5 Leaf Gas Exchange: CO₂ Response Curves

The 5th leaf was placed in the chamber of a Li-COR 6400 which was fitted with a red/blue (88 red/12 blue) LED Li-COR standard light source (2cm x 3cm). The leaf temperature was set to 24°C with a relative humidity of 55-65% and a light level of 250 µmol m⁻² s⁻¹. Four leaves from separate plants under each treatment were used at 18 and 43 DIT. Measurements were performed on cloudy days. Carbon dioxide response curves began at the ambient growth CO₂ concentration (800 µL L⁻¹) and reduced gradually to 0 µL L⁻¹. After the 0 µL L⁻¹ measurement, the CO₂ concentration was brought back to 800 µL L⁻¹ and was held steady until plant photosynthetic parameters returned to levels established during the beginning of the experiment. The CO₂ level was then increased incrementally to 2000 µL L⁻¹ at which point the CO₂ response curve was terminated. At each CO₂ concentration, the photosynthetic rate reached a steady-state then a measurement was taken to produce values for that CO₂ concentration. Photosynthetic rates were plotted against internal CO₂ concentration (Cᵢ) and fitted to the FvCB model (Farquhar et al., 1980) and temperature corrected (McMurtrie & Wang, 1993; Bernacchi et al., 2001) to determine the maximum rate of photosynthesis under Rubisco-limited and RuBP-limited conditions.

4.3.6 Chlorophyll Fluorescence Imaging

Intact leaflets were dark adapted using aluminium foil for 10 minutes. After the dark adaptation period, leaflets were detached and immediately used for chlorophyll imaging using a closed
FluorCam model FC 800-C with FluorCam v.7.0 software (FluorCam, Photon System Instruments, Brno, Czech Republic). The minimum fluorescence in a dark-adapted state (F₀) was acquired during a dark-period of 5s, after which an 800ms saturating light pulse (2400 µmol m⁻² s⁻¹) from a blue LED (peak emission of 449nm) was used to measure maximum fluorescence in a dark-adapted state (Fₘ). From F₀ and Fₘ, the variable fluorescence in a dark-adapted state (Fᵥ) was calculated (Fᵥ=Fₘ-F₀) which was used to determine the maximum photosystem II (PSII) quantum yield (Fᵥ/Fₘ). In general, the lower the value of Fᵥ/Fₘ, the more severe the photoinhibition (Baker, 2008). Typical optimum values for Fᵥ/Fₘ are 0.79-0.84 (Maxwell & Johnson, 2000).

By calculating Fᵥ/Fₘ using chlorophyll fluorescence imaging, we are able to assess not only the prevalence of injury, but also the spatial heterogeneity of Fᵥ/Fₘ from a leaflet. Eight leaflets from the 5th leaf were used for each light treatment when plants were 22 DIT. Measurements from each lighting treatment were taken again at 50 DIT with eight leaflets from the 5th and 10th leaves.

4.3.7 Carbohydrate Analysis

Eight 0.79 cm² leaf punches were taken from the 5th leaf of each lighting treatment at five different time periods. Leaf punches were taken from the most distal part of the leaf at the first time point and moved towards the base of the leaf during each time point avoiding main veins. The time points were pre-night (17:45; 54 DIT), during the night (22:00; 54 DIT), pre-day (05:45; 55 DIT), mid-day (12:00; 55 DIT), and again pre-night (17:45; 55 DIT). Each punch was immediately weighed then frozen using liquid nitrogen and kept at -80°C until analysis.

Leaf punches were extracted three times in 80% boiling ethanol until tissue was cleared (Tetlow & Farrar, 1993; Lanoue et al., 2018a). The ethanol soluble fraction was then dried and suspended in water and 99% chloroform (2:1 v/v), agitated, and centrifuged at 11,000 RPM to separate the water-soluble fraction (sugars) from chloroform soluble leaf components (chlorophyll, lipids, etc.). Soluble sugars were assayed using a Sucrose/Fructose/Glucose kit (Megazyme; https://www.megazyme.com) and analyzed using spectrophotometry at 340nm.

Ethanol insoluble fractions were dried then ground and suspended in sodium acetate. 50 µL (~150 U) of α-amylase was added and samples were vortexed then placed in a boiling water bath. Samples were vortexed every 4 minutes for 12 minutes. Samples were then placed in a 50°C water bath and allowed to equilibrate. 50 µL (~165 U) of amyloglucosidase was added to each sample and incubated at 50°C overnight. 30 µL of each sample were then assayed in duplicate using a
Total Starch Assay kit (Megazyme; https://www.megazyme.com) and analyzed using spectrophotometry at 510nm.

4.3.8 Statistical Analysis

All statistics were performed using SAS Studio 3.5. Means comparisons between 12h lighting and CL treatments were done using a one-way ANOVA with a p<0.05 indicating a significant difference.

4.4 Results

4.4.1 Effect of CL on Morphology and Pigments

At 18 DIT, plants exposed to CL were on average taller and had one more leaf than tomato plants under 12h lighting (Table 4.2). At 55 DIT, plants under CL were again observed to be taller than plants under 12h lighting indicating that CL did not hinder the plants ability to grow at a normal rate (Table 4.2). At 55 DIT, plants under 12h lighting and CL produced similar 5th leaf length and width as well as 10th leaf width. The length of the 10th leaf was observed to be higher in plants under 12h lighting than plants under CL (Table 4.2). Furthermore, in contrast to measurements at 18 DIT, both plants exposed to 12h lighting and CL produced the same number of leaves. Of note, flowers first appeared 14 DIT with no difference in flower appearance between treatments.

Table 4.2: Morphological parameters of plants grown under 12h lighting (200 µmol m⁻² s⁻¹ red + 50 µmol m⁻² s⁻¹ blue, 06:00-18:00) and CL (200 µmol m⁻² s⁻¹ red, 06:00-18:00 + 50 µmol m⁻² s⁻¹ blue, 18:00-06:00). Parameters represent measurements taken at two time points during the life cycle of the plants. Values represent the mean ± the standard error of the mean with n=4. Different letters (A,B) represents a significant difference within a time point of a given parameter at p<0.05.

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>18 DIT</th>
<th>55 DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Height (cm)</td>
<td>12h Lighting</td>
<td>12h Lighting</td>
</tr>
<tr>
<td></td>
<td>217.8±2.9 B</td>
<td>230.4±3.0 A</td>
</tr>
<tr>
<td></td>
<td>68.3±1.6 B B</td>
<td>79.7±0.9 A A</td>
</tr>
<tr>
<td>Stem Diameter (mm)</td>
<td>9.4±0.1 A A</td>
<td>9.5±0.3 A A</td>
</tr>
</tbody>
</table>
Upon destructive analysis, plants grown under supplemental CL produced more leaf area and a higher stem fresh weight at 19 DIT compared to plants grown under 12h supplemental lighting (Table 4.3). Other destructive metrics such as leaf fresh weight, leaf dry weight, specific leaf mass, and stem dry weight were similar between the two supplemental lighting treatments (Table 4.3). At 50 DIT, plants grown under CL and 12h lighting were similar with respect to most parameters measured during destructive analysis (Table 4.3).

Table 4.3: Destructive measurements of plants grown under 12h lighting (200 μmol m⁻² s⁻¹ red + 50 μmol m⁻² s⁻¹ blue, 06:00-18:00) and CL (200 μmol m⁻² s⁻¹ red, 06:00-18:00 + 50 μmol m⁻² s⁻¹ blue, 18:00-06:00). Parameters represent measurements taken at two time points during the life cycle of the plants. Total plant fresh and dry biomass include leaves and stem weights. Any fruit present during the 50 DIT were not included in analysis. Values represent the mean ± the standard error of the mean with n=4. Different letters (A,B) represents a significant difference within a time point of a given parameter at p<0.05.
At 18 DIT plants under both supplemental lighting treatments had similar concentrations of chlorophyll indicating that supplemental CL did not hinder the plants ability to produce chlorophyll or absorb light (Table 4.4). At 55 DIT the chlorophyll index of the 5th leaf was similar between both supplemental lighting treatments (Table 4.4). However, 55 DIT the 10th leaf had higher values of all chlorophyll parameters measured when plants were grown under 12h lighting compared to CL (Table 4.4). Of note, leaves from plants growing under the 12h lighting treatment were observed to have a cupping morphology not seen in the CL treatment (Appendix V).

Table 4.4: Pigment analysis of plants grown under 12h lighting (200 µmol m$^{-2}$ s$^{-1}$ red + 50 µmol m$^{-2}$ s$^{-1}$ blue, 06:00-18:00) and CL (200 µmol m$^{-2}$ s$^{-1}$ red, 06:00-18:00 + 50 µmol m$^{-2}$ s$^{-1}$ blue, 18:00-06:00). Parameters represent measurements taken at two time points during the life cycle of the plants. Values represent the mean ± the standard error of the mean with n=4. Different letters (A, B) represents a significant difference within a time point and leaf rank of a given parameter at p<0.05.

