GREENHOUSE APPLICATION OF LUMINESCENT GREENHOUSE FILMS EMBEDDED WITH QUANTUM DOTS TO IMPROVE GROWTH OF LETTUCE

by

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As members of the Master's Committee, we certify that we have read the thesis prepared by Morgan Mattingly, titled *Greenhouse Application of Luminescent Greenhouse Films Embedded with Quantum Dots to Improve Growth of Lettuce* and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

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Dedication

For my parents, for sharing a world of possibilities, and instilling in me both curiosity and drive.

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Abstract

Many industries in arid environments, especially agriculture, are facing water and energy resource challenges, such as drought, increasing costs, and worsening pollution. Controlled environment agriculture (CEA) can increase water use efficiency and reduce environmental impact by producing crops within closed loop hydroponic systems. However, energy is required to power irrigation pumps, electrical lighting, and other environmental controls to achieve optimal plant growth and production in CEA. A new luminescent agriculture film technology presents a solution for improving crop production without requiring electricity. Quantum Dot (QD) technology embedded in the luminescent films and deployed in greenhouse covers can passively alter less photosynthetically efficient ultraviolet (UV) and blue wavelengths from the sun to red wavelengths inside a greenhouse, which are more efficient in promoting plant growth and increasing biomass. Two QD films with emission peaks in orange (625 nm) and red (650 nm) were selected for study in a greenhouse to determine the growth impacts of these films. Seven repeated experiments of red romaine lettuce (*Lactuca sativa* cv. 'Outredgeous') were grown in deep water culture (floating raft) tanks under the quantum dot films and simultaneously under a control film with the similar reflective and diffusive properties as the quantum dot films (four crops were grown under the 625 nm QD film and three under the 650 nm QD film). Each experiment was replicated under each film in two tanks of 56 plants each. The air (temperature, RH) and root zone (EC, pH) environments and the light received (PPFD) were monitored during growth, and upon harvest, the wet and dry biomass and morphological properties (leaf count and length) were measured. The light use efficiency (LUE), or the grams of biomass produced per mole of light received, and the daily light integral (DLI) were calculated. The lettuce plants grown under the quantum dot films increased in fresh mass (+10 to 27%), dry mass (+15 to 24%), and LUE (+7 to 35%) compared to the plants grown under the control film. The quantum dot films have demonstrated the potential to produce increased biomass in lettuce plants grown in greenhouses without increased energy cost, and have potential for numerous future applications.

1 Introduction

1.1 Controlled Environment Agriculture

In temperate locations, controlled environment agriculture (CEA) is frequently used to improve food production. Especially in locations that are generally temperate, but have infrequent severe weather, the processes to control the environments and ensure reliable plant growth are well understood (McCartney and Lefsrud, 2018). In extreme environments, however, such as arid deserts or polar regions, there remains opportunity for improvement. The challenges are varied, and solutions are still being explored. Arid and tropical regions, though in different ways and for different reasons, share many similar barriers to agriculture. The high temperatures need to be controlled with innovative techniques due to the respective high and low relative humidity in the climates, and water and energy resources are frequently scarce and must come from unique sources (McCartney and Lefsrud, 2018). For this reason, new methods to increase crop growth without adding energy costs must be explored. Altering the quality of a the light environment of the plant can be one way to improve the light use efficiency and associated crop growth.

1.2 Light Spectral Quality and Effects on Plant Growth

In addition to plant response to the quantity of light determined by intensity (PPFD) and duration that direction promotes plant growth and biomass accumulation, the plant responds in various ways to the quality of light that it receives. The importance of light spectral quality to the improvement of plant production has been widely explored. Generally, plants rely on wavelengths in the photosynthetically active radiation (PAR) region, commonly known to range from 400-700 nm (McCree, 1972). Within this spectrum, plants are affected in unique ways by different qualities, or distributions of wavelengths, of light they receive. These qualities not only

contribute to photosynthesis and transpiration, but can also act as informational signals directing plant morphology and growth patterns (Kang et al., 2016).

1.2.1 Red Light, Far Red Light, and Phytochromes

Light is absorbed by proteins in plants called phytochromes, which primarily sense red and far-red light (Taiz and Zeiger, 2010). These proteins control carbon intake, biomass increase, and metabolism in plants (Yang et al., 2016). Red light from about 600 to 680 nm is generally recognized as the most photosynthetically efficient for plant growth (Wang et al., 2016; McCree, 1972). Light at wavelengths greater than 685 nm rapidly declines in photosynthetic efficiency (Zhen et al., 2018; Emerson and Lewis, 1943). Far red light greater than 680 nm, however, plays a role in exciting photosystem I (PS1), while shorter wavelengths between 400 and 680 nm excite photosystem II (PS2) (Zhen et al., 2018).

When both photosystems are excited simultaneously by the application of both a shorter wavelength of light less than 680 nm and a longer wavelength light greater than 680 nm, overall rate of photosynthetic activity is increased significantly in a phenomenon known as the Emerson Effect (Zhen et al., 2018; Emerson et al., 1957). Thus, both red and far-red light and the ratio between the two play a significant role in the enhancement of photosynthetic activity. It has recently been recommended that far-red light up to 740 nm should be measured to better monitor and understand photosynthetic responses, since far-red light at a wavelength of 731 nm has been demonstrated to cause photosynthetic activity in lettuce plants (Zhen et al., 2018).

In vegetative plants like lettuce and St. John's Wort, an increase in red light ratios can cause an increase in biomass (fresh and dry mass), leaf count, and leaf length (Wang et al., 2016, Karimi et al., 2022). However, a light with a high red to far-red ratio (lacking in far-red light) has also been shown to decrease lettuce leaf size (Zhen, 2018), showing that both red and far red

light are useful and necessary to effectively grow lettuce plants, and that the ratio has a significant effect.

1.2.2 Blue and UV Light

Plants grown under higher ratios of blue light have increased leaf photosynthesis rates and lower starch contents. Despite this increased photosynthesis, the plants had smaller leaves and slower growth rates. (Kang et al., 2016; Wang et al., 2016). This reduction in growth and biomass results from the smaller leaves, since blue light inhibits cell expansion and resulting leaf area (Bugbee, 2016; Dougher and Bugbee, 2004; Karimi et al., 2022). This characteristic of blue light to inhibit plant growth has been supported by numerous studies, including one observing reduced growth from blue light alone during the healing process of grafted tomato seedlings (Melissas et al., 2022). Still, red light alone is insufficient, and a combination of the both red and blue, at a minimum, is needed for effective growth (Melissas et al., 2022; Kang et al., 2016).

Ultraviolet (UV) light from the sun that passes through the atmosphere to reach earth and can affect plants includes UV-A, which ranges from 230 to 400 nm and UV-B, which ranges from 280 to 320 nm (Kang et al., 2022). High intensity of UV light can have negative effects on plant growth (Kang et al., 2022). UV-B light, specifically, has been shown to induce stress on plants and decreases their size in terms of fresh and dry weight and leaf area, although it also increases flavonoid and anthocyanin pigment development (Lee et al., 2022). However, some amount of UV-A light has beneficial effects on the quality and quantity of leafy greens production, and low levels can even improve the rate of photosynthesis (Kang et al., 2022). Additionally, it can significantly increase certain effects on photomorphogenesis of plants. Certain crops, such as ornamental flowers or red lettuce plants, require some amount of UV light

to grow properly and to produce leaves and flowers with the desired color, since it effects the creation of red anthocyanin pigment (Alwadai and El-Bashir, 2022).

1.2.3 Red and Blue Light

The ratio of the amount of red to blue light is critical. For example, when lettuce plants are grown under lamps emitting more red than blue wavelengths, the resulting plants are larger in terms of fresh weight and dry weight than those grown under lamps emitting more blue than red wavelengths (Ohashi-Kaneko et al., 2007; Lin et al., 2018). However, this must be tempered with the complementary fact that lettuce plants grown under a higher proportion of blue to red light are higher in protein content (Lin et al., 2018) and have higher rates of photosynthesis (Karimi et al., 2022).

Many morphological characteristics, such as leaf count and leaf area, and parameters such as chlorophyll per leaf area, are increased by a combination of both red and blue light, as long as the red to blue ratio is high (Wang et al., 2016). Different combinations of colors (wavelengths) of light promote distinct aspects of growth, and produce unique impacts at various phases of growth (Mickens et al., 2018).

For example, white or red light combined with blue light produced smaller plants with higher nutritional value overall for one lettuce cultivar, whereas red, green, blue, and far red light produced the largest plants, and the combination of white and green light accelerated in seedlings (Mickens et al., 2018). Likewise, Zhou et al. (2022) found that the yield of a tomato plant can be increased by red and blue light provided with a high red to blue ratio, but that the flavor was improved by providing red light alone. The application of specific wavelengths to maximize plant growth is, therefore, both crucial and complex. It depends both on the desired priorities of the growers and on the species of the plant being grown (Alwadai and El-Bashir, 2022).

1.2.4 Controlling Light Quality with LEDs

Many of the studies discussing the benefits of specific light ratios and the applications of specific wavelengths use light emitting diode (LED) lamps at the desired wavelengths. In one notable experiment, B. Matysiak et al. (2021) conducted further research into the impact of different combinations of LED wavelength outputs on Romaine lettuce in a vertical farm, and found that the quality of light has a crucial impact on the growth of lettuce in closed environments. Not only was the lettuce larger with a greater proportion of red light combined with relatively lower proportions of blue and green (specifically, RGB 70:18:12), it had reduced nitrate nitrogen content and had increased flavanol content, which are desirable in lettuce (Matysiak et al., 2021). It was clear from this study that specific control of light spectral quality had a substantial and valuable impact on improved vegetative growth in lettuce plants, but replacing light from the sun with light from electrically powered LEDs lamps requires additional hardware purchases and operating expenses, which can be prohibitive.

1.2.5 Light Quality in Greenhouses

In another study of lighting conditions for commercially grown plants, researchers contrasted the common in vitro growth of orchid seedlings in grow rooms under fluorescent lighting to germination and early growth in greenhouses under sunlight. The use of sunlight provided several benefits. It eliminated energy costs required by lamps for lighting. Seedlings were more quickly acclimated to the natural photoperiod experienced when matured and moved out of the seedling growth chamber. Additionally, this study determined that having a broader spectral light distribution greatly improved the growth of plants, since each color of light in the PAR region, as well as some wavelengths outside of it, serves a purpose for plant growth (Ferreira et al., 2021).

Although plants typically need much higher amounts of blue and red light to increase successful germination and biomass production, the addition of green light can have benefits to plants and to growers. It has been shown to be increase plant biomass and be aesthetically useful to growers by making the light appear more white and thus allowing growers to more easily identify issues in plant growth (Kim et al., 2004; Orlando et al., 2022), to increase plant height, and to increase phytonutrient content above plants grown with only red and blue lights (Orlando et al., 2022).

This observation with broad-spectrum lighting is one of the reasons that growing plants in greenhouses with sunlight can be preferable to growing them in growth chambers, which invites investigation into the modification, rather than replacement, of natural sunlight.

1.3 Greenhouse Covering Film Selection

1.3.1 UV Blocking Greenhouse Covering Films

Many greenhouse-covering films have been researched that modify the transmission of wavelengths. One very common spectrum change is the limitation or complete block of ultraviolet (UV) radiation. In 2006, it was demonstrated that eggplants in soilless cultures respond negatively to light in the UV region by analyzing greenhouse covering films with UV blocking from 5% down to 0%. Generally, however, the effects of the UV blocking film on the greenhouse microclimate were neutral or even positive. There was no environmental impact to air temperature, relative humidity, or PAR radiation using UV blocking films, but the plants were taller with larger leaf dimensions, and the fruit had increased weight and better color (Kittas et al., 2006).

However, there are numerous benefits to UV light in a greenhouse. These benefits include that bees, which are required for pollination for fruiting plants, need UV radiation to

navigate, that UV can increase vitamin D, phenolics, and other active substances in plants, and that UV light can be required for some photomorphogenic responses, such as intensity of color in some types of crops. The level of UV light blocking in a greenhouse covering film must be based on the physiological needs of the crop, and on the types of beneficial insects required to pollinate the plants and control pests (Alwadai and El-Bashir, 2022). Although UV light blocking films are commonly used, well-studied, and can be beneficial in the right situations, additional types of films must be explored that shift, rather than block, specific light wavelengths, to improve plant quality as well as biomass development.

1.3.2 Photoselective Greenhouse Films

A different approach to greenhouse films is to selectively alter the light quality of the PAR waveband entering a greenhouse to optimize the plant response. In 2006, photoselective films dyed with fluorescent pigments were developed and tested by monitoring strawberries grown under light conditions with varying red to blue ratios. One primary concern with growing plants in a greenhouse covered by photoselective films is that the light intensity in the PAR region transmitted through the film was reduced to as low as 57% (Hemming et al., 2006).

Multiple films were created using fluorescent dyes that increased the red or blue ratio of the light and tested against a reference film . Ultimately, the red films decreased PAR transmission without providing a measurable benefit to plant growth, while the blue film slightly increased PAR transmission and increased the fruit output of the strawberry plants (Hemming et al, 2006). This early attempt at passively modifying the ratio of red to blue wavelengths using specialized greenhouse films could not passively modify light quality without lowering intensity.

More recently, an experiment was performed using a similar theoretical principle, though with more advanced resources. S. M. El-Bashir et al., in 2016, proposed the use of luminescent

solar concentrator (LSC) films, which are polymethylmethacrylate (PMMA) nanohybrid films mixed with a fluorescent dye, and for a preliminary experiment, covered outdoor potted soybean seedlings during the summer growing season to observe the plant growth patterns. The goal of these films was to change green and yellow wavelengths, which the researchers proposed were less useful for plant growth and photosynthesis, into red wavelengths, which are essential for photosynthesis, plant growth, and increased biomass production. The transmission of PAR through the PMMA nanohybrid film was significantly greater compared with previous materials, the seedlings responded positively to the new film, and the films degraded slowly, providing a significantly longer useful life. While the addition of nano-oxide materials improved tolerance of the film to damage from weather and UV radiation, and the PAR transmission was relatively high, the measurement of plant response to the modified light spectrum was limited to preliminary studies on the soybean seedlings (El-Bashir et al, 2016).

1.3.3 Barriers to Photoselective Film Use

Two barriers, then, to the use of photoselective films for improving light use efficiency in a greenhouse, are the lowering of light intensity in the PAR region (Hemming et al., 2006) and the relatively short lifespan compared with other films (El-Bashir et al., 2016).

Another challenge with using the films described is the impact of the quality of the transmitted light on the plants being grown, and the potential negative effects of filtering out specific wavelengths in the PAR region. For example, although red and blue wavelengths are generally most important for lettuce biomass production, green light can be beneficial for biomass production and nutritional qualities (Matysiak et al., 2021; Kim et al., 2004). Furthermore, the addition of green light makes plants appear more naturally colored, which assists growers to more effectively evaluate and provide care to produce quality crops (Kim et

al., 2004). Also, a more complete light spectrum is likely beneficial to plants compared with a limited spectrum that removes most or all of a specific wavelength (Ferreira et al., 2021).