<table>
<thead>
<tr>
<th></th>
<th>18 DIT</th>
<th>55 DIT</th>
<th>18 DIT</th>
<th>55 DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf Fresh Weight</strong></td>
<td>76.46±4.57 A</td>
<td>82.05±8.05 A</td>
<td>484±35 A</td>
<td>470±59 A</td>
</tr>
<tr>
<td><strong>Leaf Dry Weight</strong></td>
<td>9.02±0.52 A</td>
<td>10.12±1.42 A</td>
<td>47.90±3.02 A</td>
<td>46.35±5.83 A</td>
</tr>
<tr>
<td><strong>Specific Leaf Mass</strong></td>
<td>31.43±0.34 A</td>
<td>30.70±2.77 A</td>
<td>38.45±0.76 A</td>
<td>35.71±2.12 A</td>
</tr>
<tr>
<td><strong>Stem Fresh Weight</strong></td>
<td>116±5 B</td>
<td>128±4 A</td>
<td>620±29</td>
<td>649±33</td>
</tr>
<tr>
<td><strong>Stem Dry Weight</strong></td>
<td>6.75±0.46 A</td>
<td>7.85±0.73 A</td>
<td>40.55±2.57 A</td>
<td>41.03±2.55 A</td>
</tr>
<tr>
<td><strong>Total Plant Fresh Biomass</strong></td>
<td>193±6 A</td>
<td>211±8 A</td>
<td>1104±63 A</td>
<td>1118±90 A</td>
</tr>
<tr>
<td><strong>Total Plant Dry Biomass</strong></td>
<td>15.75±0.63 A</td>
<td>17.85±1.40 A</td>
<td>88.45±4.92 A</td>
<td>87.38±8.36 A</td>
</tr>
</tbody>
</table>

At 18 DIT plants under both supplemental lighting treatments had similar concentrations of chlorophyll indicating that supplemental CL did not hinder the plants ability to produce chlorophyll or absorb light (Table 4.4). At 55 DIT the chlorophyll index of the 5th leaf was similar between both supplemental lighting treatments (Table 4.4). However, 55 DIT the 10th leaf had higher values of all chlorophyll parameters measured when plants were grown under 12h lighting compared to CL (Table 4.4). Of note, leaves from plants growing under the 12h lighting treatment were observed to have a cupping morphology not seen in the CL treatment (Appendix V).
<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>18 DIT</th>
<th>55 DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h Lighting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous Lighting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h Lighting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous Lighting</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leaf Rank</strong></td>
<td>5th</td>
<td>5th</td>
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<tr>
<td>5th</td>
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<td></td>
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<td>5th</td>
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<tr>
<td>10th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10th</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll a (µg cm(^{-2}))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 DIT</td>
<td>49.79±0.78 A</td>
<td>49.09±0.71 A</td>
</tr>
<tr>
<td>55 DIT</td>
<td>49.09±0.71 A</td>
<td>44.34±2.58 A</td>
</tr>
<tr>
<td><strong>Chlorophyll b (µg cm(^{-2}))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 DIT</td>
<td>14.41±0.15 A</td>
<td>14.47±0.17 A</td>
</tr>
<tr>
<td>55 DIT</td>
<td>14.47±0.17 A</td>
<td>13.36±0.49 A</td>
</tr>
<tr>
<td><strong>Chlorophyll a+b (µg cm(^{-2}))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 DIT</td>
<td>64.21±0.93 A</td>
<td>63.56±0.88 A</td>
</tr>
<tr>
<td>55 DIT</td>
<td>63.56±0.88 A</td>
<td>57.71±3.01 A</td>
</tr>
<tr>
<td><strong>Chlorophyll a:b</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 DIT</td>
<td>3.45±0.02 A</td>
<td>3.39±0.01 A</td>
</tr>
<tr>
<td>55 DIT</td>
<td>3.39±0.01 A</td>
<td>3.30±0.08 A</td>
</tr>
<tr>
<td><strong>Carotenoids (µg cm(^{-2}))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 DIT</td>
<td>12.16±0.29 A</td>
<td>11.74±0.19 A</td>
</tr>
<tr>
<td>55 DIT</td>
<td>11.74±0.19 A</td>
<td>10.24±0.94 A</td>
</tr>
</tbody>
</table>
4.4.2 Physiological Responses of Tomato to CL

At 22 DIT, the 5th leaf of plants exposed to CL produced higher Fv/Fm values than did leaves from 12h lighting (Figure 4.2A). This result indicates that leaves under supplemental CL were as healthy as leaves under 12h supplemental lighting after more than three weeks of exposure to CL. Furthermore, Fv/Fm was assessed at 50 DIT on both 5th and 10th leaves from both supplemental light treatments. Again, leaves under CL were determined to be as healthy as leaves grown under 12h lighting (Figure 4.2B). Furthermore, using chlorophyll imagining, no obvious spatial difference pertaining to Fv/Fm were observed between the two lighting treatments (Figure 4.3). Under both lighting treatments, Fv/Fm values in the 5th leaf decreased from analysis at 22 DIT to 50 DIT (p<0.05). While values determined during 22 DIT and 50 DIT Fv/Fm measurements are slightly lower than optimum (0.79-0.84), our focus was to determine differences between the two light treatments used and not compare leaves to optimum Fv/Fm values. The differences between optimum and measured Fv/Fm values could be due to leaf position during growth (i.e., mutual shading, sun fleck occurrence) or other environmental parameters not attributed to the light treatments which did not confound the aforementioned comparison.

Day-time net carbon exchange rate (NCER) at both 20 DIT and 53 DIT from leaves grown under 12h lighting or CL produced similar values (Figures 4.4A & 4.4B). However, during the night-time (18:00h-06:00h) measurements, leaves under CL produced a higher NCER at both 20 DIT and 53 DIT (Figures 4.4A & 4.4B). Of note, during analysis at the 53 DIT period, NCER of leaves under CL during the night-time was a positive value indicating photosynthesis and a net gain in carbon instead of net loss which was seen during 12h lighting due to respiration (Figure 4.4B). Using the NCER averages in Figure 4.4, a prediction of the total carbon gain during a 24h period can be made. Of note, these calculations are assuming little variation in NCER over each 12h day and night period. Nonetheless, using the day-time and night-time averages, at both 20 DIT and 53 DIT CL leaves assimilate approximately 550 mg C m⁻² and 800 mg C m⁻² more respectively over a 24h period.
Figure 4.2: Maximum efficiency of PSII (Fv/Fm) from tomato leaves grown under either 12h lighting (200 µmol m⁻² s⁻¹ red + 50 µmol m⁻² s⁻¹ blue, 06:00-18:00) and CL (200 µmol m⁻² s⁻¹ red, 06:00-18:00 + 50 µmol m⁻² s⁻¹ blue, 18:00-06:00) at 22 DIT (panel A) and 50 DIT (panel B). Of note, measurements taken at 50 DIT (panel B) were taken on both 5th and 10th leaf. Error bars represent the standard error of the mean of n=4. Letter groups (A,B) represent significant difference between the lighting treatments at a specific time point and leaf position at p<0.05.
Figure 4.3: Spatial response of $F_v/F_m$ from tomato leaves grown under either 12h lighting (200 $\mu$mol m$^{-2}$ s$^{-1}$ red + 50 $\mu$mol m$^{-2}$ s$^{-1}$ blue, 06:00-18:00) and CL (200 $\mu$mol m$^{-2}$ s$^{-1}$ red, 06:00-18:00 + 50 $\mu$mol m$^{-2}$ s$^{-1}$ blue, 18:00-06:00) at 22 DIT (A and B) and 50 DIT (C-F). Chlorophyll fluorescence images of the 5$^{th}$ leaf are labelled A-D and images of the 10$^{th}$ leaf are labelled E and F.
**Figure 4.4:** Net carbon exchange rate (NCER) of the 5th leaf from tomato plants grown under 12h lighting (200 µmol m⁻² s⁻¹ red + 50 µmol m⁻² s⁻¹ blue, 06:00-18:00) and CL (200 µmol m⁻² s⁻¹ red, 06:00-18:00 + 50 µmol m⁻² s⁻¹ blue, 18:00-06:00) at 20 DIT (panel A) and 53 DIT (panel B) during the day-time and night-time. Measurements were performed using a Li-COR 6400 fitted with a clear top chamber on a cloudy day or night and thus represent the NCER driven by the supplemental lighting. Error bars represent the standard error of the mean of n=4. Letter groups (A,B) represent significant difference between the lighting treatments at a specific data collection period at p<0.05.
During day-time NCER measurements at 20 DIT, stomatal conductance, $C_i$, transpiration rate, and water-use efficiency (NCER/transpiration) were similar between treatments (Figures 4.5A, 4.5C, 4.5E, & 4.5G). During the night-time measurements, stomatal conductance and transpiration were similar between the lighting treatments while $C_i$ was lower in CL leaves (Figure 4.5C) which is reflective of a higher night-time NCER (Figure 4.4A). Night-time WUE was calculated as NCER/transpiration, similar to day-time WUE (Figure 4.5G) with negative values indicating a negative rate of NCER (i.e., respiration occurring). Night-time WUE were increased in CL plants due to the increase in NCER during the subjective night period in which plants were exposed to blue light.

At 53 DIT, day-time stomatal conductance and transpiration rate were higher in leaves grown under 12h supplemental lighting than leaves grown under supplemental CL (Figures 4.5B & 4.5F). Both $C_i$ and water-use efficiency during the day-time of leaves at 53 DIT were similar between both treatments (Figures 4.5D & 4.5H). Similar to leaves at 20 DIT, leaves at 53 DIT grown under CL had a lower night-time $C_i$ (Figure 4.5D) reflective of a higher night-time NCER (Figure 4.4B). Night-time stomatal conductance and transpiration rates were similar between the two lighting treatments when leaves were at 53 DIT (Figures 4.5B & 4.5F). Similar to WUE values at 20 DIT, night-time WUE was calculated as NCER/transpiration, similar to day-time WUE (Figure 4.5H) with negative values indicating a negative rate of NCER (i.e., respiration occurring). Night-time WUE were increased in CL plants due to the increase in NCER (i.e., plants photosynthesizing during the subjective night period) in which plants were exposed to blue light.

All parameters from light response curves (Figure 4.6), both measured (respiration) and calculated (light compensation point, quantum yield, and photosynthetic maximum ($P_{n\text{max}}$)) were similar between lighting treatments at both time points studied (Table 4.5). Furthermore, all relevant photosynthetic parameters (day-time NCER, quantum yield, and $P_{n\text{max}}$) were quantified on a chlorophyll basis, again producing similar values between the two lighting treatments ($P<0.05$; data not shown).

Figure 4.7 represents the CO$_2$ response curve at two time points from leaves of plants grown under 12h supplemental lighting or supplemental CL. These response curves were specifically run at a light level of 250 µmol m$^{-2}$ s$^{-1}$ to determine the response of leaves from both lighting treatments at
or near their growth conditions (Figure 4.7). Similarities between 12h lighting and CL treatments indicate no affect of CL on photosynthesis.

### 4.4.3 Carbohydrate Analysis of Tomatoes Under CL

Carbohydrate status (Figures 4.8A-C) shows a rapid decrease of glucose, fructose, and sucrose respectively during the initial 4h during the dark period of the 12h lighting treatment and the blue light period of the CL treatment. A decrease in these three sugars continues until 05:45 when the lights were turned on in the 12h lighting treatment or the blue light becomes red light in the CL treatment (Figures 4.8A-C). Upon illumination or a change in wavelength accompanied by an increase in light intensity, glucose, fructose, and sucrose levels accumulate during the initial 6h to levels comparable with pre-night levels (Figures 4.8A-C). Both glucose and fructose levels remain steady from 12:00-17:45 (Figures 4.8A & B). It is important to note that while supplemental lighting treatment photoperiods were 06:00-18:00 for 12h lighting or continuous lighting, the natural solar photoperiod during the time of carbohydrate measurements was 08:00-17:12. Similarly, patterns of glucose and fructose, sucrose levels were similar between sampling at 12:00 and 17:45 in leaves grown under 12h lighting (Figure 4.8C). However, leaves grown under CL continued to accumulate sucrose during the final hours under red light and had higher sucrose levels at 17:45 at 55 DIT compared to leaves exposed to 12h supplemental lighting (Figure 4.8C).

Throughout the night period of the 12h supplemental lighting treatment, starch levels within leaves decreased during the first 4h (Figure 4.8D). Interestingly, during the initial 4h of the blue light period of the CL treatment, starch levels remained similar to those from leaves sampled before the light spectral switch (17:45; Figure 4.8D). From 22:00 at 54 DIT to 05:45 at 55 DIT starch levels in both 12h lighting and CL treatments decreased (Figure 4.8D). During the subsequent light period, starch levels of leaves from both 12h lighting and CL treatments increased at similar rates returning to levels comparable to those from leaves sampled at 17:45 at 54 DIT (Figure 4.8D).
**Figure 4.5:** Stomatal conductance (panels A and B), C\(_i\) (panels C and D), transpiration (panels E and F), and water-use efficiency (panels G and H) of the 5\(^{th}\) leaf from tomato plants grown under 12h lighting (200 µmol m\(^{-2}\) s\(^{-1}\) red + 50 µmol m\(^{-2}\) s\(^{-1}\) blue, 06:00-18:00) and CL (200 µmol m\(^{-2}\) s\(^{-1}\) red, 06:00-18:00 + 50 µmol m\(^{-2}\) s\(^{-1}\) blue, 18:00-06:00) at 20 DIT (panels A, C, E, and G) and 53 DIT (panels B, D, E, and H) during the day-time and night-time. Measurements were performed using a Li-COR 6400 fitted with a clear top chamber on a cloudy day or night and thus represent values driven by the supplemental lighting. Error bars represent the standard error of the mean of n=4. Letter groups (A,B) represent significant difference between the lighting treatments at a specific data collection period at p<0.05.
Figure 4.6: Photosynthetic light response curves from leaves grown under either 12h lighting (200 µmol m⁻² s⁻¹ red + 50 µmol m⁻² s⁻¹ blue, 06:00-18:00) and CL (200 µmol m⁻² s⁻¹ red, 06:00-18:00 + 50 µmol m⁻² s⁻¹ blue, 18:00-06:00) at 18 DIT (panel A) and 43 DIT (panel B) as determined using a Li-COR 6400 with a red/blue standard Li-COR light source. Measurements were made at a CO₂ concentration of 800 µL L⁻¹, leaf temperature of 24°C, and a relative humidity of 55-65%. Regression lines were fit to \( y=y_0+a(1-e^{(b*x)}) \) for each light treatment. Insert A’ and B’ are magnifications of 0-100 µmol m⁻² s⁻¹ PAR regions fit to the regression line \( y=mx+b \).
Table 4.5: Summary of the major physiological traits as determined by leaf light response curves
(Figure 4.6) from tomatoes grown under 12h lighting (200 µmol m\(^{-2}\) s\(^{-1}\) red + 50 µmol m\(^{-2}\) s\(^{-1}\) blue, 06:00-18:00) and CL (200 µmol m\(^{-2}\) s\(^{-1}\) red, 06:00-18:00 + 50 µmol m\(^{-2}\) s\(^{-1}\) blue, 18:00-06:00). Respiration values were the averages of NCER when the light level was 0 µmol m\(^{-2}\) s\(^{-1}\). The light compensation point and quantum yield were calculated from a regression line (y=mx+b) fitted to the values between the PAR values of 0-100 µmol m\(^{-2}\) s\(^{-1}\). The photosynthetic maximum (P_{n_{max}}) was calculated from y=y_o+a(1-e^{(b+x)}). Values in parentheses represents ± the standard error of the mean with n=4. Similar letters (A) represents a no significant difference within a time point and of a given parameter at p<0.05.
<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Light Treatment</th>
<th>Respiration ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>Light Compensation Point ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>Quantum Yield ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$/ $\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$P_{n\text{max}}$ ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 DIT</td>
<td>12h Lighting</td>
<td>-2.03±0.38 A</td>
<td>28.83±5.98 A</td>
<td>0.068±0.001 A</td>
<td>30.61±1.11 A</td>
</tr>
<tr>
<td></td>
<td>Continuous Lighting</td>
<td>-2.36±0.23 A</td>
<td>35.62±3.81 A</td>
<td>0.066±0.001 A</td>
<td>27.94±1.21 A</td>
</tr>
<tr>
<td>43 DIT</td>
<td>12h Lighting</td>
<td>-2.96±0.19 A</td>
<td>40.45±3.11 A</td>
<td>0.070±0.001 A</td>
<td>32.06±1.83 A</td>
</tr>
<tr>
<td></td>
<td>Continuous Lighting</td>
<td>-2.94±0.15 A</td>
<td>46.84±3.44 A</td>
<td>0.063±0.005 A</td>
<td>29.98±2.24 A</td>
</tr>
</tbody>
</table>
Figure 4.7: Photosynthetic CO$_2$ response curve from leaves grown under either 12h lighting (200 $\mu$mol m$^{-2}$ s$^{-1}$ red + 50 $\mu$mol m$^{-2}$ s$^{-1}$ blue, 06:00-18:00) and CL (200 $\mu$mol m$^{-2}$ s$^{-1}$ red, 06:00-18:00 + 50 $\mu$mol m$^{-2}$ s$^{-1}$ blue, 18:00-06:00) at 18 DIT (panel A) and 43 DIT (panel B) as determined using a Li-COR 6400 with a red/blue standard Li-COR light source. Measurements were made at 250 $\mu$mol m$^{-2}$ s$^{-1}$ PAR, a temperature of 24°C, and relative humidity of 55-65%. Rubisco and RuBP limited fit lines were determined using temperature corrections from McMurtrie & Wang, (1993) and Bernacchi et al., (2001).
Figure 4.8: Diurnal pattern of glucose (panel A), fructose (panel B), sucrose (panel C), and starch (panel D) from the 5th most fully expanded leaf from plants grown under either 12h lighting (200 µmol m$^{-2}$ s$^{-1}$ red + 50 µmol m$^{-2}$ s$^{-1}$ blue, 06:00-18:00) and CL (200 µmol m$^{-2}$ s$^{-1}$ red, 06:00-18:00 + 50 µmol m$^{-2}$ s$^{-1}$ blue, 18:00-06:00) on the 54th and 55th DIT. The dark grey area represents the period of darkness during the 12h lighting treatment and the period of blue light during the CL treatment. The light grey area represents the natural photoperiod. Eight 0.79cm$^2$ leaf punches were taken from the 5th most fully expanded leaves during different time points over a 24h period. Error bars represent the standard error of the mean of n=4. An asterisk (*) represents a significant difference between the lighting treatments at a specific time point within a graph at p<0.05.
4.4.4 Yield