Therefore, it may be more valuable to transform a portion of UV wavelengths into the red spectrum to decrease UV light without omitting it entirely, and to intentionally tailor the red to blue light ratio. There is an opportunity here for further research into different types of photoselective films.

1.4 Quantum Dot Films

There are several luminescent materials, containing organic fluorescent dyes, quantum dots, or rare earth complexes, that can be added to greenhouse covering films to change the spectral quality (Alwadai and El-Bashir, 2022).

One type of very promising additive is the quantum dot. A quantum dot is a small particle of nano-scale that can alter the wavelength of a photon as when absorbed and re-emitted. A new type of non-toxic, copper-zinc quantum dot developed by UbiQD, Inc. has been studied for its use in agricultural films (Parrish et al., 2021). This type of quantum dot is a colloidal, semi-conductor nanocrystal approximately five to ten nanometers in diameter which can modify the wavelength of light if absorbed by the dot (Parrish, 2020).

The output spectrum of these quantum dots is centered around a red or orange (590-700 nm) emission waveband and is determined by the size of the quantum dot (Makarov et al., 2019). The efficiency, defined as the percentage of available higher energy wavelengths of UV and blue that are absorbed and re-emitted to lower energy, longer wavelengths, is greater than 85 percent (Parrish et al., 2021). This efficiency is defined as the quantum yield (QY) (Parrish, 2020). The high quantum yield of these quantum dots, along with their increased stability and longevity

compared with fluorescent dyes, overcome the barriers faced by other types of photoselective films. These characteristics make quantum dots a practical addition to agricultural films, which could have numerous applications, from commercial use over greenhouses to maximize light use efficiency to applications in space, where growing efficiency is crucial and spatial capacity and energy are limited (Parrish et al., 2021).

1.5 Growth Chamber Experiments – UbiQD, Inc-NASA STTR

A NASA STTR grant with UbiQD, Inc. of Los Alamos, New Mexico and University of Arizona Controlled Environment Agriculture Center (CEAC) of Tucson, Arizona included a study of plastic films made of a layer of acrylic resin embedded with this new type of quantum dot encased in two layers of polyethylene terephthalate (PET) plastic (Parrish et al., 2021). The quantum dots in this film passively modified UV and blue light to red wavelengths. This research was performed on a small scale by growing 'Outredgeous' red romaine lettuce under the quantum dot film in an indoor growth chamber (1.8×1.0×2.2 m, L×W×H) (Parrish, 2020). The growth chamber was lit by metal-halide (MH) lamps with a light spectrum similar to that from the sun, providing greater amounts of blue and UV light than possible with typical MH lamps (Parrish et al., 2021).

In this study, the efficiencies of quantum dot films at two different peak emission wavelengths (orange, 590 nm and red, 630 nm) were compared against each other and against a reference film with the same light diffusing qualities, but not embedded with quantum dots. The initial experiments determined that although the quantum dot films did reduce the overall PPFD compared to the reference film (the orange film by 3% and the red film by 12%), the light use efficiency was significantly increased under each quantum dot film. Lettuce grown under the

quantum dot films had greater fresh weight, dry weight, and leaf area than the lettuce grown under the reference film (Parrish et al., 2021).

Additionally, a study was supported by UbiQD, Inc. to compare the relative efficiencies of several quantum dot films that modified UV and blue light to several different emission wavelengths in the orange and red spectrums. Red romaine lettuce was grown in the same growth chambers used by Parrish under electrical lighting from the metal-halide lamps using a different film in each chamber, and the biomass and morphology of the lettuce was measured. The lettuce grown under each quantum dot treatment film was found to have higher light use efficiency (LUE) and biomass output than the lettuce grown under the control film. The two quantum dot films providing the most improvement in terms of LUE and biomass were selected for future study, and new films were manufactured for the following experiments (Blum et al., in review). The new quantum dot films had the same peak emissions (±10 nm) as these two selected films, with outputs at 625 nm and 650 nm.

This indoor trial also incorporated the study of 'Red Robin' dwarf cherry tomato plants, grown under two different quantum dot films (orange, 620 nm and red, 660 nm) along with a control. The tomato plants grown under each quantum dot treatment film showed improvement in light use efficiency (LUE) and, for the 620 nm film, significant increases in total tomato fruit biomass compared with the plants grown under the control film (Blum et al., in press).

The improved growth of lettuce and cherry tomatoes within the plant growth chambers in this previous research has great potential for use by organizations like NASA to grow food in space by modifying light from the sun and directing it into growth chambers. It should also be encouraging to commercial growers as an energy effective method to improve biomass production by allowing plants to use light more efficiently. However, the film that passively

modifies light quality will realistically be used by growers inside of greenhouses rather than growth chambers or with electrical lighting. Therefore, the efficiency of the film must be investigated in the context of a greenhouse with a more complete light spectrum from the sun, more seasonally variable environmental factors, and a naturally variable daily light intensity and DLI experienced in greenhouse crop production. The following document describes the subsequent greenhouse experiments completed with the quantum dot films for the production of lettuce.

1.6 Objective and Hypothesis

The objective of the project was to research the impact on the growth of red romaine lettuce (*Latuca sativa* L. cv. 'Outredgeous') within a greenhouse of quantum dot films that passively altered some incoming UV and blue wavelengths from the sun to red or orange wavelengths inside the greenhouse. The research provided study at a larger, more practical scale, which was more applicable to commercial growers than study in a growth chamber. Light from the sun rather than electrical light, and the natural environmental variability would provide commercial-like conditions. The hypothesis was that the films embedded with quantum dots would increase the biomass production and light use efficiency in the production of red romaine lettuce.

2 Methodology

2.1 Greenhouse Location and Design

The experiments were performed in an A-frame greenhouse compartment (7.3 x 14.6 m) at the University of Arizona's Controlled Environment Agriculture Center (CEAC). The bay was 2.6 m high at the gutter and 4.4 m high at the gable, with a 373 m^3 volume. The greenhouse was at the east end of a north-south oriented, gutter-connected row of greenhouses, sharing its west wall with another greenhouse and having its east wall whitewashed to create a more equal light environment from each side wall.

The greenhouse side walls were rigid, double-layer polycarbonate panels, and the roof was covered with a single layer of Ethylene Tetrafluoroethylene (ETFE) greenhouse film (Tradename F-Clean™ Diffused, UV-Open). This material was selected for this experiment because it is long-lasting and it allowed 80 – 90% of UV light to pass through (Brownell, n.d.), which is crucial for an experiment that relies on the transformation of UV radiation into red light to increase light use efficiency.

The greenhouse was divided into two 3.7 x 14.6 meter sections down the middle using an opaque divider wall mounted vertically from the roof gable and reaching the floor below. This allowed crops to be grown on each side within the greenhouse to experience the same environment, except that one received sunlight that passed the ETFE roof cover and through the standard control film, and the other received sunlight through the roof and the QD treatment film under test. A control film was suspended internally and overhead near to the ETFE roof cover within the west half of the greenhouse and a quantum dot (QD) treatment film was similarly suspended over the east half of the greenhouse. The control film was a clear polyethylene film with similar reflective and diffusive qualities as the treatment films, but with no wavelength-

changing properties. The QD treatment film was an acrylic resin layer embedded with quantum dots encased in two layers of polyethylene terephthalate (PET) plastic film.

The greenhouse was cooled using an evaporative cooling system with two exhaust fans at the north end of the greenhouse, one on each side of the space separated by the divider wall, and a 1.25 x 6 meter louvred ventilation opening on the south wall, containing an evaporative cooling pad on the interior of the opening, and an insect exclusion screen on the exterior (Alcorn, 2021). Heating was provided with a gas-fired hot air unit heater located near the south wall. Heated air temperature uniformity throughout the greenhouse was maintained with HAF fans that circulated the air around the divider wall and to each side of the greenhouse.

The cooling was achieved by ambient air added to and indoor air exhausted from both sides of the divider wall simultaneously. The heating or cooling systems were activated by a control sensor that sampled air from both sides of the divider wall and activated the heating or cooling system based on the setpoint value existing on either of the two sides. Once activated, both sides of the greenhouse received the same temperature modification.

The heating and cooling setpoints were controlled by a Wadsworth EnviroStep Control system, which activated the evaporative cooling pad pump, exhaust fans, heater, and HAF fans. The greenhouse was set to 27.2 (minimum) to 30 ℃ (maximum) during the photoperiod and 16.7 (minimum) to 23.8 ℃ (maximum) during the dark period.

From January through June 2022, the QD film was embedded with quantum dots modifying blue and UV wavelengths to 625 nm (625 nm QD treatment film), and from July through October 2022, another QD film was tested with quantum dots modifying the light to 650 nm (650 nm QD treatment film).

A spectroradiometer (Apogee Instruments, PS-300) was used to measure the light spectrum at solar noon of the light environment outside the greenhouse, and of the light environment inside the greenhouse under the control film and of the light environment inside the greenhouse under each quantum dot film. The light spectrum during the 625 nm QD film experiments is in Figure 1, and the light spectrum during the 650 nm QD film experiments is in Figure 2. The percent of light in the wavebands in each of the light environment was calculated for each of the light profiles and is in Table 1 and Table 2.

The spectral irradiance graphs below may vary, given the precise time of day at which the data was collected. However, they were collected at the same location within the greenhouse, and at approximately the same time. The quantum dot films do not significantly reduce the total DLI compared with the control films, but shift the wavelengths to a higher value. Thus, while the intensity of the light under each side of the greenhouse may vary as a result of the time of day the measurements were taken, the percent values in the associated tables show the relative differences between the daylight spectrum and that under each film. Additionally, the sunlight and control spectrums below are different for the 625 and 650 nm QD film plots, since they were measured at different times of year and slightly different times of day.

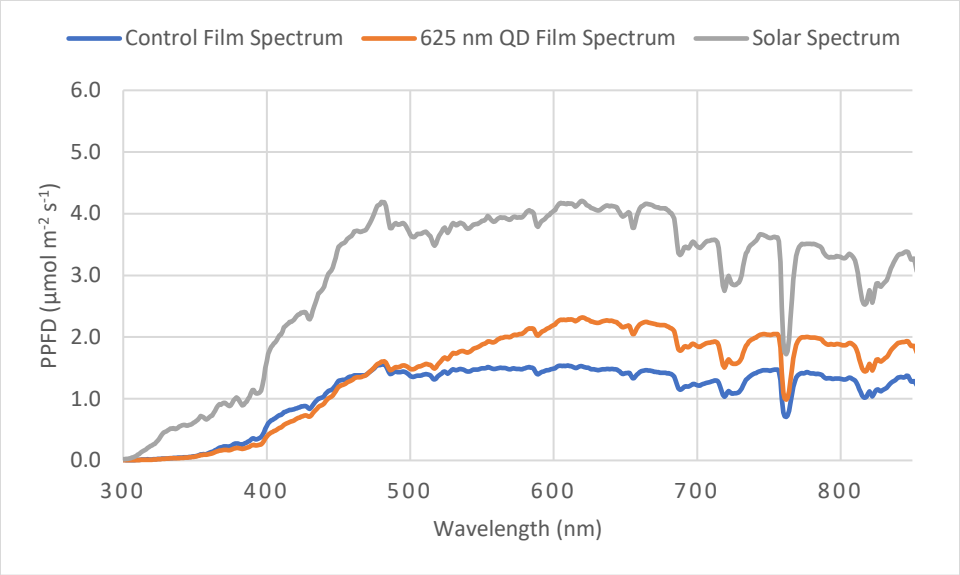


Figure 1. Light Spectrum Outside, Under the Control Film, and Under the 625 nm QD Film

Table 1. Percent of Light in Each Waveband for 625 nm QD film Experiments: Taken Outside the Greenhouse, Under the Control Film, and Under the 625 nm QD Film

	300-400 nm	400-500 nm	500-600 nm	600-700 nm
Sun	5%	27%	33%	35%
Control	3%	28%	35%	34%
625 nm	2%	21%	35%	42%

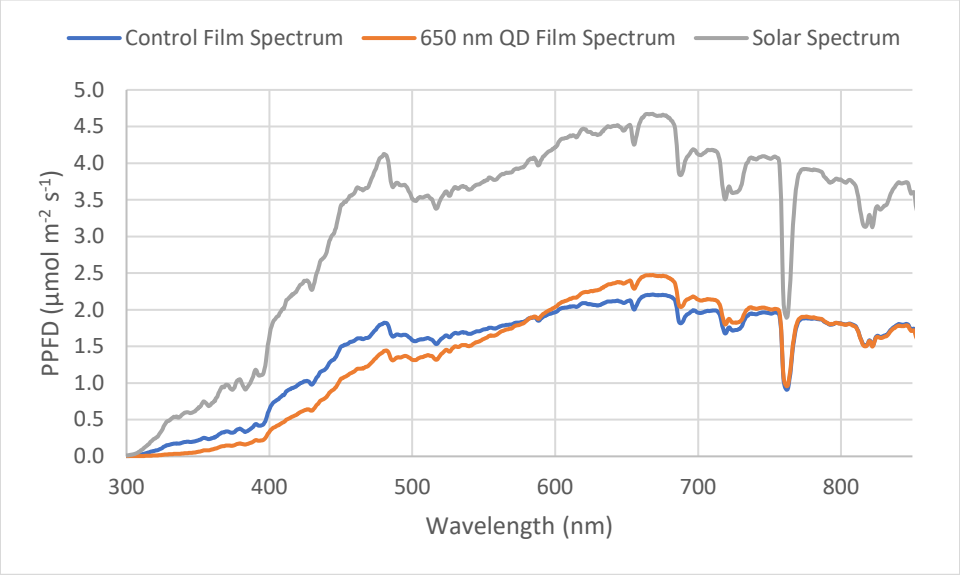


Figure 2. Light Spectrum Outside, Under the Control Film, and Under the 650 nm QD Film

Table 2. Percent of Light in Each Waveband for 650 nm QD film Experiments: Taken Outside the Greenhouse, Under the Control Film, and Under the 650 nm QD Film

	300-400 nm	400-500 nm	500-600 nm	600-700 nm
Sun	5%	26%	32%	37%
Control	4%	25%	32%	39%
650 nm	2%	19%	33%	46%

High-wire tomato plants were grown on north-south oriented aluminum benches in the north part of the greenhouse. An ebb and flow table with an associated reservoir tank and pump to fill with a manually mixed nutrient solution was located in the south part of the greenhouse and used to grow tomato and lettuce seedling transplants. Four one hundred gallon deep water culture (DWC) tanks were also located in the south part of the greenhouse and used to grow lettuce plants to maturity. Two DWC tanks were located on each side of the divider wall, and the nutrient solutions were manually mixed and pH balanced to the required pH (6.0) and EC (1.8 dS m^{-1}). The DWC tanks were filled with nutrient solution with no additional added through harvest. An air bubbler stone and associated air pump were included with each tank. The layout of the tomatoes, ebb and flow table, and DWC tanks is shown in Figure 3.