During the initial sampling period (January 28th, 2019 – February 21st, 2019) 75 – 99 DIT, Total fruit fresh weight and fruit weight per unit area was higher when plants were grown under CL (Table 6). During this same sampling period, fruit number and specific fruit weight were similar between treatments. During the remainder of the sampling period (February 22nd, 2019 – May 16th, 2019) fruit number, total fruit fresh weight, fruit weight per unit area, and specific fruit weight did not differ between treatments within a sampling period (Table 4.6).

**Table 4.6:** Tomato fruit marketable yield from plants grown under 12h supplemental lighting or supplemental continuous lighting from January 28th, 2019 to May 16th, 2019. Sampling periods correspond to January 28th, 2019 – February 21st, 2019 (1), February 22nd, 2019 – March 21st, 2019 (2), March 22nd, 2019 – April 23rd, 2019 (3), and April 24th, 2019 – May 16th, 2019 (4). Within each parameter and sampling period, different letter groups (A,B) represent statistical difference at p<0.05.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Sampling Period</th>
<th>Fruit Number</th>
<th>Total Fruit Fresh Weight (kg)</th>
<th>Fruit Weight per unit area (kg m⁻²)</th>
<th>Specific Fruit Weight (g fruit⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12h Lighting</td>
<td>1</td>
<td>375±18 A</td>
<td>43.27±1.57 B</td>
<td>6.49±0.13 B</td>
<td>115.88±3.55 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>492±7 A</td>
<td>60.76±3.16 A</td>
<td>9.09±0.49 A</td>
<td>123.35±5.15 A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>566±15 A</td>
<td>79.26±1.70 A</td>
<td>11.88±0.24 A</td>
<td>140.43±5.18 A</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>532±15 A</td>
<td>76.36±4.39 A</td>
<td>11.94±0.51 A</td>
<td>143.42±4.20 A</td>
</tr>
<tr>
<td>Continuous Lighting</td>
<td>1</td>
<td>414±20 A</td>
<td>50.70±1.14 A</td>
<td>7.49±0.22 A</td>
<td>123.15±6.02 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>494±10 A</td>
<td>57.28±2.50 A</td>
<td>8.55±0.47 A</td>
<td>116.16±5.47 A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>540±16 A</td>
<td>72.87±4.15 A</td>
<td>10.93±0.64 A</td>
<td>134.71±4.27 A</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>559±7 A</td>
<td>80.27±2.52 A</td>
<td>11.71±0.60 A</td>
<td>143.68±6.30 A</td>
</tr>
</tbody>
</table>
4.5 Discussion

Plant biomass during controlled environment production is largely dictated by the total amount of the light intercepted by the plant (daily light integral (DLI) – intensity \times photoperiod). Continuous light (CL, 24h lighting) could increase plant biomass and yield if CL does not cause any injury. It is also more economical because the costs of the light fixtures stay the same, cost of electricity in Ontario, Canada at night is lower, and heat released by the light fixtures help to meet the heating requirement during the night. In this study, we set out to determine the effects of alternating spectrum supplemental CL on greenhouse grown tomatoes. Overall, the results presented in this paper indicate that tomato plants grown under supplemental CL with alternating red and blue spectrum do not show signs of injury such as the leaf chlorosis observed in other studies investigating CL (Hillman, 1956; Matsuda et al., 2014; Haque et al., 2015; Haque et al., 2017; Velez-Ramirez et al., 2017b). Furthermore, the morphological and physiological parameters examined were similar to tomato plants grown under a conventional 12h supplemental lighting regime.

Most studies investigated the effect of CL on tomato were preformed inside growth chambers which lack the normal light/dark cycle of natural lighting (Matsuda et al., 2014; Haque et al., 2015; Haque et al., 2017; Velez-Ramirez et al., 2017b). However, Arthur et al., (1930), Demers et al. (1998) and Hao et al. (2017a) studied tomatoes produced in greenhouses under supplemental MH or HPS lights and still observed CL-injury. This indicates that the oscillations present during a natural light/dark period do not reduce the presence of CL-injury in tomato plants.

The introduction of cost-effective LEDs has allowed for the wavelength specific modification of morphology and physiology in tomatoes (Hernández and Kubota, 2012; Liu et al., 2012; Lanoue et al., 2018a). In our study, wavelength specific LEDs were utilized to create an alternating spectrum CL regime which produced injury-free tomato plants. The utilization of LEDs to produce an alternating spectrum during CL has been previously documented in lettuce (Ohtake et al., 2018). In this study using lettuce, an alternating CL spectrum with red light during the day (12h) and blue light during the night (12h) produced the highest dry weight and leaf area compared to a CL with a constant red/blue spectrum and a CL provided by fluorescent lights (Ohatke et al., 2018).
During the initial stages of our experiment (late November – early December), the natural light intensity was low, and the natural photoperiod is short, thus, supplemental lighting had the largest effect. Similar to results in Ohatke et al., (2018) using lettuce, during this period, plants grown under CL produced a larger leaf area and taller stem height than plants grown under 12h lighting. Since day-time NCER are similar between the two treatments, it is likely that elevated NCER between 18:00-06:00 from the CL treatment allocated more carbon for vegetative growth (Figure 4). As previously theorized, growing tomatoes under CL during the light limiting months of production can lead to increased biomass accumulation when CL-injury is not present (Velez-Ramirez et al., 2012).

During a similar early stage of the experiment, total fruit fresh weight and fruit weight per unit area increased in plants grown under CL (Table 4.6). During the subsequent months, total fruit fresh weight and fruit weight per unit area were similar between the two treatments. As noted above, this period of production was characterized with low natural light intensity and short photoperiod, allowing supplemental light to have a maximized effect. Plant grown under CL during this period had a higher initial leaf area and higher stem height (Tables 4.2 & 4.3). These characteristics can lead to a higher light capture which may be able to support enhanced fruit production. Furthermore, a recent study indicated that sole blue light was able to increase carbon export, the process responsible for transporting photo-assimilate from the leaf to growing sinks such as fruit (Lanoue et al., 2018a). Thus, the sole blue light during the CL night period may allow for increased carbon export leading to enhanced fruit production. As the natural DLI increased throughout the experiment, the advantage from growth under CL may diminish leading to comparable fruit production later in the experiment (Table 4.6). Thus, CL may be most beneficial during periods of low natural lighting and less beneficial as solar DLI increases.

Leaf carbohydrate status plays an important role in regulating photosynthetic performance (Azcón-Bieto, 1983). Continuous lighting has been shown to down-regulate genes related to photosynthesis within tomato leaves (Velez-Ramirez et al., 2014). The down-regulation has been associated with excess starch and sucrose levels leading to oxidative stress within the leaf causing CL-injury (Velez-Ramirez et al., 2017a). The excess accumulation is likely due to the constant energy influx due to light availability during what would be the night period during a non-CL photoperiod.
Within our CL regime, leaves produced similar concentrations, as well as diurnal patterns, of glucose, fructose, sucrose, and starch as leaves grown under a 12h photoperiod (Figure 8). These results may be explained by one of two factors. Potentially, plants grown under CL with alternating red and blue LEDs are able to control their carbohydrate metabolism similarly to plants under a 12h photoperiod. Or, the low light intensity used during the subjective night period did not drive photosynthesis at high enough levels for carbohydrates to build up within the leaf. Regardless of the mechanism at work, excess starch and sucrose were not able to build up in the leaf, end product feedback inhibition of photosynthesis was alleviated, and CL-injury symptoms were not apparent.

Furthermore, parameters related to photosynthetic performance (i.e. Pnmax, quantum yield, and Fv/Fm) of leaves grown under CL were similar to leaves grown under 12h lighting. Similar results were observed in other studies when a temperature drop was performed, which reduced or alleviated CL-injury (Haque et al., 2015; Hao et al., 2017a; Hao et al., 2017b; Haque et al., 2017; Hao et al. 2018b). Thus, alternating the light spectrum during a CL regime maintains normal leaf function, a result which has not previously been reported during CL under constant spectrum within tomatoes unless a temperature drop was present (Arthur et al., 1930; Demers et al., 1998; Matsuda et al., 2014; Haque et al., 2015; Haque et al., 2017; Velez-Ramirez et al., 2017a).

Unlike other studies (Velez-Ramirez et al., 2014; Haque et al., 2015; Matsuda et al., 2016) our study used a low blue light level during the subjective night period of the CL treatment. The light level used produced a photosynthetic level near the compensation point alleviating excess carbohydrate accumulation (Figure 4.8). Furthermore, low blue light level may initiate gene turnover similar to what would occur during the shift to a night period in a conventional light treatment (Velez-Ramirez et al., 2014). Perhaps the drastic shift from red to blue light reduces photo oxidative stress caused by continuous illumination with one wavelength via phytochrome and cryptochrome genetic regulation (Casal, 2000). However, exact mechanisms involved require further experimentation pertaining to wavelength specific genetic regulation.

The importance of understanding the role of photoreceptors in physiological responses has increased since the introduction of wavelength specific LED lighting options. A recent study suggests the role of phytochrome (PHY) A in eradicating CL-injury in tomato (Velez-Ramirez et al., 2019). Phytochromes respond to red and far-red light while cryptochromes perceive blue light. In our study, we showed that the implementation of an alternating spectrum CL using red LED
light during the daytime and blue LED light during the night, allowed for injury-free tomato production. Thus, the use of low intensity blue light during the night might not disturb circadian rhythms. Phytochromes are activated by red light and have been shown to play a key role in anthocyanin biosynthesis (PHY A at low light levels and PHY B1 at higher) in tomato (Weller et al., 2000). Anthocyanins have been shown to have a photoprotective role and help re-establish balance between light capture and CO₂ fixation, thus reducing the potential for photo-oxidative damage (Steyn et al., 2002). Furthermore, under low irradiance blue light, similar to that used in this study, PHY A retains its function and continues to promote anthocyanin biosynthesis (Weller et al., 2000). Taken together, the role of PHY B1 during high light period and PHY A during low light promote anthocyanin biosynthesis in tomato leading to a potential photoprotection mechanism during our CL regime alleviating injury.

Another hypothesis could be that an alternating spectrum CL regime could maintain normal circadian rhythms, something which probably did not occur when no change in spectrum was used (Velez-Ramirez et al., 2017a). The drastic shift in spectrum from red to blue may initiate gene turn over via photoreceptor signal transduction including important genes involved in carbohydrate status or light harvesting such as *CAB13* (Velez-Ramirez et al., 2014). In this way, an alternating spectrum during CL may act in similar ways to a drastic temperature drop as seen in other studies (Haque et al., 2015; Haque et al., 2017). However, the role of photoreceptors in CL-injury is interesting and requires further research.

### 4.6 Conclusion

Taken together, the results indicate that using alternating spectrum CL allows for injury-free tomato production. Morphologically, plants grown under CL were similar to plants grown under the 12h lighting treatment in most respects. All physiological parameters (*Pn*ₘₐₓ, *Fᵥ/Fₘ*, respiration rates, and stomatal conductance) were similar between leaves grown under CL and 12h lighting. One noticeable difference was the NCER between the 18:00-06:00. During this period, leaves under the CL treatments produced higher NCER values and produced positive values at 53 DIT indicating carbon gain during the subjective night period. Carbohydrate diurnal patterns were similar between both light treatments displaying a drop during between 18:00-06:00 and a rise between 06:00-18:00. This result indicates that the CL with alternating red and blue allows for a normal circadian rhythm with regards to carbohydrate metabolism. The effects of spectral quality
and timing of light quality during CL is poorly understood. Our study indicates that an alternating red (200 µmol m\(^{-2}\) s\(^{-1}\)) and blue (50 µmol m\(^{-2}\) s\(^{-1}\)) CL regime allow for injury-free tomato production. Thus, the need for further research pertaining to spectral quality and the role of photoreceptors during CL tomato production is needed.

This work has been prepared for submission as a mini review to Frontiers in Sustainable Food Systems: Crop Biology and Sustainability section.

The manuscript has been altered to adhere to the University of Guelph thesis format.

Contributions:

Jason Lanoue prepared the manuscript. Jason Lanoue, Dr. Bernard Grodzinski, and Dr. Xiuming Hao reviewed and edited the manuscript.
5.1 Abstract

To meet the high consumer demand for fresh vegetables year-round, greenhouse production must maintain high yields during the light limiting winter months. In order to do so, the implementation of supplemental lighting fixtures is needed to provide an adequate growth environment to produced high yields and fruit quality. The light environment largely depends on five aspects: light intensity, the photoperiod length, light distribution, light quality, and microclimate. Traditionally, high pressure sodium (HPS) fixtures were implemented in order to supplement ambient light levels during light limiting months to improve growth. However, due to their high heat emission, fixed spectral quality, and low photosynthetic photon efficacy (PPE), HPS lights are less than optimal fixtures for supplemental greenhouse lighting. Advances in light-emitting diode (LED) technology, low heat emission, small fixture size, ability to emit wavelength specific light, and higher PPE have made them a viable and more sustainable choice for supplemental greenhouse lighting. This mini review examines the science related to light intensity, photoperiod length, light distribution, light quality, and microclimate and the affect alterations to these parameters can have on plant growth. The practical implications of implementing LEDs as supplemental lighting will also be examined as a way to improve and develop new lighting strategies to meet ever increasing consumer demand for fresh vegetables.

5.2 Introduction

Consumer demand for fresh vegetables is high year-round. However, in many northern regions around the world (i.e., Canada, northern Europe), low solar radiation limits production of greenhouse vegetables during the winter months. To ensure a steady supply of greenhouse vegetables to meet consumer demand and to maintain or increase market share, artificial lighting is needed to supplement natural sunlight during winter production. The implementation of supplemental lighting has been shown to increase growth and yield of many vegetable crops (Hao & Papadopoulos, 1999; Demers & Gosselin, 2002).

Traditionally, the addition of high pressure sodium (HPS) lighting fixtures provided the addition light desired. These high output HPS fixtures provide ample light but have many draw backs. While the most advanced double-ended HPS fixtures have photosynthetic photon efficacy (PPE) of 1.7 µmol J⁻¹, older models more likely to be found in commercial production are much lower
(~1.3 \mu\text{mol J}^{-1}). Thus, a lot of energy is converted to heat, making HPS lights inadequate for implementation in close proximity to the crop. Additionally, the light spectrum emitted from HPS fixtures is high in the orange and yellow spectral components which are not optimum for plant growth (Hogewoning et al. 2012). Light-emitting diodes (LEDs) have evolved quickly to be a viable option for supplemental lighting in greenhouses (Lanoue et al., 2019a). LEDs have many advantages over traditionally supplemental lighting fixtures. Namely, LED fixtures have a reduced heat output allowing them to be placed close to vegetation without harm. With this, the PPE of LED fixtures is higher than traditional supplemental lighting being around or above 2 \mu\text{mol J}^{-1} with a theoretical maximum around 5.0 \mu\text{mol J}^{-1} (Pattison et al., 2018). Additionally, the ability to alter the spectral output via manipulation of the semiconducting materials provides the flexibility for multiple applications within commercial production (Singh et al., 2015).