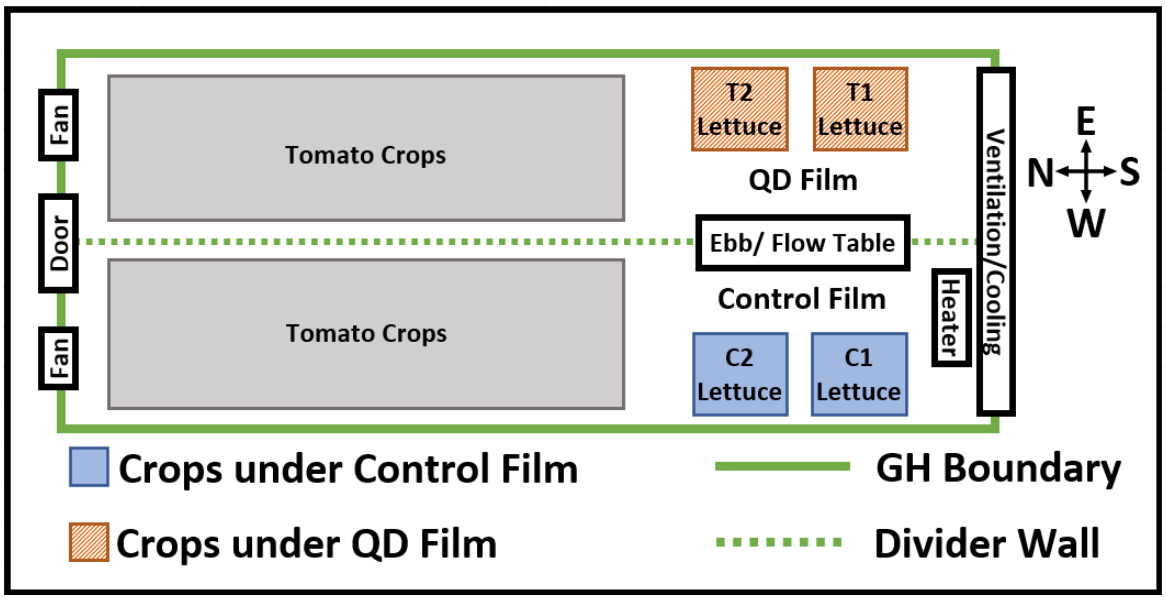


Figure 3. Greenhouse Layout of Divider Wall, Test and Control Film, and Environmental Controls.

2.2 Environmental Data Collection

Environmental data used for the lettuce experiment was monitored and stored using a WayBeyond wireless sensor system (WayBeyond, Folium, New Zealand) which measured PPFD, air temperature, and relative humidity (RH). The air temperature and RH were monitored by a Folium sensor near the plant canopy height adjacent to the DWC lettuce tanks in the south end of the greenhouse. At the end of each experiment, the average daytime and nighttime temperature and RH were calculated throughout the experiment. Daytime (photoperiod) temperature and RH were calculated by averaging data throughout each experiment where the PPFD was greater than 5 µmol m^{-2} s^{-1}. Nighttime (scotoperiod) temperature and RH were calculated by averaging data throughout each experiment where the PPFD was less than 5 µmol m^{-2} s^{-1}. Vapor pressure deficit (VPD) was calculated using the College of Agriculture and Life Sciences (CALS) and Controlled Environment Agriculture Center (CEAC) VPD calculator (*VPD Calculator*, accessed 2023).

DWC nutrient solution water, representative of the root zone data, was collected for lettuce plants by measuring the pH, electrical conductivity (EC), depth, and temperature within each of the four tanks once weekly from transplant to harvest.

Throughout each experiment, Photosynthetic Photon Flux Density (PPFD) was monitored with an Folium quantum sensor (WayBeyond wireless system) for light in the photosynthetically active radiation (PAR, 400 – 700 nm) spectrum, as well as a Campbell Scientific system using Li-Cor Quantum sensors (400 – 700 nm). This data was used to determine the daily light integral (DLI), or the total PAR provided to the plants throughout the lettuce test between uncovering seedlings and harvest of mature plants. The Folium sensor was mounted adjacent to the four DWC tanks at 1 m above the lettuce canopy, as shown in Figure 4.

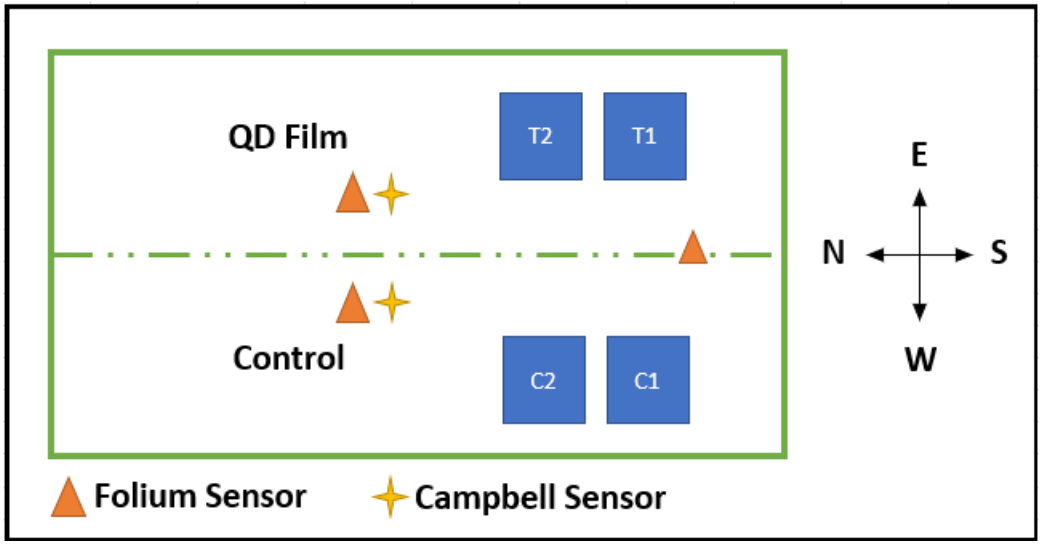


Figure 4. Folium and Campbell Sensor Locations in the Greenhouse.

Daily Light Integral (DLI) was calculated from 10 minute averages of PPFD µmol m^{-2} s^{-1}. The average DLI (Equation 1) for each crop from uncovering of the germinated seedlings through harvest was calculated from the sum of the 24-hour DLI (mol m^{-2}) divided by the number of days for the test period (34 for first harvest or 41 for second harvest). Note that two

successive harvests were completed for each of the lettuce production experiments, as will be described later.

$$(1)\ DLI\ (Mol\ m^{-2}\ day^{-1}) = \frac{\frac{\Sigma PPFD\ (\mu mol\ m^{-2} s^{-1}) \times 600\ s}{1{,}000{,}000}}{Days\ from\ uncovering\ to\ harvest}$$

Fresh mass light use efficiency (LUE) is a measure of the grams fresh weight that was produced per mole of light received during each experiment. Light use efficiency was calculated using Equation 2 using the PPFD data measured throughout the experiment, the fresh weight of each lettuce plant, and the planting density.

$$(2)\ LUE = \frac{FW\left(\frac{g}{plant}\right) \times (Plant\ Density\left(\frac{plant}{m^2}\right)/\#plants)}{Avg\ DLI\left(\frac{mol}{m^2 day}\right) \times Days\ after\ Treatment\ (days)}$$

These procedures were used to determine the estimated total DLI and total LUE received for the east and west Folium sensors within the Control and the QD treatment film sections of the greenhouse located over the tomato growing area. Because Folium quantum sensors were not located directly above the lettuce tanks, the DLI and LUE calculations under the control and QD film treatments are included because they provide a ratio of the light quality difference between east and west sides of the greenhouse, although they are not the actual values seen at the lettuce canopy. Therefore, the LUE throughout the results and analysis is referred to as "relative" LUE.

2.3 Lettuce Experiment Overview

From January through June 2022, four lettuce experiments were performed under a QD test film with a peak fluorescence centered around 625 nm. These are referred to as the 625 nm Film Experiments 1, 2, 3, and 4. From July to October 2022, three lettuce experiments were completed with a QD test film with peak fluorescence centered around 650 nm. These are

referred to as 650 nm Film Experiments 1, 2, and 3. Each experiment followed the same methodology for growth, data collection, harvest, and analysis.

2.4 Lettuce Experiment Methodology

2.4.1 Lettuce Growth and Procedures

2.4.1.1 Lettuce Seeding

For each experiment, 224 plus 15% extra 2.54 cm (one-inch) rockwool cubes were double-seeded and placed in black trays. The trays were placed side by side onto a flood irrigation tray and covered with opaque plastic film. The seeded cubes were irrigated three times per day with fresh water having no nutrients by flooding the tray to a depth of 2.5 cm and draining excess water to the storage tank.

2.4.1.2 Germination

For each experiment, the trays were covered and irrigated until all seeds had germinated, which was indicated by the root radicles emerging from the seed and shoots appearing. At germination (between three and five days from seeding, depending on the season), the trays were uncovered and the seedlings were divided into two groups: one half of the seedlings were moved to the control side of the ebb and flow tray, and the other was moved to the QD film treatment side of the ebb and flow tray.

The lettuce nutrient solution formulation, shown in Table 3, was the lettuce formula used in the Plant Growth Test Chamber (PGTC) in the Phase I part of the research program, and was provided at one-half strength, resulting in a pH of 6.0 and an EC of 0.9 mS/cm. The seedlings within their rockwool cubes were fertigated by flood irrigation 3 to 4 times per day.

Table 3. Lettuce Nutrient Formula.

Recommended Lettuce Nutrient Solution (CEAC, 2/22/2008)								
		Elements (ppm)						
Tank A		N	P	K	Ca	Mg	S	Cl
Ca(NO3)2	Calcium Nitrate	134			165			
CaCl2.6H2O	Calcium Chloride				35			60
Tank B								
KNO3	Potassium Nitrate	46		137				
KH2PO4	Potassium Phosphate		50	61				
MgSO4.7H2O	Magnesium Sulfide					40	52	
Total ppm		180	50	198	200	40	52	60

2.4.1.3 Thinning

At 8 days from seeding, the plants had a small first true leaf and they were thinned to one seedling per rockwool cube (Figure 5). The seedlings removed were measured for fresh weight.

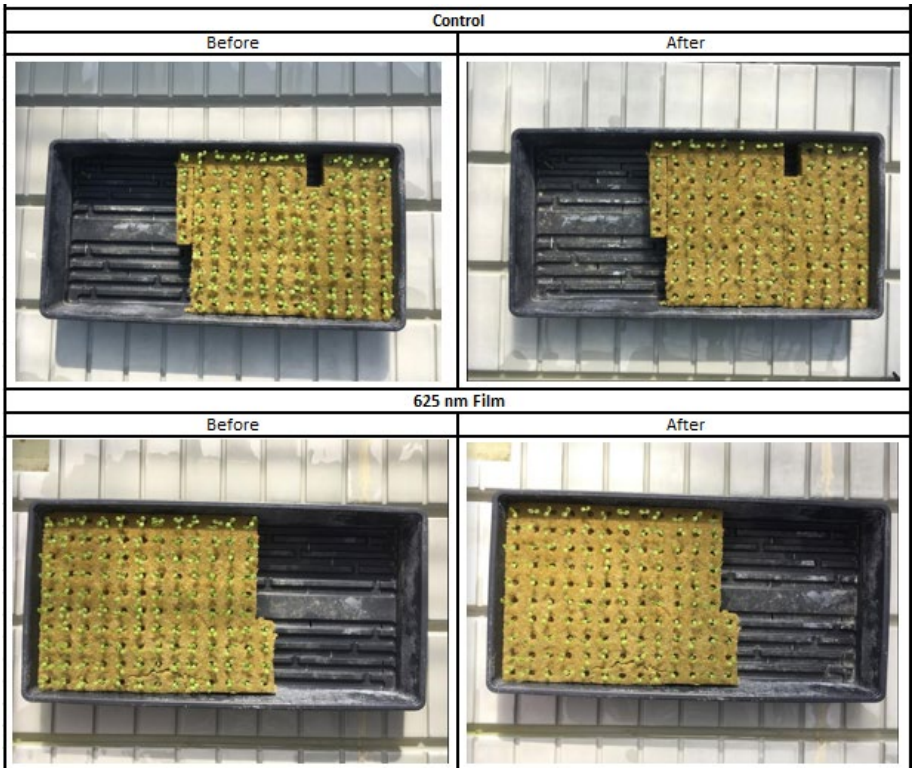


Figure 5. Lettuce Seedlings Before and After Thinning to One Seedling Per Rockwool Cube (625 nm QD Film Experiment 1: 1/13/22).

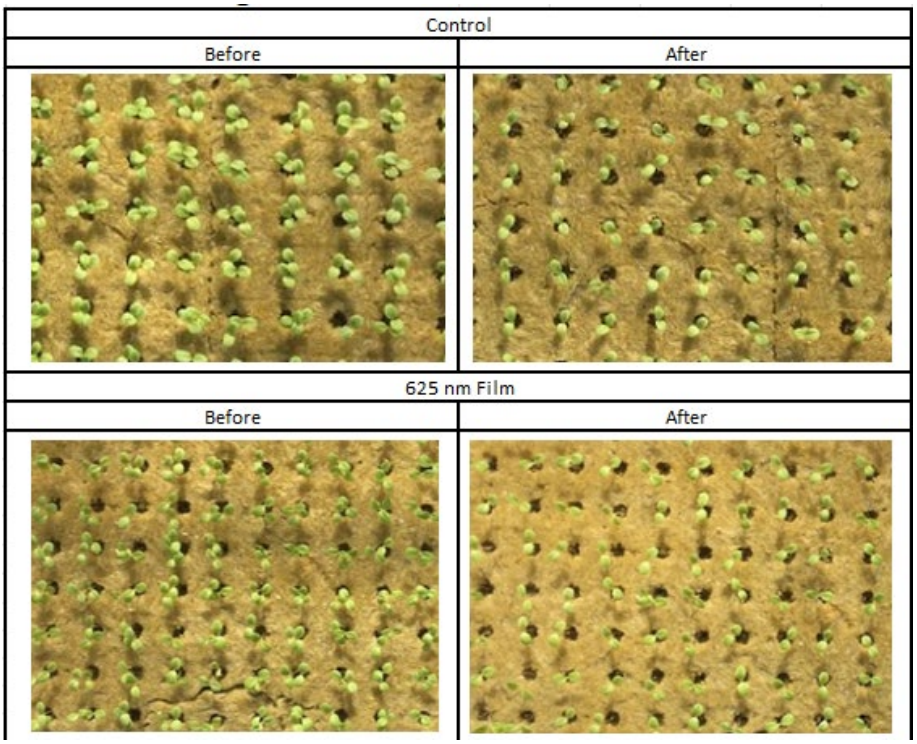


Figure 6. Close View of Lettuce Seedlings Before and After Thinning to One Seedling Per Rockwool Cube (625 nm QD Film Experiment 1: 1/13/22).

2.4.1.4 Transplanting

Transplanting occurred 14 days from seeding for each experiment, when the plants each had a single unfolded true leaf in addition to the two cotyledons. Four deep water culture (DWC) floating raft tanks (1.33 X 1.33 X 0.3 m, L:W:H) were cleaned and sanitized with 10% bleach solution and positioned with two tanks on the control side and two on the QD film side of the greenhouse. The tanks were filled with pH-balanced (6.0), full-strength (1.8 mS/cm) EC nutrient solution to a depth of 30 cm so that the rafts (1.22 X 0.61 m) would float at the top. The 112 seedling cubes were placed into the holes in the rafts on the control and the QD film side of the dividing wall with a plant density of 31.7 plants per meter squared. The seedlings were planted into each of the four tanks in the staggered configuration shown in Figure 7.

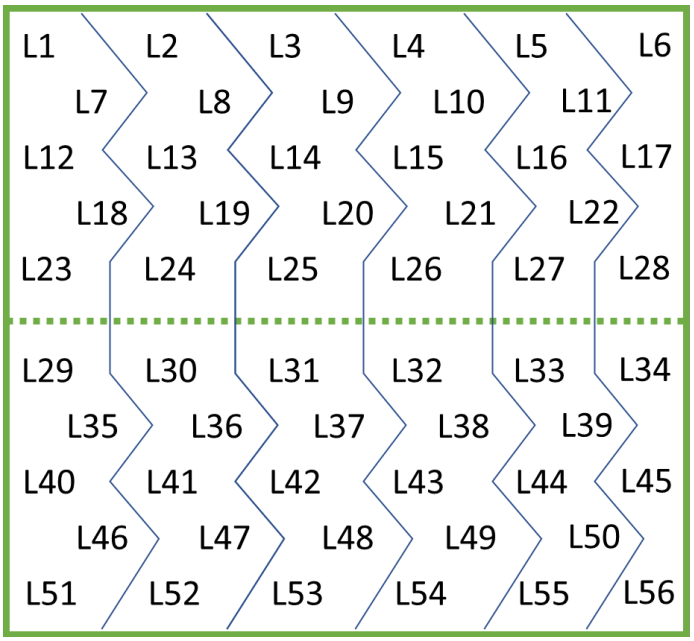


Figure 7. Lettuce Layouts in the DWC Tanks.

The fresh and dry weights of ten individual seedlings, representative of those transplanted, were selected from the control group and from the QD film group. The dry weights were measured after 94 hours at 45°C in a drying oven.

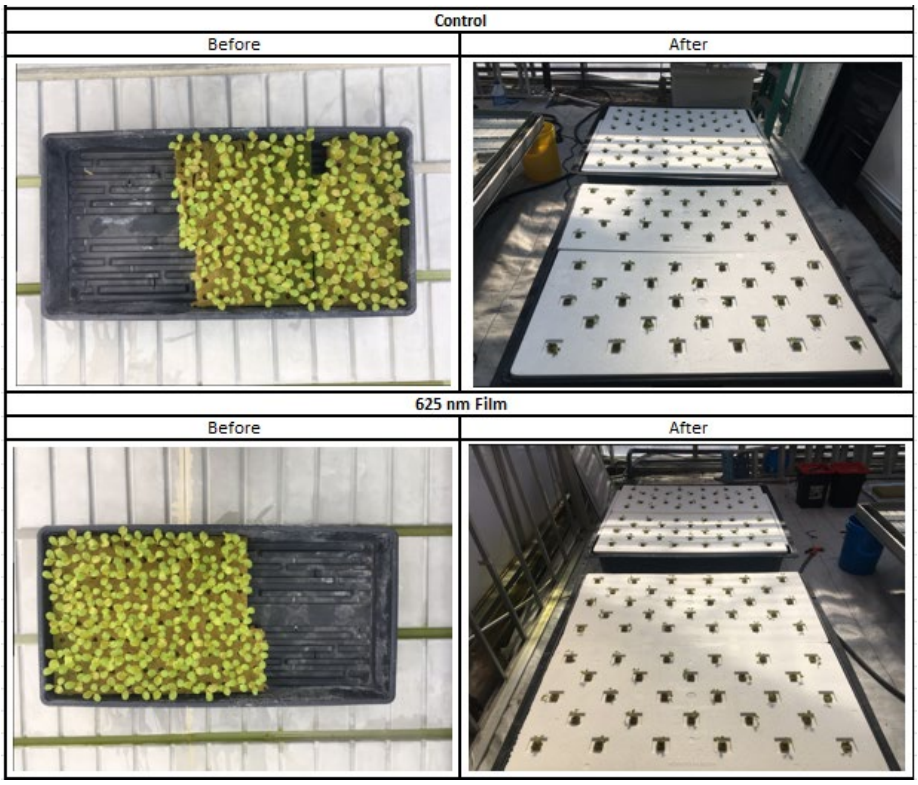


Figure 8. Lettuce Seedlings Before and After Transplant from Ebb and Flow Table to Deep Water Culture Tanks (625 nm QD Film Experiment 1: 1/19/22).

2.4.1.5 Harvesting

For each experiment, an initial harvest was performed at 36 days from seeding (23 days from transplant). These days were selected during Experiment 1 according to maturity and canopy closure, and were used again for the remainder of the experiments. This growing period length was preferred because it was the same as the growth period for the PGTC from the initial experiments, but due to the winter temperature and light environment of Experiment 1, the plants were small at 23 days. An additional seven days was provided for the remaining lettuce plants after the initial harvest at 23 days from transplant. This second harvest is referred to as the second harvest, and the two-harvest methodology was followed throughout the experiment.

This first harvest was performed by removing twenty plants from each DWC tank in one day (rows shown in blue in Figure 9). The order of harvest was a QD film tank (T2), a control

tank (C2), then the other 625 nm tank (T1), and remaining control tank (C1). One week later, the remaining plants again reached canopy closure (43 days from seeding, 30 days from transplant), and a second harvest was performed on all remaining non-border plants (rows shown in red). None of the border plants in rows R1 and R10 and in columns C1 and C6 were harvested for data.

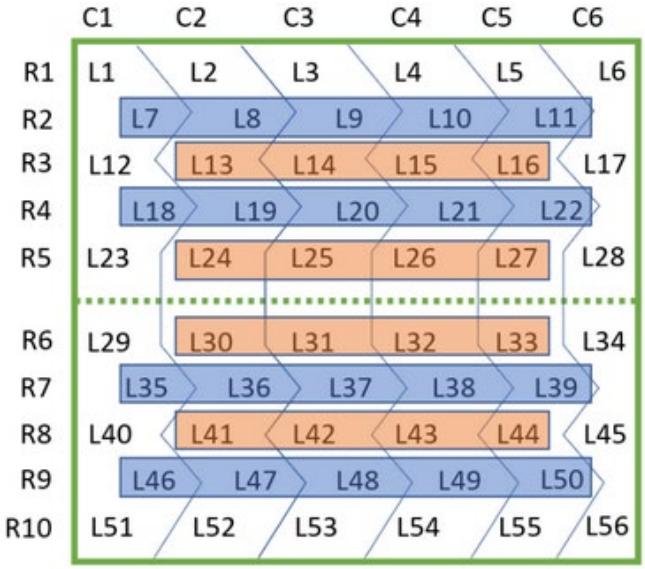


Figure 9. For each of the four DWC tanks, the first group of lettuce plants (Initial Harvest) was harvested 23 days after transplanting and were the rows highlighted in blue (R2, R4, R7, R9), and the second group (Final Harvest) was harvested 30 days after transplanting and were the rows highlighted in red (R3, R5, R6, R8).

2.4.2 Lettuce Data Collection

2.4.2.1 Plant Data Collection

Throughout each lettuce experiment, images were taken regularly from above the replicated Control and Treatment DWC tanks. The purpose was to monitor the rate of canopy closure of the lettuce plant growth within each DWC tank.

For each harvest and within all experiments, the aerial portion (leaves) of ten data plants from each of the four DWC tanks was weighed (g, fresh weight, FW), leaves longer than one centimeter were counted (cm, leaf count), and the longest leaf was measured (leaf length, mm).

In addition, for five randomly selected data plants per DWC tank per harvest, each leaf was laid flat on a white posterboard under a clear plastic sheet. An image was taken from a camera suspended at a fixed position above the leaves. From these pictures, a leaf area analysis program developed by KC Shasteen was used to calculate the total leaf area of each plant (Shasteen, 2020).

Finally, each of 20 randomly selected data plants per control or treatment group per harvest was placed in a paper bag and then a drying oven at 45 C for at least 168 hours until leaves were dry, and each plant was weighed again for dry weight (g, dry weight, DW). The ratio of DW to FW for each dried plant was calculated as percent dry weight.

2.4.3 Experimental Design for Statistical Analysis

Checking for data outliers was completed before statistical analysis was performed on each set of data using a z-score test with a threshold of +/- 3 standard deviations. If any fresh weights were determined to be outliers, all data from the plant was removed from analysis. Any data passing the threshold was removed from the averages, standard deviations, and plots included in this report, and was not included in any further analysis.

ANOVA tests were performed for each individual set of data, and where necessary, a transformation was applied or, when a transformation was not sufficient to mitigate challenges with normality, a Kruskal-Wallis test was performed. Significance is included for each experiment. A p-value less than 0.05 was deemed significant using a 95% confidence interval.

Each of the processed datasets was analyzed using a two-factor factorial ANOVA test procedure (alpha = 0.05) to determine the impact on each of the primary measured parameters of the treatment (i.e., the control film versus the 625 nm film), the DLI (per trial, as measured at the pad in the greenhouse in between the control film and the treatment film), and the interaction between the two factors using Equation 3. Analysis was performed using SAS Analysis Software (*SAS On Demand for Academics*, 2022).

(3) $measured\ variable = treatment + DLI + (treatment \times DLI)$

The following measured datasets were analyzed with respect to the two factors using SAS code: fresh weight, dry weight, leaf count, and longest leaf length per plant throughout experiments 1 – 4 under the 625 nm film.

During initial analyses for several factors, the residuals plots showed a significant skew, indicating that the data was not a normal distribution, and the results may not be reliable. In order to correctly analyze the data, a transformation in the form *log(response variable)* was performed in each required instance to aid in analysis. The transformation was applied to: fresh weight (initial and final harvests), dry weight (initial and final harvests), leaf count (final harvest only), and longest leaf length (initial and final harvests). The results discussed below were determined after the transformation was applied.

3 Results

3.1 Lettuce Experiment Results

3.1.1 Lettuce Environmental Data Summary

3.1.1.1 Lettuce Aerial Environment

The average greenhouse air temperatures for each experiment are shown in Figure 10.The average photoperiod air temperature increased throughout the 625 nm QD film experiments as the season changed from January through July with longer photoperiods and warmer photoperiod air temperature. The average air temperature was more consistent throughout the 650 nm QD film experiments compared to the 625 nm QD film experiments.

Typically, warmer air temperatures are less ideal for lettuce crops compared with cooler temperature (Hernandez et al., 2017). This could indicate that the high photoperiod temperatures experienced by the lettuce in the summer were less advantageous for growth. However, lettuce plants grown in DWC tanks have been demonstrated to be healthy, even in warm summer weather in Tucson (Hernandez et al., 2017).

Additionally, although the air temperatures did change throughout the experiments, the colocation of the four DWC tanks at the south end of the greenhouse caused the temperature of the four tanks to be maintained at air temperatures the same or very close to each other. Therefore, the effect of the seasonally changing air temperature affected both the control and treatment groups to the same extent.

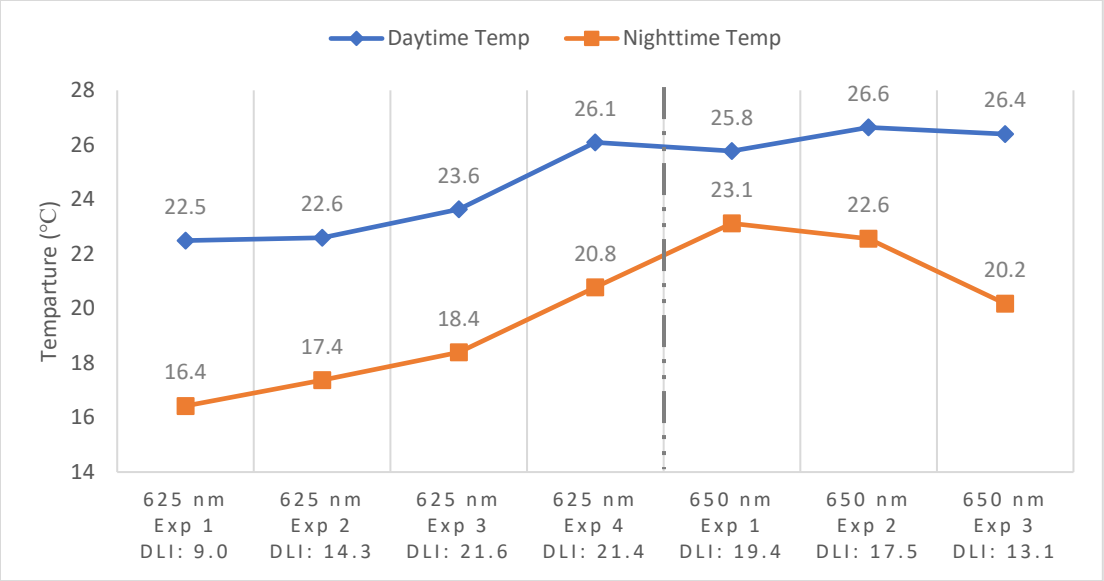


Figure 10. Average Photoperiod and Scotoperiod Air Temperature (°C) for both Control and Treatment Groups for each of the 625 and 650 nm QD Film Experiments. Each experiment listed with average daily light integral (DLI).

The average photoperiod relative humidity (RH) in the greenhouse was not controlled, but was continuously monitored. Average photoperiod RH per experiment was the average of RH while PPFD was greater than 5 µmol m^{-2} s^{-1}, and average scotoperiod RH per experiment was the average of RH while PPFD was less than 5 µmol m^{-2} s^{-1}. RH

The greenhouse relative humidity (RH) was influenced by the seasonal climate humidity of southern Arizona. During the 625 nm QD Film experiments, the photoperiod RH decreased from Experiments 1 through 3, as the winter rain period became the spring and summer arid climate. The RH increased for Experiment 4 during the beginning of the summer monsoon season, which is a period of time near the end of the summer in Southern Arizona when rainstorms become frequent and intense, and RH increases significantly. For the 650 nm film, the RH was at a maximum during Experiment 1 at the peak of the monsoon season and then decreased for Experiments 2 and 3 as the monsoon season ended. The average day and night RH are shown for each experiment in Figure 11.