Year-round greenhouse fruiting vegetable production with supplemental lighting is different from production without lighting. Many aspects of the crop production systems from cultivars, crop schedules, crop pruning and training systems, to climate control and fertigation management need to be adjusted to maximize the benefits of the lighting (Dorais, 2003; Hao and Papadopoulos, 2005; Hao, 2016a; Hao et al., 2016b). Given the high cost of electricity, energy efficient lighting strategies must be developed and optimized for the year-round production with supplemental lighting (Nelson and Bugbee, 2014). Within these lighting strategies, five important aspects must be carefully thought out in order to optimize lighting for tall greenhouse fruiting vegetables: light intensity, photoperiod, light distribution, light quality (spectrum), and microclimate (Figure 5.1). While each of the five aspects are individually important in controlling yield, it is optimizing the interaction among them which can lead to the largest marketable gains.
Figure 5.1: Proper utilization of the five important aspects related to lighting strategies (light intensity, photoperiod, light distribution, light quality, and microclimate) can lead to optimized production.
5.3 Light Intensity & Photoperiod

Light intensity and photoperiod (duration of light) together define the daily light integral (DLI) which is the amount of light exposed to a plant over a given 24h period (mol m\(^{-2}\) d\(^{-1}\)). In greenhouse plant production, supplemental lighting is only used during low natural solar radiation. Generally, at natural solar radiation levels under 300 W m\(^{-2}\), supplemental lighting is used. However, this threshold can vary depending on the species and cultivar being grown, temperature, local climate, and overall growth technique (Hao et al., 2018a). When supplemental lighting is deployed, typical light intensities range between 100-300 µmol m\(^{-2}\) s\(^{-1}\) with photoperiods generally being shorter than 18h. However, like the threshold for the use of supplemental lighting both light intensity and photoperiod vary with species, cultivar, temperature, and local climate.

From financial point of view, it would be more economical to use low light intensity and a long photoperiod to achieve the target DLI due to the need for less lighting fixtures and thus a lower capital cost (Singh et al., 2015; van Iersel & Gianino, 2017). From a physiological viewpoint, low light intensities also make sense. For traditional greenhouse crops such as tomato, cucumber, and pepper the light saturation point is achieved between a light intensity of 600-800 µmol m\(^{-2}\) s\(^{-1}\) (Ögren & Evans, 1993; Lanoue et al., 2018b). The response to high light can be seen on virtually every level of the plant, from organismal to molecular (Szymańska et al., 2017). Visually, leaves under high light will begin to droop causing less light absorption. Similarly, chloroplast will begin stacking and movement of the light harvesting complex will occur, causing less excitation of the photosystems (Kirchhoff, 2014). On a molecular level, photoinhibition of photosystem II leads to an increase in reactive oxygen species and hydrogen peroxide (Pospišil, 2016). Taken together, at high light intensities, the photosynthetic efficiency (ratio of carbon fixed per photon) is drastically reduced (Lanoue et al., 2017).

The photoperiod requirements for greenhouse crops vary drastically. For example, lettuce grows well under long photoperiods even under continuous light (CL; Koontz & Prince, 1986; Ohtake et al., 2018). However, excessively long photoperiods cause leaf injury characterized by mottled interveinal chlorosis in greenhouse fruiting vegetables such as tomatoes, sweet peppers and cucumbers (Demers and Gosselin, 2002; Sysoeva et al., 2011; Velez-Ramirez et al., 2011; Haque et al., 2017). Long photoperiods (>17h) of supplemental lighting reduce plant growth and yield because leaf chlorosis can lead to reductions in leaf photosynthesis, chlorophyll content, and
phosphate synthase activity (Demers and Gosselin, 2002). The underlying mechanism of photoperiod related injury has yet to be determined. However, the role of the type III light harvesting chlorophyll a/b binding protein 13 (CAB13) gene has been demonstrated to play an important role in photoperiod related injury (Velez-Ramirez et al., 2014). CAB13 plays a role in light harvesting and under extended photoperiods or CL, expression levels are reduced leading to photoinhibition and photoperiod related injury (Kouřil et al., 2011; Velez-Ramirez et al., 2014).

The implementation of extended photoperiods such as CL has the potential to be beneficial on both a yield and economic basis (Velez-Ramirez et al., 2012). Economically, the use of extended period of low levels of light can translate to the need for less fixtures. Further, extending the period illumination into the night period can also have implications on energy use. Illuminating plants during the typical night periods can use energy during non-peak hours when rates of electricity are lower (i.e., such is the case in Ontario, Canada; Independent Electricity System Operator, 2020). By needing less fixtures to achieve the desired light level and using non-peak electricity hours, extended photoperiods can lead to economic gain for growers.

The implementation of CL strategies can hypothetically lead to increased plant growth and yield (Velez-Ramirez et al., 2012). Illuminating a plant with light for a 24-hour period means that there is a constant influx of energy into the photosystem which can drive photosynthesis and produce the carbon building blocks needed for plant growth (Lanoue et al., 2019b). Indeed, a recent study using alternating red and blue LEDs (12h red followed by 12h blue) observed no photoperiod related injury and an increase in tomato yield during the winter months compared to a photoperiod consisting of 12h of light and 12h of darkness (Lanoue et al., 2019b). This study represented the first studying able to produce mature, fruiting plants free of photoperiod related injury and demonstrated that CL can lead to increased plant growth and yield. Interestingly, this study also suggests that spectral quality may play a key role in eliminating photoperiod related injury.

The interaction between spectral quality and photoperiod length may be important in avoiding photoperiod related injury. Tomatoes grown under 23h illumination with red supplemental light produced at a higher rate over a 4-month period compared to plants grown under a 23h mixed (76% red, 16% blue and 8% white) spectrum supplemental light treatment (Lanoue et al., 2019c). Furthermore, Velez-Ramirez et al., (2019) determined that phytochrome A plays an important role
in photoperiod related injury. Together, these studies indicate that light quality may play an important role in reducing injury when plant are exposed to extended photoperiods.

5.4 Light Distribution & Light Quality

LED lights have the advantage of being able to emit wavelength specific light. Plants have photoreceptors such as cryptochrome and phytochrome, which absorb blue and red light respectively and play pivotal roles in everything from photomorphogenesis, photosynthetic performance, and genetic regulation (Folta & Carvalho, 2015). The ability of LEDs to emit wavelength specific light can be utilized within greenhouse production systems to regulate plant growth. For example, blue light activation of cryptochrome has been observed to play a role in hypocotyl growth regulation, chloroplast movement, flower timing, and stomatal function (Yu et al., 2010) while red light responses mediated by phytochrome including root phototropism, hypocotyl elongation, and flowering time (Lin, 2000b; Kiss et al., 2003). Certain wavelengths, such as green light, can affect plant growth without being mediated through a specific photoreceptor (Folta & Maruhnich, 2007). The addition of green light, a wavelength which is often overlooked as being useful, can increase the biomass and yield of a variety of crops when added to a red and blue light spectrum (Kim et al., 2004; Wang & Folta, 2013; Kaiser et al., 2019a). This occurs because although red and blue light are highly absorbed by a leaf (Cui et al., 1991), green light is able to penetrate deeper within leaf tissue and deeper within the plants driving photosynthesis in places neither red or blue light can reach (Sun et al., 1998; Terashima et al., 2009).

The light quality can not only have an influence on the CO2 gas exchange but also H2O gas exchange. The affect of light quality on stomatal function has been greatly studied. Blue light has been shown to increase stomatal opening (Kinoshita & Shimazaki, 1999). Upon absorption of blue light by phototropin, H+ ions efflux from the stomatal guard cells causing K+ ions to enter the cells and lead to stomatal opening (Kinosita et al., 2001; Kinoshita et al., 2003). Green light has been shown to be an antagonist to this process, however, the mechanism of this has yet to be determined (Talbott et al., 2002; Eisinger et al., 2003). The control of stomatal opening via light quality can also have an effect on water loss via transpiration and over all water-use-efficiency (WUE). Thus, the light quality used during commercial production must be carefully thought out as its affect on
stomatal function and thus transpiration and WUE means altered irrigation schedules will be needed for optimal growth.

In vining greenhouse crops such as tomato, cucumber, and pepper, the light intensity can drastically decrease within the plant canopy. This light gradient results in a lower photosynthetic capacity from leaves deep within the canopy and thus a lower overall whole plant photosynthetic rate (Besford, 1993; Lanoue et al., 2018b). Traditional supplemental lighting strategies for greenhouse vegetable production employ high power HPS fixtures as top lighting. While these types of fixtures are adequate for providing supplemental lighting, they are not suitable for illuminating lower leaves via inter-lighting due to their high heat output. LEDs, due to their low heat emittance, are able to be used as inter-lighting and increase the light intensity deep within the canopy (Figure 5.2). The introduction of inter-canopy LED lighting, either in a sole application or coupled with top lighting, has improved the canopy light environment and thus increased yield in vining greenhouse crops (Hovi et al., 2003; Kumar et al., 2016). Furthermore, Gomez et al., (2013), showed that the use of LED inter-lighting was more economical than overhead HPS lights for greenhouse tomato production.