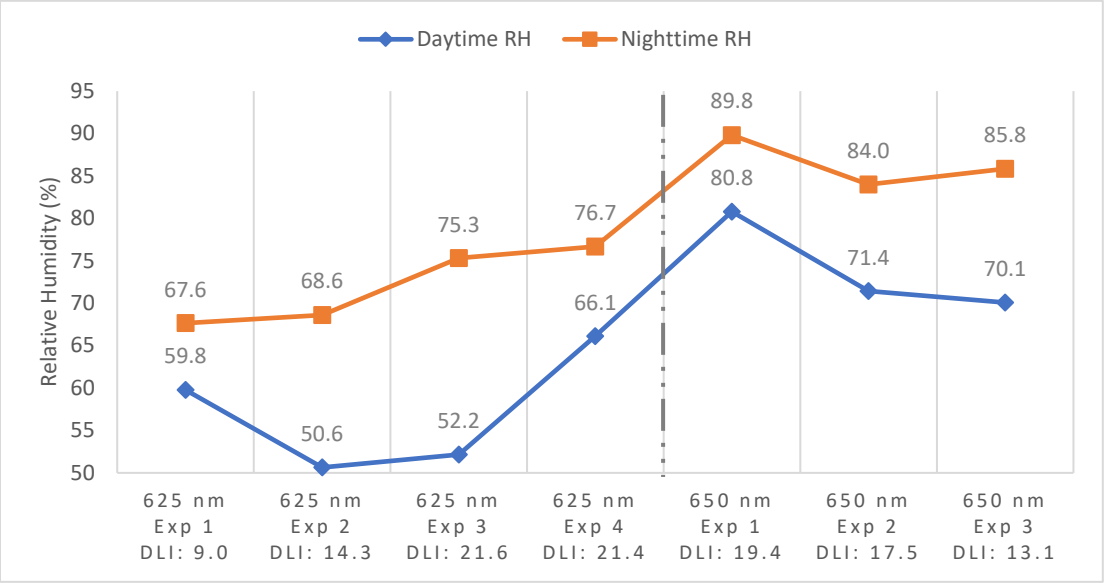


Figure 11. Average Photoperiod and Scotoperiod Relative Humidity (%) for both Control and Treatment Groups during each of the 625 and 650 nm QD Film Experiments. Each experiment listed with average daily light integral (DLI).

The vapor pressure deficit (VPD) was calculated using the average air temperature and relative humidity for each experiment. The average photoperiod VPD was relatively constant during the 625 nm QD film experiments, and dropped significantly at the 650 nm QD Experiment 1, which occurred during Tucson's monsoon season, as a result of the increased humidity (Figure 12). As the daily humidity and air temperatures decreased, the VPD increased again, though not to the levels of the early 625 nm QD film experiments. The scotoperiod VPD followed the same trend.

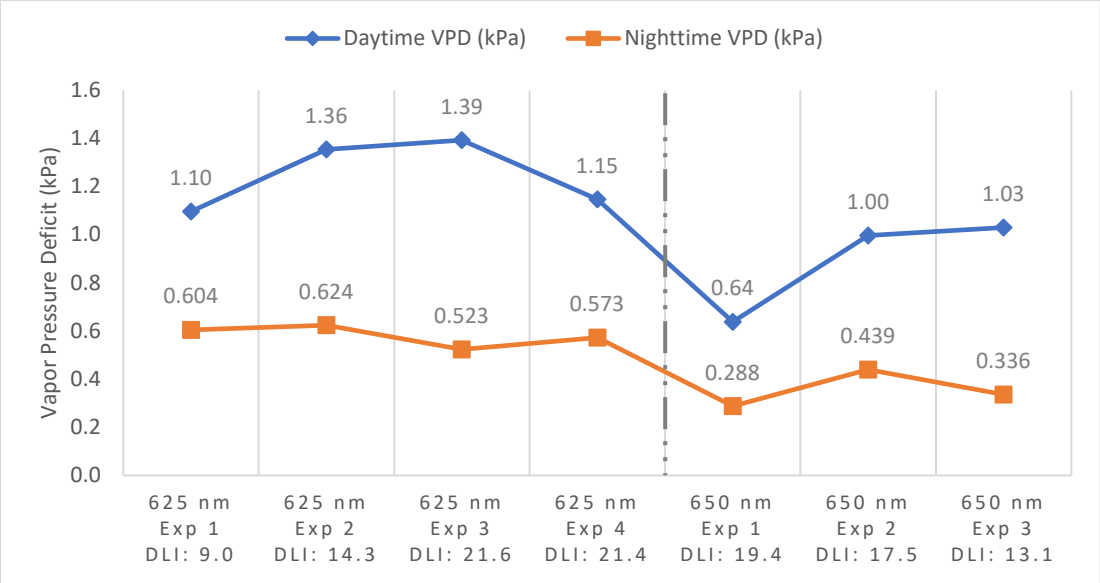


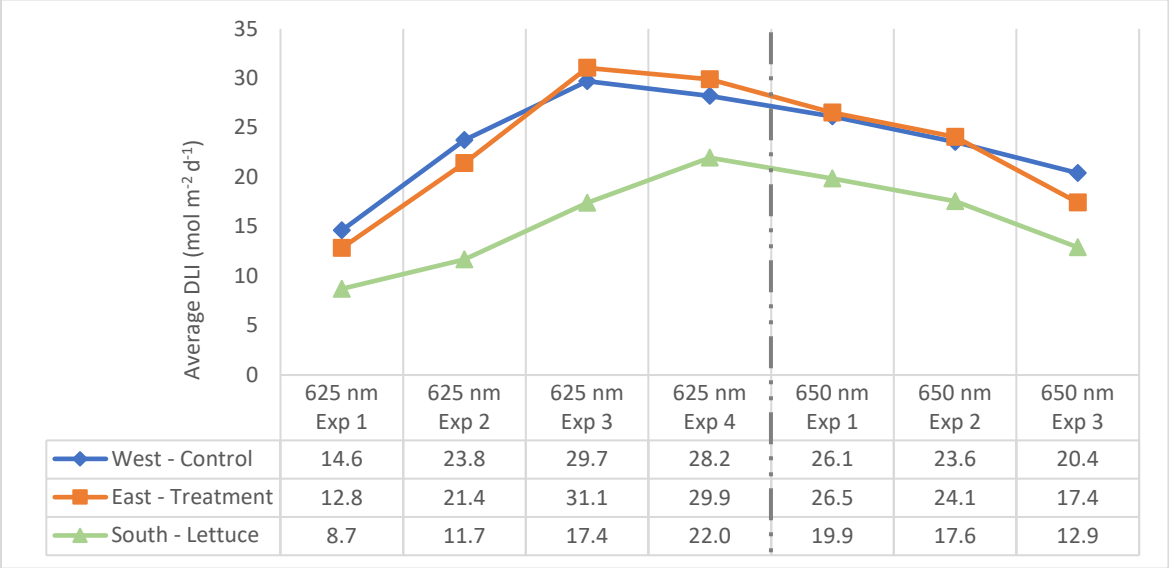
Figure 12. Average Photoperiod and Scotoperiod Vapor Pressure Deficit (VPD, kPa)) for both Control and Treatment during each of the 625 and 650 nm QD Film Experiments. Each experiment listed with average daily light integral (DLI).

3.1.1.2 Lettuce Light Environment

The average DLI are displayed for the initial (Figure 13) and final (Figure 14) harvests from the Folium sensors located in the greenhouse per Figure 4. The average DLI followed a seasonal trend, increasing as the experiments progressed from January through June, and decreasing gradually until the final experiment in October.

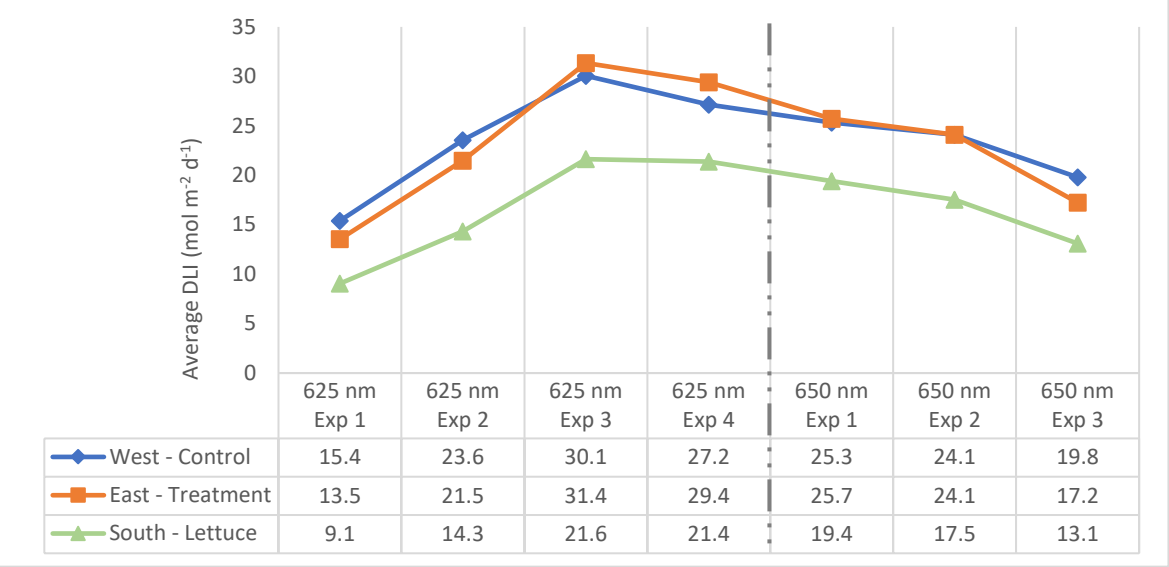
The DLI at the south end of the greenhouse, closest to the lettuce canopies, was lower than those by the tomato canopies, since that sensor was closer to the evaporative cooling pad. The average DLI was similar under each treatment, but not exact for the control and treatment for each experiment. Energy was neither gained nor lost as it passed through the film. The differences the DLI figures for control and treatments were a result of the differences of sunlight received at the greenhouse cover (ETFE film) on the associated side of the greenhouse. These were influenced by the sun angle to the glazings during the changing seasons, and to the divider

wall which caused the treatment on the east side to receive primarily morning sunlight and less afternoon sunlight, and the opposite for the west side.



	625 nm Exp 1	625 nm Exp 2	625 nm Exp 3	625 nm Exp 4	650 nm Exp 1	650 nm Exp 2	650 nm Exp 3
West - Control	14.6	23.8	29.7	28.2	26.1	23.6	20.4
East - Treatment	12.8	21.4	31.1	29.9	26.5	24.1	17.4
South - Lettuce	8.7	11.7	17.4	22.0	19.9	17.6	12.9

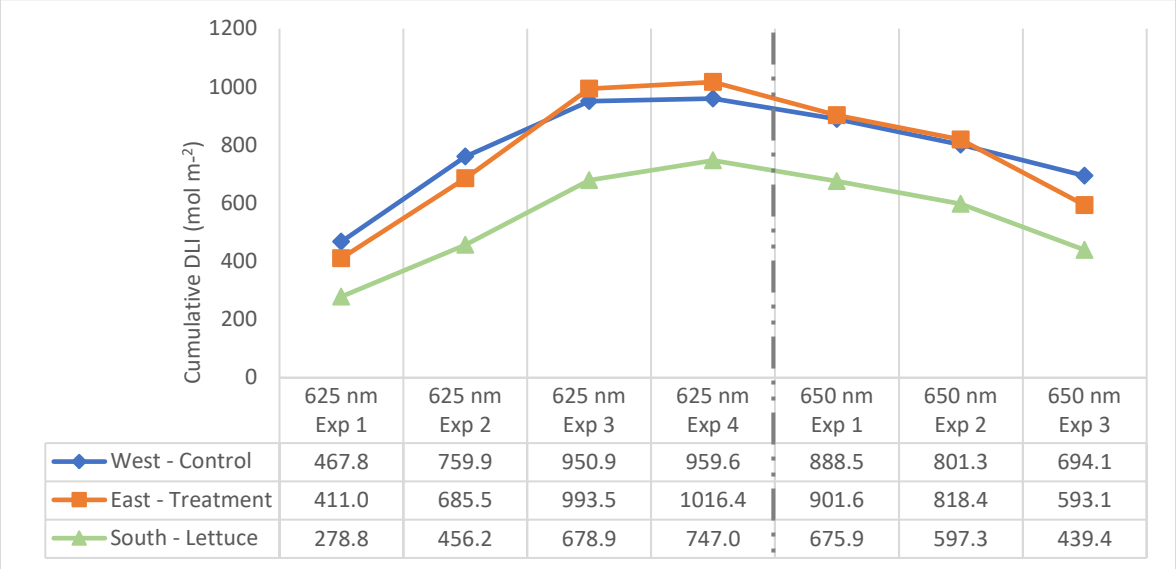
Figure 13. Average DLI (mol m^{-2} d^{-1}) from Control Film (West), QD Film (East), and Lettuce (South) Folium Sensors from Seedling Uncovering through Initial Harvest for four 625 nm and three 650 nm QD Film Experiments.



	625 nm Exp 1	625 nm Exp 2	625 nm Exp 3	625 nm Exp 4	650 nm Exp 1	650 nm Exp 2	650 nm Exp 3
West - Control	15.4	23.6	30.1	27.2	25.3	24.1	19.8
East - Treatment	13.5	21.5	31.4	29.4	25.7	24.1	17.2
South - Lettuce	9.1	14.3	21.6	21.4	19.4	17.5	13.1

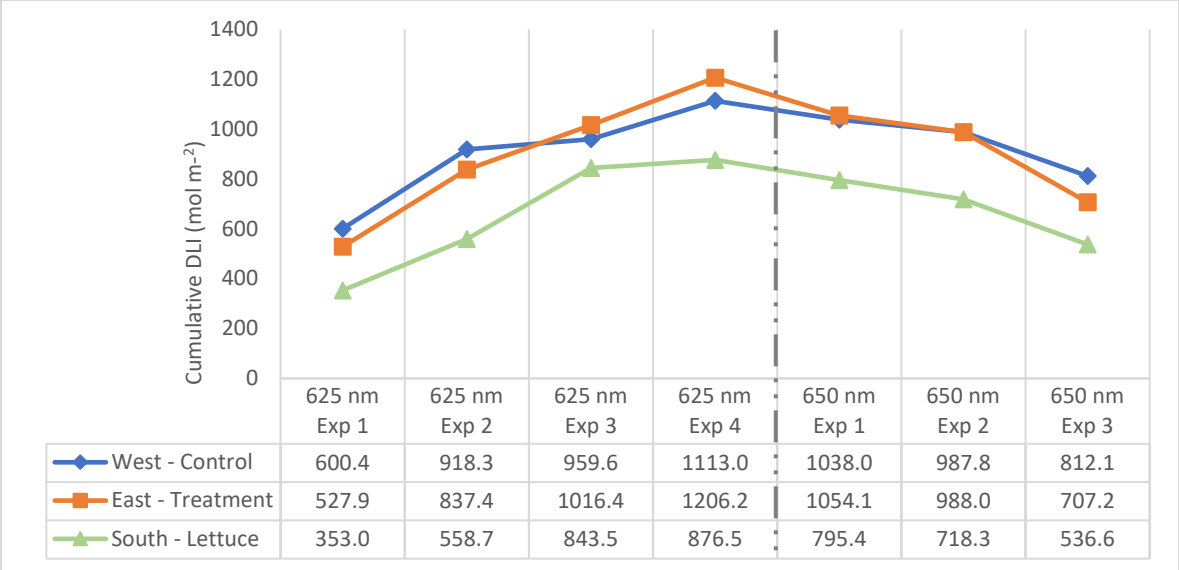
Figure 14. Average DLI (mol m^{-2} d^{-1}) from Control Film (West), QD Film (East), and Lettuce (South) Folium Sensors from Seedling Uncovering through Final Harvest for four 625 nm and three 650 nm QD Film Experiments.

The cumulative DLI are displayed for the initial (Figure 15) and final (Figure 16) harvests are from the Folium sensors in the greenhouse. They followed generally the same trend as the average DLIs. The cumulative lettuce DLI were used for the statistical analyses, since they are the most indicative of the total amount of sunlight received throughout the course of the experiment.



	625 nm Exp 1	625 nm Exp 2	625 nm Exp 3	625 nm Exp 4	650 nm Exp 1	650 nm Exp 2	650 nm Exp 3
West - Control	467.8	759.9	950.9	959.6	888.5	801.3	694.1
East - Treatment	411.0	685.5	993.5	1016.4	901.6	818.4	593.1
South - Lettuce	278.8	456.2	678.9	747.0	675.9	597.3	439.4

Figure 15. Cumulative DLI (mol m^{-2}) from Control Film (West), QD Film (East), and Lettuce (South) Folium Sensors from Seedling Uncovering through Initial Harvest for four 625 nm and three 650 nm QD Film Experiments.



	625 nm Exp 1	625 nm Exp 2	625 nm Exp 3	625 nm Exp 4	650 nm Exp 1	650 nm Exp 2	650 nm Exp 3
West - Control	600.4	918.3	959.6	1113.0	1038.0	987.8	812.1
East - Treatment	527.9	837.4	1016.4	1206.2	1054.1	988.0	707.2
South - Lettuce	353.0	558.7	843.5	876.5	795.4	718.3	536.6

Figure 16. Cumulative DLI (mol m^{-2}) from Control Film (West), QD Film (East), and Lettuce (South) Folium Sensors from Seedling Uncovering through Final Harvest for four 625 nm and three 650 nm QD Film Experiments.

Near Infrared (NIR) wavelengths (greater than 800 nm) from sunlight were transmitted by both the control and the QD films into the greenhouse. Some of the sunlight in the blue region was been shifted by the QD films to the Near Infrared (NIR) region. However, this increase was not large for either QD film. The increase of NIR under the 625 nm QD film compared with the control film was 3% (from 28% under the control film to 41% under the treatment film, shown in Figure 17), and the increase under the 650 nm QD film compared with the control film was 2% (from 38% under the control film to 39% under the treatment film, shown in Figure 18).

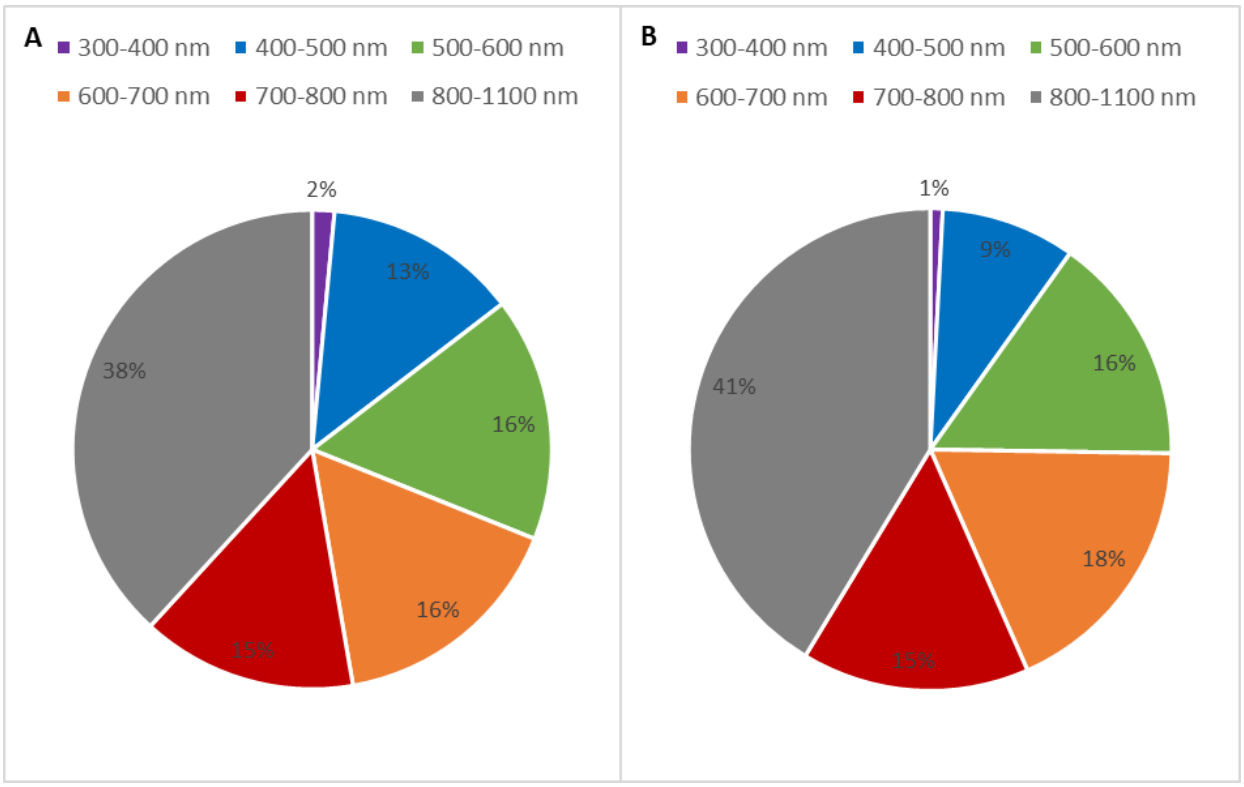


Figure 17. Proportion of Light in Each Waveband Including: Ultraviolet (300-400 nm), Blue (400-500 nm), Green/Yellow (500-600 nm), Orange/Red (600-700 nm), Far Red (700-800 nm), and NIR (800-1100 nm) as Measured Using a Spectroradiometer During 625 nm QD Film Experiments. (A) Proportions of Light Under Control Film. (B) Proportions of Light Under 625 nm QD Film.

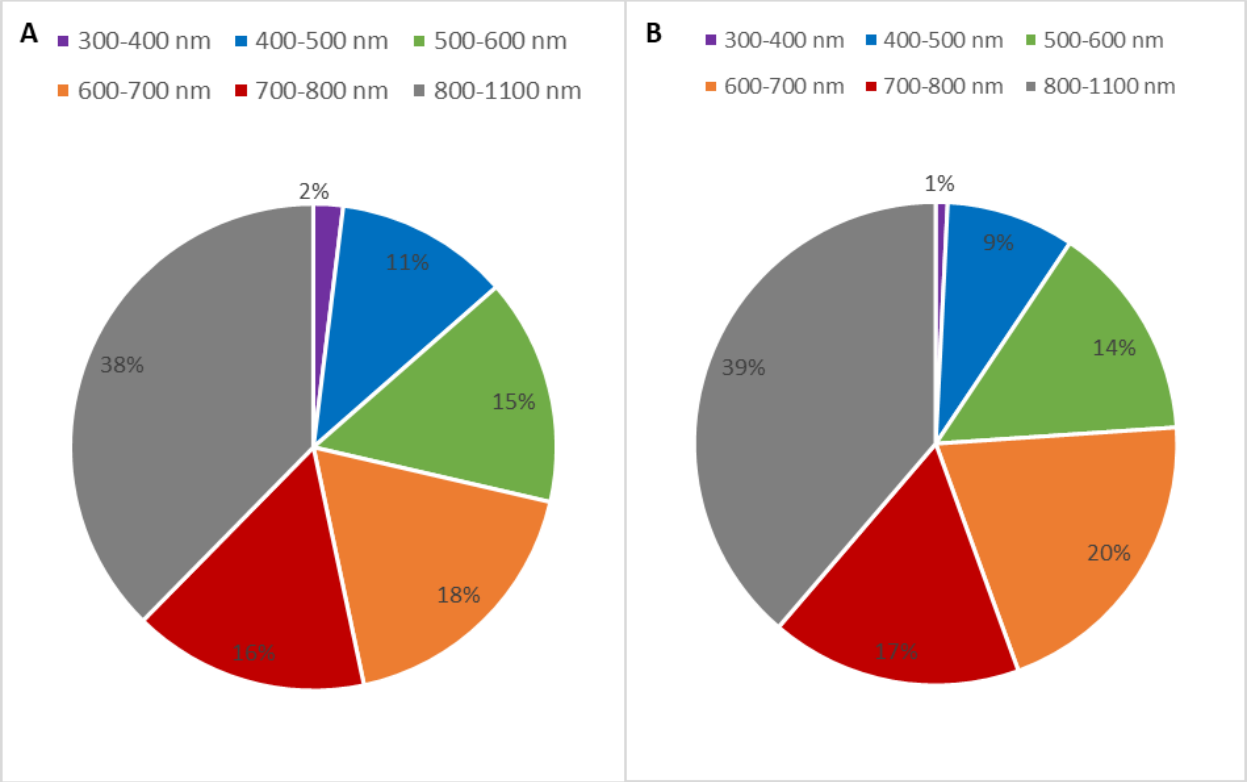


Figure 18. Proportion of Light in Each Waveband Including: Ultraviolet (300-400 nm), Blue (400-500 nm), Green/Yellow (500-600 nm), Orange/Red (600-700 nm), Far Red (700-800 nm), and NIR (800-1100 nm) as Measured Using a Spectroradiometer During 650 nm QD Film Experiments. (A) Proportions of Light Under Control Film. (B) Proportions of Light Under 650 nm QD Film.

This small increase in NIR likely did not have a substantial impact on leaf temperature or resulting transpiration, although leaf temperature was not measured during this experiment. Water use throughout each experiment was measured, and can be ascertained from the water depth in the DWC tanks (plots in Figure 19C through Figure 25C). The amount of water used by the plants for transpiration was not consistently different between the control and treatment groups for either of the QD test films.

3.1.1.3 Lettuce Root Zone Environment

Beginning at week 1 (transplant), the pH, EC, depth, and temperature of the nutrient solution in each of the four tanks were measured weekly using handheld sensors (Figure 19 through Figure 25).

The pH and EC were balanced to 6.0 ± 0.2 and 1.8 ± 0.1 dS/m, respectively, before transplant at each experiment, after which they were measured consistently throughout each experiment. They generally stayed constant, though there were some variations since they were not adjusted throughout the experiments. The depth of the nutrient solution decreased slowly in the beginning each experiment as plant growth was slower and water use was more gradual. Near the end of each experiment, the water use increased rapidly and the water level dropped quickly. The roots of the lettuce plants grew along with the water level decrease, so that by the time of the harvest, all plants were still able to access the nutrient solution.

Water temperature varied between experiments, depending on the season. Typically, measurements were taken at approximately the same time each week, but in some cases they were taken early or late in the day, causing lower temperatures for some measurements (Figure 23D, Figure 24D). Additionally, the water temperature sometimes differed between tanks on the same day as a result of measurement at a time of day when one side of the greenhouse experienced more direct sunlight than the other, heating one set of tanks over the other at that time of day (Figure 19D).

Consistency was found among the DWC control and treatment tanks within each experiment, as shown by the average value per tank measured during each experiment. For example, the tanks during 625 nm QD film Experiment 4 had average pH ranging from 6.1 to 6.3, EC from 1.8 to 1.9 dS m^{-1}, water temperature from 24.3 to 25.6 ℃, dissolved oxygen

content from 7.2 to 7.9 mg L^{-1}, and nutrient solution consumption measured as the change of water depth in the tank 2.8 to 3.2 cm. During 650 nm QD film Experiment 1, the tanks had average pH ranging from 6.4 to 6.8, EC from 1.8 to 1.9 dS m^{-1}, water temperature from 24.8 to 25.5 ℃, dissolved oxygen content from 6.2 to 6.9 mg L^{-1}, and nutrient solution consumption measured as the change of water depth in the tank x to xx. Average data for 625 nm Experiment 4 and 650 nm Experiment 1 is included in Appendix B Table B 2 and Table B 3.

During 625 nm Experiment 1, a leak was identified in tank C2; the roots were still able to reach the water, but the depth dropped faster and more in this tank than in the others (Figure 19C). The leak was repaired before beginning Experiment 2. At the start of 625 nm Experiment 2, an air stone with associated pump was added to each tank, and dissolved oxygen was measured. During 625 nm Experiment 3, the pump in tank C2 failed near the end of the experiment, causing the drop in dissolved oxygen content (Figure 21E) and was replaced, after which point the DO began to rise.

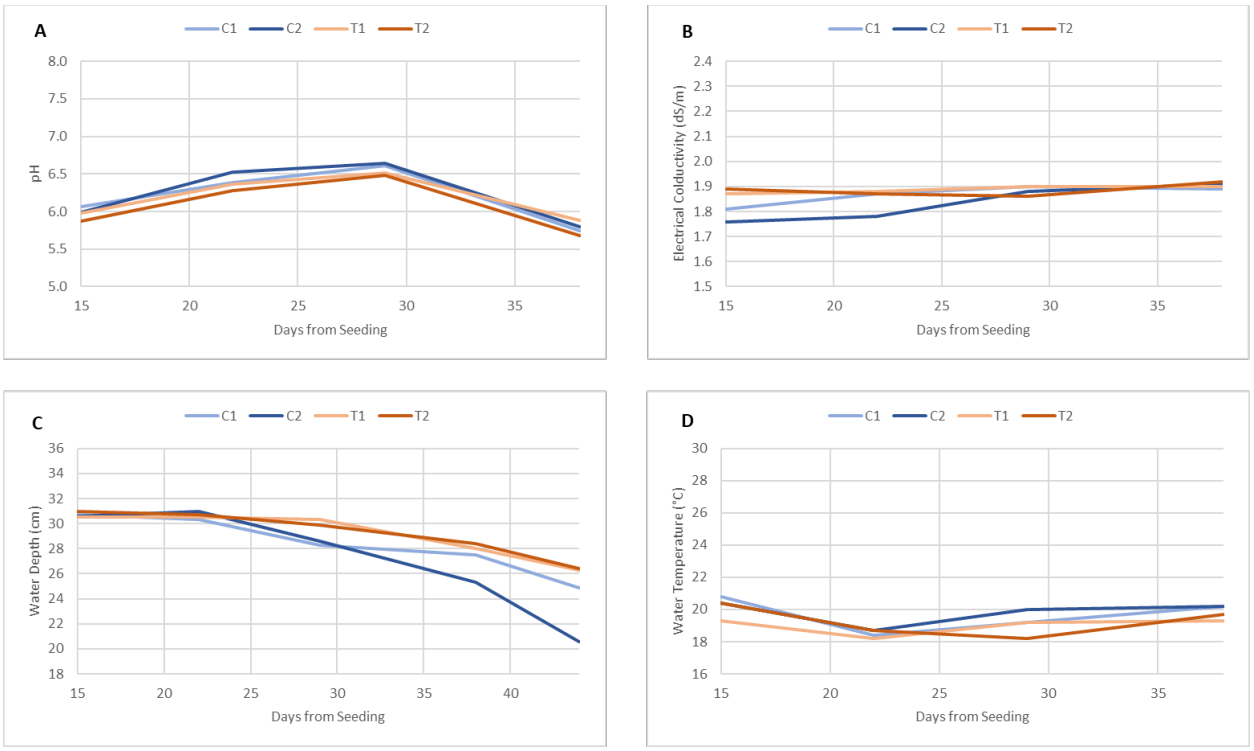


Figure 19. DWC Nutrient Solution Measurements during 625 nm QD Film Experiment 1. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C).