The interaction between light distribution and light quality may also play a pivotal role in greenhouse crop production. While inter-canopy lighting is generally used to increase the light intensity deep within the plant canopy and thus increase overall plant photosynthetic rates, inter-lighting may also be able to play a key role in another fundamental pathway controlling yield. Carbon export is the process by which photo-assimilates (i.e., sugars) which are made in the leaf move from the leaf to the fruiting body (Lemoine et al., 2013; Osorio et al., 2014). The rate of carbon export has been shown to increase with increasing light and subsequent photosynthetic levels (Grodzinski et al., 1998). Furthermore, spectral quality has also been observed to play a role in controlling the rate of photo-assimilate efflux from a source leaf. A recent study showed that tomato leaves illuminated with either a blue or orange LED increased the export rate compared to a leaf illuminated with a white LED (Lanoue et al., 2018a). These studies suggest that the introduction of inter-canopy lighting can affect more than just the photosynthetic rate. With the implementation of LED inter-lighting and correct selection of wavelength, both the photosynthetic rate and the rate of carbon export can be increased leading to greater biomass accumulation and yield.
Secondary metabolites such as antioxidants and anthocyanins play a role in plant biology but have also been linked to human health benefits (Ristow, 2014). By using wavelength specific light emitted by LEDs, the biosynthesis of these beneficial compounds can be increased. For instance, Lefsrud et al., (2008) found that lutein and β-carotene concentrations were increased in kale grown under red light and blue light respectively. Furthermore, anthocyanin synthesis has also been linked to light quality. Examining anthocyanin concentrations in baby leaf lettuce, Li and Kubota (2009) found that both supplemental blue and UV-A light increased concentrations by 31% and 11% respectively.
**Figure 5.2:** Light distribution patterns from three common greenhouse lighting strategies: Top lighting, inter-lighting, and top + inter-lighting. These distribution patterns illustrate the light gradient created from top lighting fixtures and which can be alleviated by the introduction of inter-lighting.
5.5 Microclimate

While the climate (air temperature, air circulation pattern and speed, humidity, supplemental light intensity, etc.) within a greenhouse are meticulously thought out and controlled, what happens at a leaf surface and in the surrounding air are usually of less concern to growers. However, differences between the set macroclimate within a greenhouse and the microclimate surrounding the leaf can be significant (Zhang et al., 2002). Traditionally supplementary lighting fixtures (HPS) not only emit high amounts of energy as light, but also high amounts as radiant heat. Newer LED luminaries used for supplemental lighting within greenhouses contribute a much lower heat load (approximately 50% less than HPS) to the surrounding environment. Although having similar air temperature set points, greenhouses using HPS lights can have a much higher leaf temperature than plants under LEDs (Figure 5.3).

The physical properties of the lights being used can have an affect on the microclimate and leaf boundary layer. Altering the leaf boundary layer can affect the thermal exchange between the leaf surface and air (Schuepp, 1993). This, in turn, can affect gas exchange properties such as photosynthesis, transpiration, and WUE through modulation of the stomatal aperture. For instance, the high radiant heat from HPS luminaries can elevate the leaf temperature and humidity of the boundary layer inadvertently placing the leaf outside of the optimal temperature range for photosynthesis. Furthermore, elevated humidity can leave the plant susceptible to increased disease prevalence. Taken together, decreased leaf photosynthesis and increased disease pressure can lead to a reduction in yield, especially during months of high natural air temperature, which further exacerbate the difference between the leaf microclimate and greenhouse macroclimate. Thus, one must be cognisant of the potential effect on the microclimate when introducing supplementary lighting.
Figure 5.3: Thermal image of pepper plants grown under HPS lights (A) and a red/blue LED light (B) and the thermal properties associated with the upper leaves when grown at the same air temperature and humidity.
5.6 Optimizing Greenhouse Lighting for Increased Yield

Light is one of the most important inputs controlling plant growth and yield. Indeed, the implementation of supplemental lighting during greenhouse production has allowed plants to be produced year-round to meet consumer demand. The introduction of LED fixtures as a viable source of supplemental lighting has allowed for the fine-tuning and spectral manipulation of lighting strategies for increased crop growth. Today, virtually every aspect of plant growth can be affected by differences in light intensity, photoperiod duration, light distribution, light quality, and microclimate from plant morphology to fruit nutrient concentration and taste. While plant biology and growth strategies have come a long way since the introduction of supplemental lighting, much research remains as lighting technology itself continues to advance. Furthermore, investigation into the interactions between light and other abiotic factors (such as temperature, CO₂ concentration, and nutrient availability) is needed to create optimal growing environments within greenhouses and other controlled environment production systems.
Chapter 6: Summary and Future Directions

6.1 Carbon Export

Carbon export is a complicated, multi-cellular pathway which is fundamentally important to plant yield. Essentially, carbon export in vascular plants such as tomato, controls the movement of sugar molecules from source tissue (leaf) to sink tissue (growing tip, roots, and fruits). In tomato, the process of carbon export is regulated by many transporters and enzymes. For this reason, we hypothesized that the carbon export process may be affected by light intensity and spectral quality.

Our experiments with both ‘Bonny Best’ and ‘Foronti’ at ambient and elevated CO$_2$ showed that as the photosynthetic rate was increased, the percentage of newly fixed carbon which was exported decreased. This observation led to two symbiotic hypotheses. Firstly, as the rate of sugar production is increased with an increasing photosynthetic rate, relatively more sugar is stored in the form of starch or sugars in the vacuole to be used at a later time instead immediately exported. Secondly, the increase in sugar production and subsequent decrease in relative export percentage could be due to a bottleneck within the carbon export process. Since this process is regulated by enzymes and transports in tomatoes, an increase in carbon assimilation could be above the functioning of one of these regulatory steps. Having a rate limiting step as such would decrease the relative export and lead to this observation.

More interestingly was the effect of spectral quality on carbon export. Our experiments were specifically designed to control the influx of carbon by setting the photosynthetic rate. At a medium photosynthetic rate (~8 µmol m$^{-2}$ s$^{-1}$) leaves which were exposed to a blue LED produced a higher export rate than did leaves exposed to a white LED. This novel finding was ubiquitous and occurred in experiments using both ‘Bonny Best’ and ‘Foronti’ at an ambient CO$_2$ level and when ‘Foronti’ plants were grown and analyzed under elevated CO$_2$ conditions. This finding of short-term exposure to blue light increasing the carbon export rate from tomato leaves has led to the hypothesis that blue light is able to independently affect a regulatory point within the carbon export pathway without affecting the CO$_2$ assimilation process.

While this novel result is extremely interesting, the underlying mechanism behind it is still unknown. Due to the complicated nature of the carbon export pathway, an RNA sequencing (RNA-seq) experiment is currently underway in hopes of identifying the effect of blue light on the
regulatory transporters and enzymes. This experiment was set up to analyze tomato leaves exposed to either white, red, or blue LEDs after 8h and 16h of exposure. Light intensities from each LED were set using knowledge from photosynthetic light curves to achieve photosynthetic levels of approximately $8 \mu\text{mol m}^{-2} \text{s}^{-1}$. The use of RNA-seq will allow for the detection of the mRNA transcripts related to the enzymes and transporters within the carbon export pathway as well as other known proteins within the tomato genome. Using this unbiased method and analyzing the leaves under different wavelengths we can begin to unravel which step within the carbon export is being affected by the blue light, leading to the increase in relative export at the medium photosynthetic rate.

6.2 Whole Plant Analysis

Whole plant analysis adds an additional layer of information to plant studies. Whereas leaf level analysis provides data for the main photosynthetic organ of a plant, whole plant analysis provides information on the organism as a whole. Doing so, we can acquire information at how plant gas exchange is affected by plant architecture, mutual shading, micro-climates, and leaf age. Coupling this additional layer of information with different abiotic factors can aid in the implementation of supplementary or sole lighting strategies for production purposes.

The experiment in Chapter 3 builds off a previous experiment (Lanoue et al., 2017) and aimed at expanding the knowledge of whole plant gas exchange by examining the interaction between spectral quality and CO$_2$ concentration. Experiments in Chapter 3 confirmed the findings in Lanoue et al., (2017) where exposing plants to either HPS lighting, red-blue LEDs, or red-white LEDs had no affect on the whole plant photosynthetic level over the course of a day. However, when exposed to either LED light treatment, the whole plant WUE was observed to be lower than plants exposed to the HPS luminaries. This finding was ubiquitous under all CO$_2$ conditions tested. These finding indicate that altering the lighting strategies during plant production can influence the irrigation strategies being used.