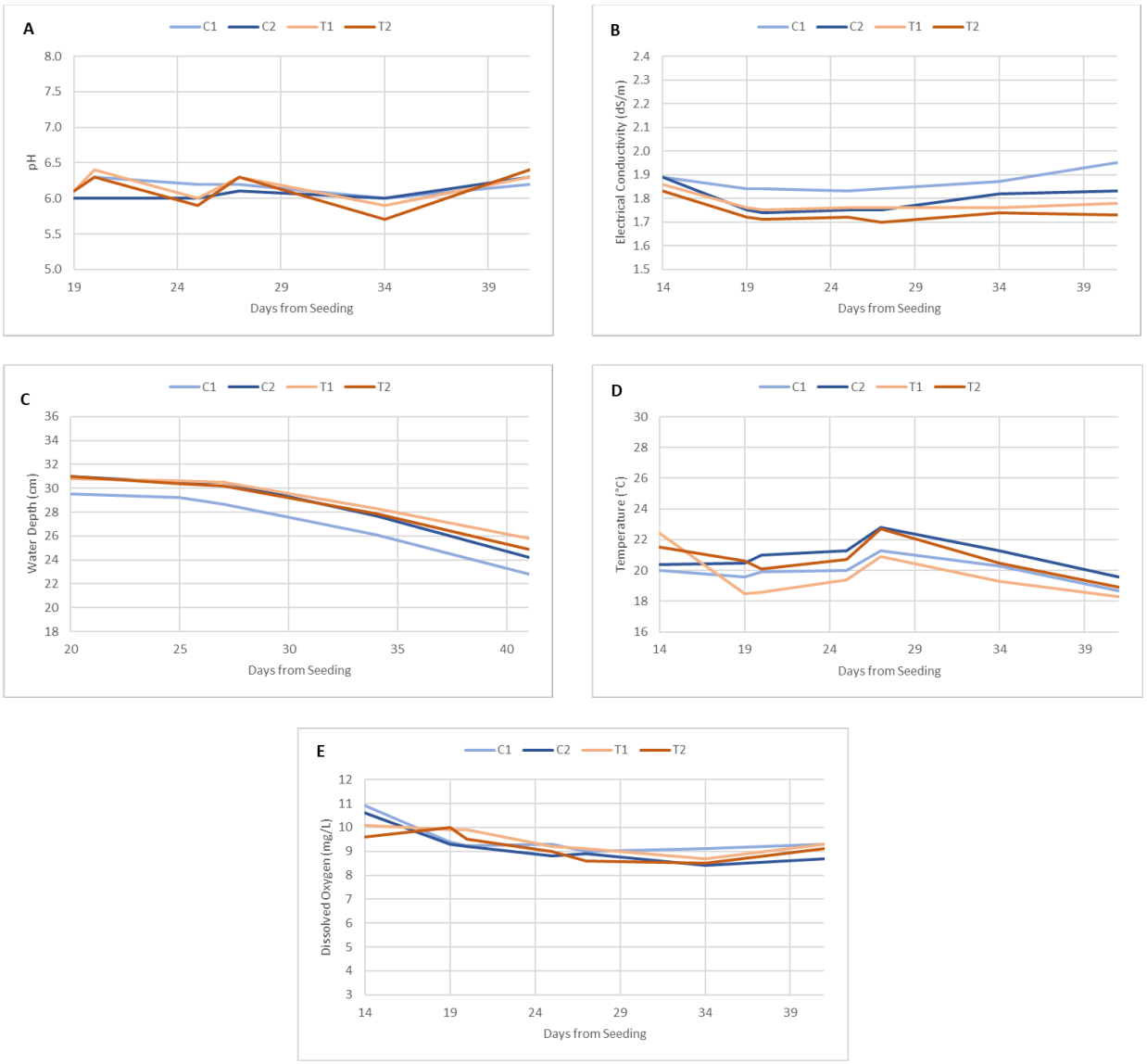


Figure 20. DWC Nutrient Solution Measurements during 625 nm QD Film Experiment 2. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C); I Dissolved Oxygen (mg/L).

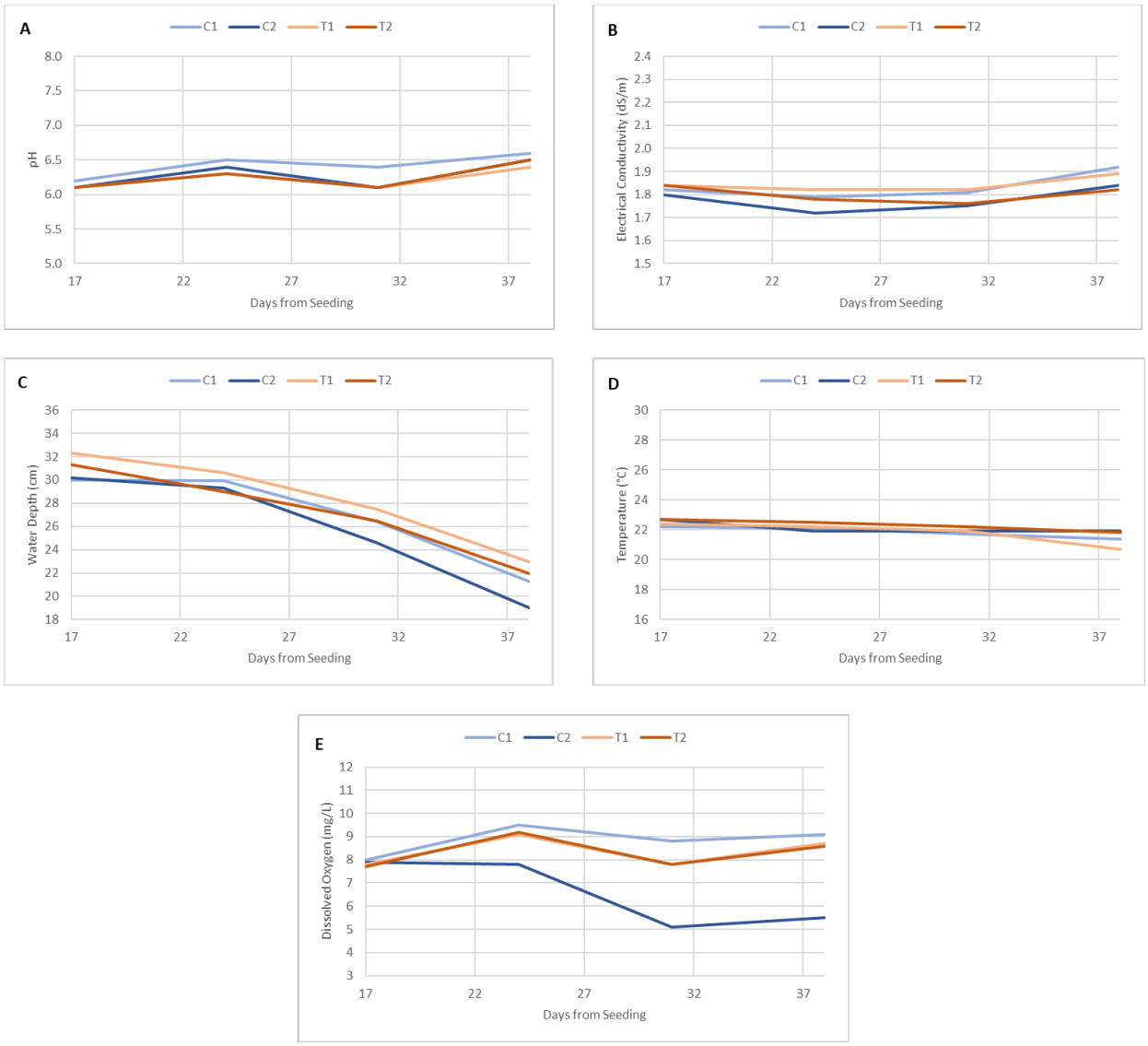


Figure 21. DWC Nutrient Solution Measurements during 625 nm QD Film Experiment 3. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C); (E) Dissolved Oxygen (mg/L).

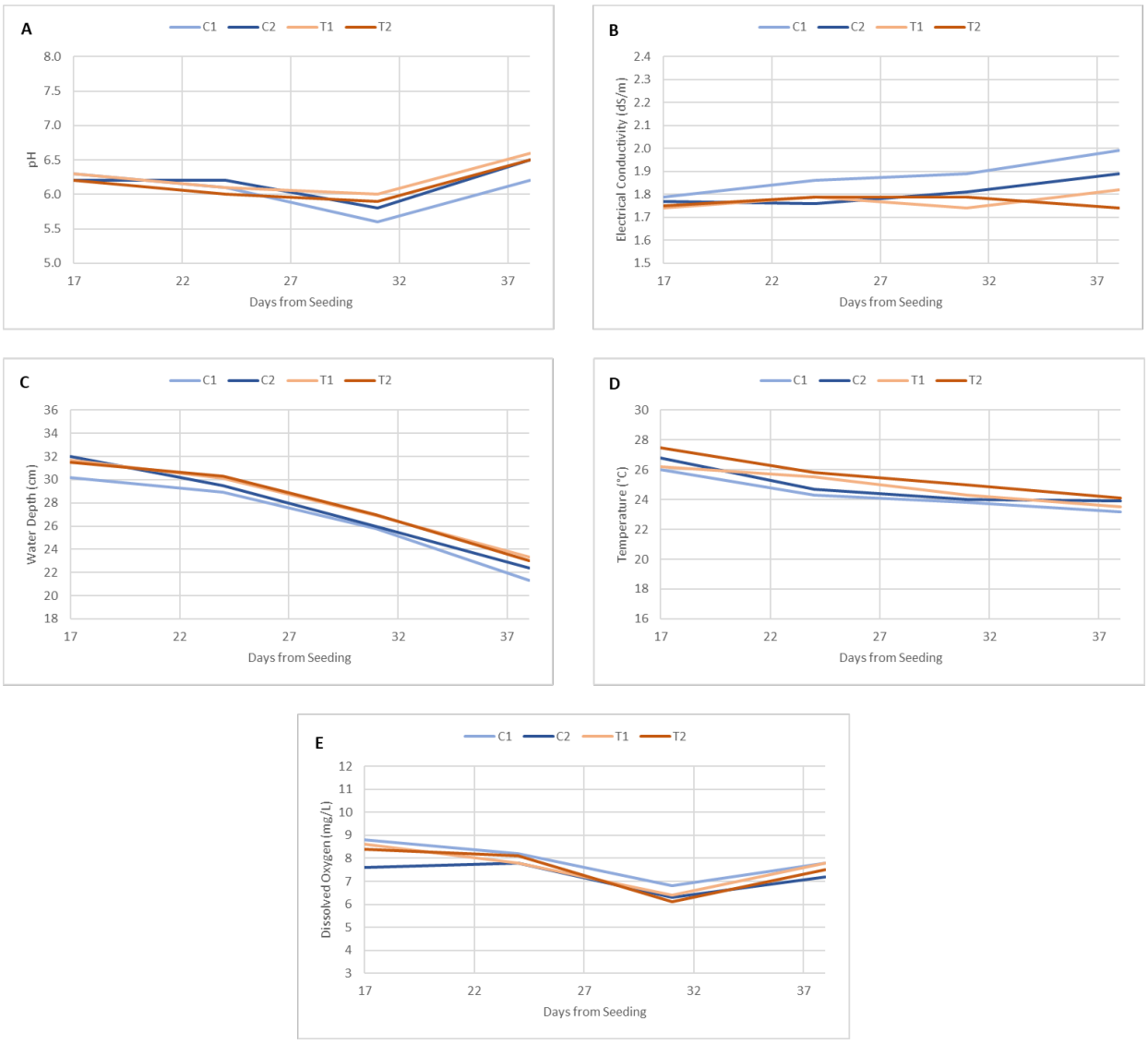


Figure 22. DWC Nutrient Solution Measurements during 625 nm QD Film Experiment 4. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C); (E) Dissolved Oxygen (mg/L).

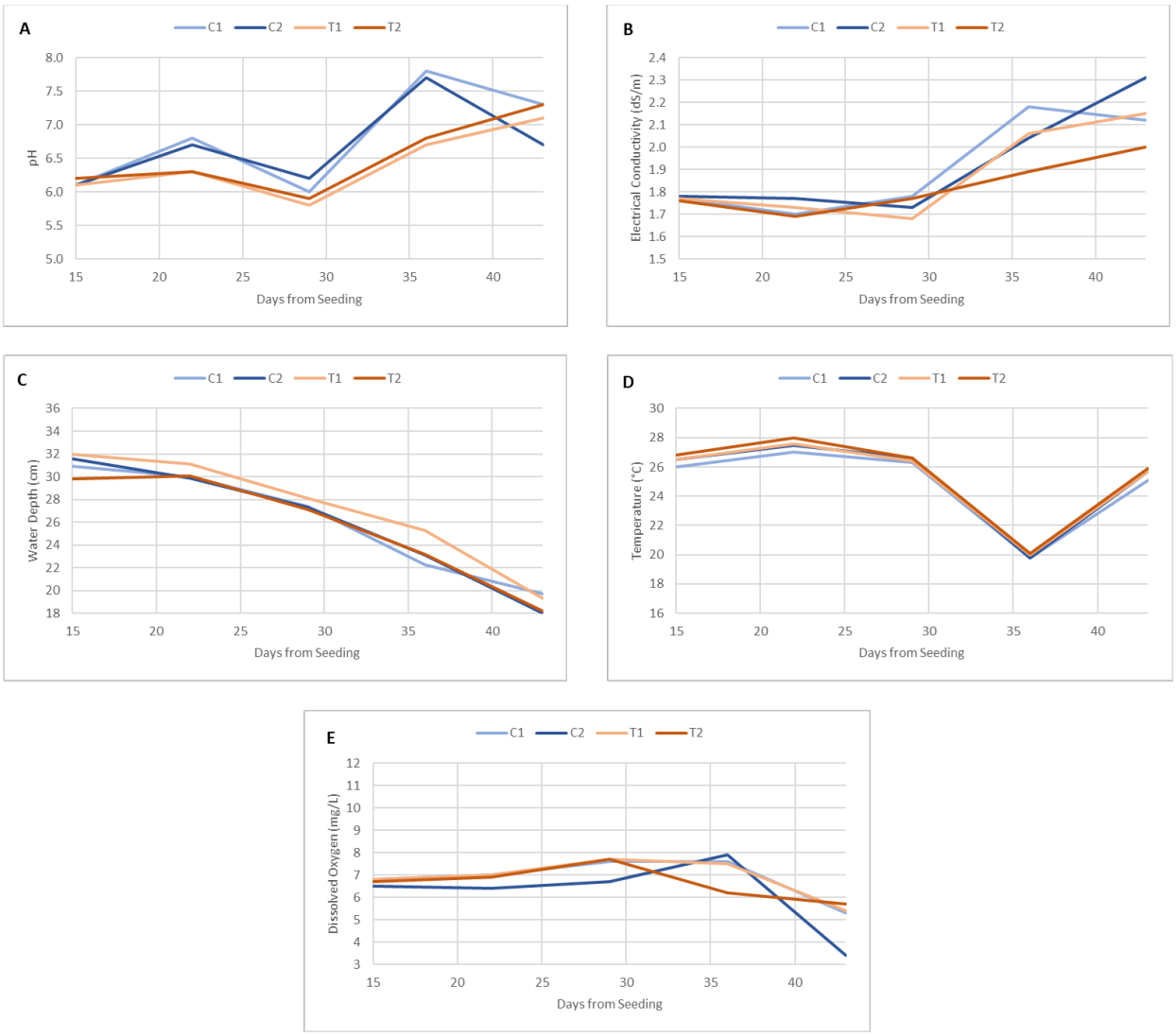


Figure 23. DWC Nutrient Solution Measurements during 650 nm QD Film Experiment 1. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C); (E) Dissolved Oxygen (mg/L).