The use of whole plant analysis can give additional information into the inner workings of plant production. Building on the current research, additional abiotic factors can be added to the whole plant analysis. Temperature is easily controlled during greenhouse production and can have a major influence on plant growth. Future experimentation should focus on the interactions between
multiple abiotic factors such as light spectrum, CO₂ concentration, and temperature. Doing such will allow for optimization of the abiotic environments during production as current temperature suggestions for use during HPS lighting may not be optimal during the use of LEDs. Further, with the advancements in LED lighting technology, new wavelength recipes can be examined using whole plant analysis to see how they affect gas exchange while taking into account plant architecture. This will allow for analysis of light penetration which can increase light available for leaves deep within the canopy and have a positive effect on whole plant photosynthesis and thus growth.

6.3 Continuous Lighting

Continuous lighting during greenhouse production of tomatoes has been hypothesized to increase yield if photoperiod related injury can be avoided. This is due to the constant light availability to drive photosynthesis and thus plant growth. However, many studies have shown that during CL in growth chambers, plants begin to show photoperiod related injury after only 10 days of CL. It has been hypothesized that a downregulation of the gene type III light harvesting chlorophyll a/b binding protein 13 (CAB13) during CL causes injury by affecting photosystem function.

In our experiment, we compared two different lighting strategies: 12h of red and blue light at 250 µmol m⁻² s⁻¹ followed by 12h of darkness (control) and 12h of red (200 µmol m⁻² s⁻¹) followed by 12h of blue (50 µmol m⁻² s⁻¹; CL) as supplemental lighting during greenhouse production of tomatoes during the light limiting months of winter. Our results showed that our CL treatment did not cause any photoperiod related injury as determined by chlorophyll fluorescence imaging. Furthermore, the carbohydrate status of the leaves under CL, a trait which is often altered during CL, was similar to leaves under the control light treatment.

During the first month of harvest, our CL treatment produced a higher fruit fresh weight than did the control treatment. Over a 4-month harvest period, plants grown under CL produced similar production numbers to plants under the control treatment. While there was no increase in production, it is hypothesized that the utilization of CL could have an economic benefit to growers. The use of a lower light intensity during the day means growers would need to purchase less lighting fixtures to achieve the desired light level and thus utilize less energy. Furthermore, the use
of light during the night, a period which is associated with off-peak electrical use and thus lower electrical prices, will further reduce the cost of production.

Further analysis is already underway with respect to photoperiod length as well as light intensity during the night period. An experiment is currently running in which the length of red light during the day is lengthened. This experiment is designed to see how long plants are able to sustain injury-free growth under a higher light intensity. This will maximize the time plants are under higher rates of photosynthesis. Doing so also has a further economic benefit. By keeping the DLI the same, a longer day-time period allows for a reduction in the day-time supplemental light intensity needed. By lengthening the day-time period, less lighting fixtures will need to be purchased to achieve the desired light intensities.

An experiment is also currently planned to assess how plants will handle a higher light intensity during the night period. In the experiment from Chapter 4, not only was a spectral shift utilized (red during the day followed by blue at night), the light level was also reduced from 200 µmol m\(^{-2}\) s\(^{-1}\) to 50 µmol m\(^{-2}\) s\(^{-1}\). By having both a spectral shift and reduction in light intensity, the exact mechanism responsible for injury-free tomato production under CL can not be determined. By running an experiment using higher blue light intensities during the night, we hope to identify if there is a point at which CL-related injury occurs. Doing such will allow for further analysis and aid in determining if the injury-free production during CL is due to a spectral shift or a reduction in light intensity.

### 6.4 Conclusion

Understanding the inner workings of gas exchange is of vast importance to predicting plant production during controlled environment cultivation. Due to the technological advancements surrounding LED manufacturing, the ability to manipulate the spectral composition during controlled environment production has never been easier. In this thesis, it was observed that manipulating the spectral quality leaves and plants were exposed to, carbon export and whole plant gas exchange can be affected, and results were consistent at both ambient and elevated CO\(_2\) levels. Both carbon export and whole plant gas exchange can drastically affect the overall yield during production and thus are important aspects when designing lighting strategies. Furthermore, utilizing the ability to manipulate the spectral quality during controlled environment production,
it was determined that a continuous alternating red and blue light spectrum can support injury-free tomato production while having economic benefits. While this research provides significant advancements in understand both the theoretical and applied implications of manipulating spectral quality, the introduction of LEDs in plant biology has opened the door for a variety of precision agricultural applications.
References


Appendices

Appendix I: Leaf NCER of tomato plants (cv. ‘Bonny Best’) under AC (A), SEC (B), and EC (C) exposed to various spectral qualities expressed on an area basis. Leaf temperature was set to 22°C with an RH of 55-65%. The regression lines are fit to \( f = y_0 + a(1-e^{-b \cdot x}) \) where \( y_0 \) is the respiration rate at a light level of 0 μmol m\(^{-2}\) s\(^{-1}\), \( a \) is the maximum photosynthetic rate (μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), and \( b \) is a constant. Each regression line is fitted to \( n = 3 \) leaves.

Appendix II: Leaf NCER of tomato plants (cv. ‘Bonny Best’) under AC (A), SEC (B), and EC (C) exposed to various spectral qualities expressed on a chlorophyll basis. Leaf temperature was set to 22°C with an RH of 55-65%. The regression lines are fit to \( f = y_0 + a(1-e^{-b \cdot x}) \) where \( y_0 \) is the respiration rate at a light level of 0 μmol m\(^{-2}\) s\(^{-1}\), \( a \) is the maximum photosynthetic rate (μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), and \( b \) is a constant. Each regression line is fitted to \( n = 3 \) leaves.
Appendix III: Principle component analysis of the impact of CO$_2$ condition and light quality on whole plant gas parameters such as the average photosynthesis, transpiration, and WUE of a tomato (cv. ‘Bonny Best’) at the first flower developmental stage. Plants were analyzed under either HPS, red-blue LED, or red-white LED during a 16/8h photoperiod with a day-time light intensity of 1000±10 µmol m$^{-2}$ s$^{-1}$. Temperature was 22/18°C and RH was 60±5%. Values identified by a circle (●) indicates plants grown and analyzed at AC, a triangle (▼) indicates plants grown and analyzed at EC, and a square (■) indicates plants grown at ambient CO$_2$ then analyzed under SEC. Panel A represents all values normalized on a leaf area basis. Panel B represents photosynthesis on a chlorophyll basis (µmol CO$_2$ g Chl$^{-1}$ s$^{-1}$), transpiration on an area basis (mmol H$_2$O m$^{-2}$ s$^{-1}$), and the resulting WUE (µmol CO$_2$ g Chl$^{-1}$/ mmol H$_2$O m$^{-2}$).
Appendix IV: Principle component analysis of the impact of CO₂ condition and light quality on leaf gas parameters such as average photosynthesis, transpiration, and WUE of a tomato (cv. ‘Bonny Best’) at the first flower developmental stage and a light level of 500 µmol m⁻² s⁻¹. Leaf temperature was set to 22°C with an RH of 55-65%. Values identified by a circle (●) indicates plants grown and analyzed at AC, a triangle (▼) indicates plants grown and analyzed at EC, a square (■) indicates plants grown at ambient CO₂ then analyzed under SEC, a diamond indicates plants grown under elevated CO₂ then analyzed under SAC. Panel A represents all values normalized on a leaf area basis. Panel B represents photosynthesis on a chlorophyll basis (µmol CO₂ g Chl⁻¹ s⁻¹), transpiration on an area basis (mmol H₂O m⁻² s⁻¹), and the resulting WUE (µmol CO₂ g Chl⁻¹ / mmol H₂O m⁻²).

Appendix V: Leaf morphology of plants (cv. ‘Endeavour’ grafted on ‘Maxifort’) under 12h supplemental lighting (red + blue at 250 µmol m⁻² s⁻¹; panel A) and CL (red during the day (200 µmol m⁻² s⁻¹) and blue at night (50 µmol m⁻² s⁻¹); panel B). Application of both supplemental lighting treatments began on November 15th, 2018 and continued to May 16th, 2018 with harvest beginning on January 28th, 2019. Throughout the experiment, supplemental lighting remained on regardless of ambient DLI to ensure both treatments received the same total DLI. The daytime temperature was held between 21-24°C depending on the ambient solar radiation while night temperature was maintained at 20±0.5°C. Relative humidity of 70±10% was maintained during both day and night periods. Greenhouses were CO₂ enriched to approximately 800 µL L⁻¹. The red circle highlights leaf cupping of leaflets grown under 12h supplemental red/blue LED lighting while leaves under CL remain flat.