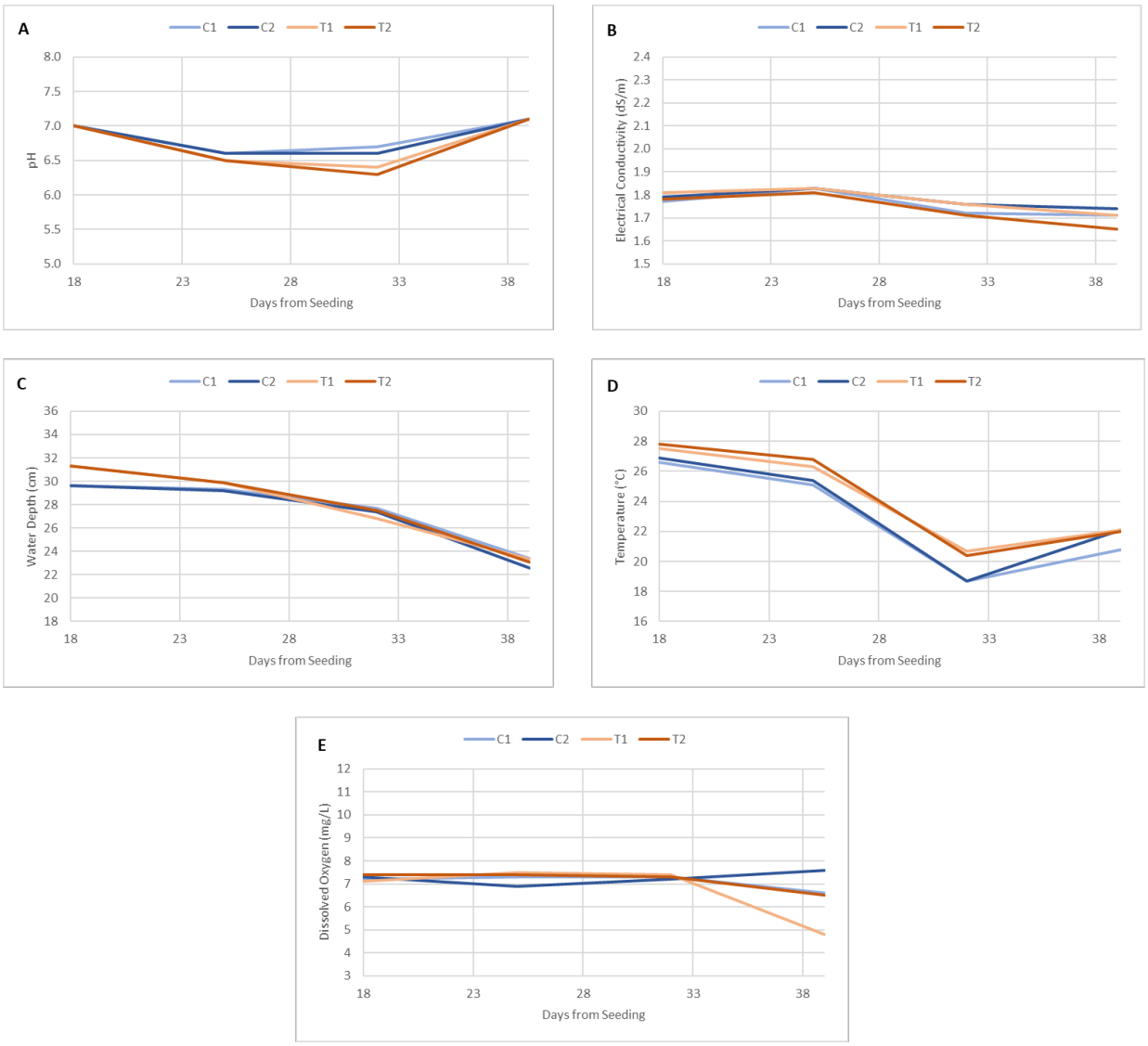


Figure 24. DWC Nutrient Solution Measurements during 650 nm QD Film Experiment 2. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C); (E) Dissolved Oxygen (mg/L).

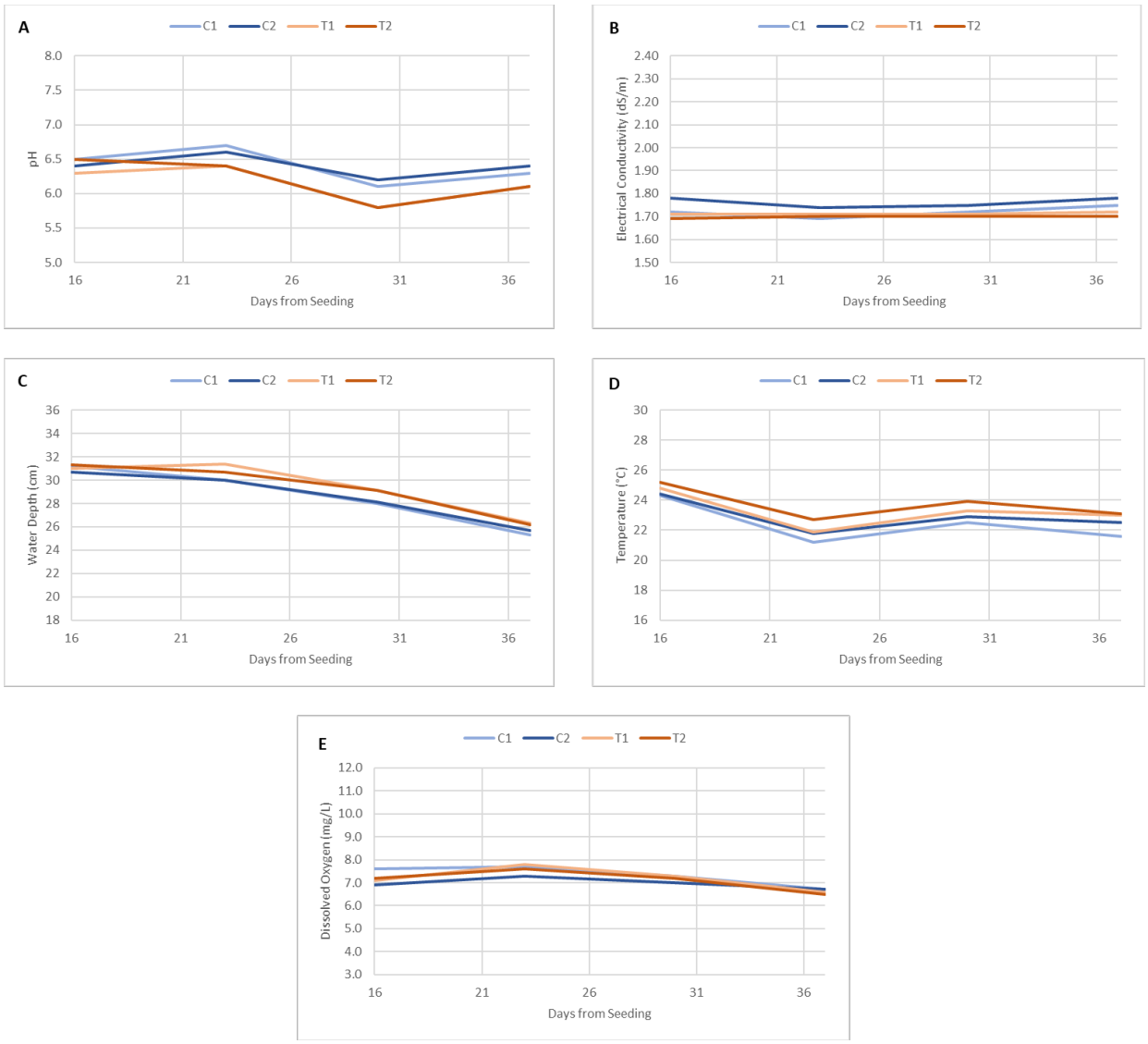


Figure 25. DWC Nutrient Solution Measurements during 650 nm QD Film Experiment 3. (A) pH; (B) Electrical Conductivity (EC, mS/cm); (C) Depth (cm); (D) Temperature (°C); (E) Dissolved Oxygen (mg/L).

3.1.2 Lettuce Plant Data Summary

3.1.2.1 Lettuce Thinning Data

At thinning, the average fresh weight per seedling was essentially equal between the control and the 625 nm QD treatment groups of lettuce (Table 4, Figure 26). There were no statistical design and analyses completed for this data, which was monitored only to determine

visual differences during germination caused by the QD films. There were no visual differences between the groups.

Table 4. Average Fresh Weight (g) of Thinned Lettuce Seedlings under Control and 625 nm QD Films.

	Control	625 nm	% Change from Control
Experiment 1 Avg	0.0096	0.0090	-6.3%
Experiment 2 Avg	0.0134	0.0131	-2.2%
Experiment 3 Avg	0.0127	0.0139	+9.4%
Experiment 4 Avg	0.0203	0.0189	-6.9%

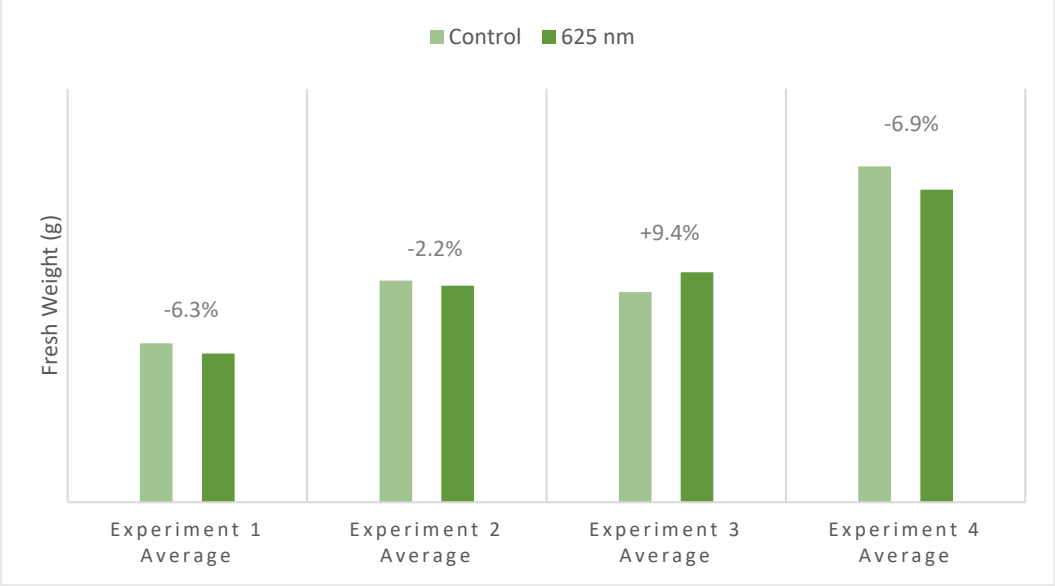


Figure 26. Average Fresh Weight (g) of Thinned Lettuce Seedlings (7 Days from Seeding) under Control and 625 nm QD Films.

During the 650 nm QD film experiments, there was no consistent trend of average seedling weight between the lettuce grown under the control and treatment films (Table 5, Figure 27). There was no observable visual difference in appearance, although color measurements were not collected during this measurement.

Table 5. Average Fresh Weight (g) of Thinned Lettuce Seedlings under Control and 650 nm QD Films.

	Control	650 nm	% Change from Control
Experiment 1 Avg	0.0297	0.0254	-14.5%
Experiment 2 Avg	0.0228	0.0257	+12.8%
Experiment 3 Avg	0.0195	0.0202	+3.8%

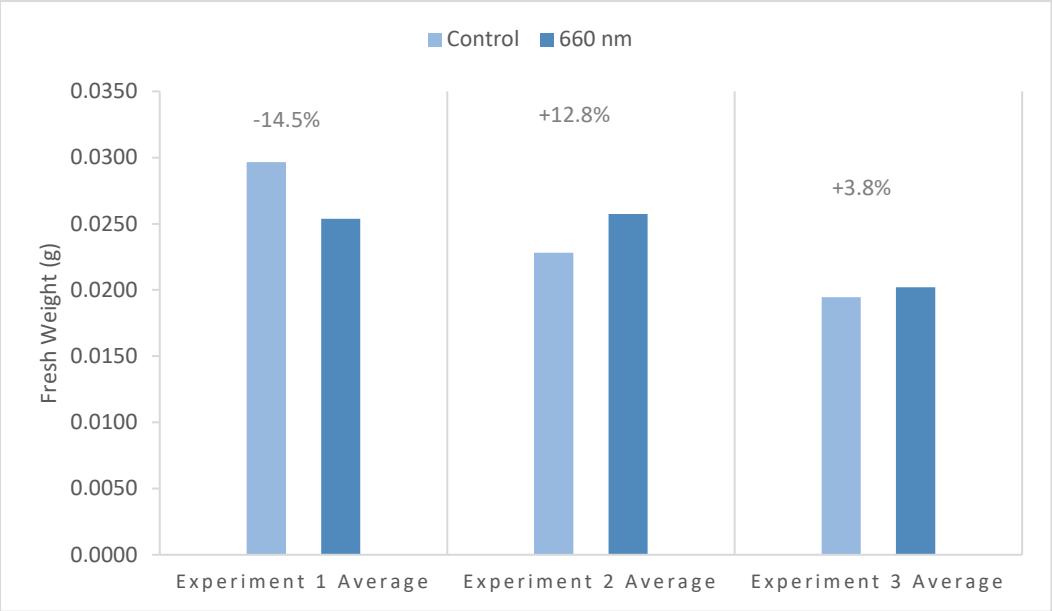


Figure 27. Average Fresh Weight (g) of Thinned Lettuce Seedlings (7 Days from Seeding) under Control and 650 nm QD Films.

3.1.2.2 Lettuce Data at Transplant

At the transplant stage of lettuce development during the 625 QD nm film experiment, the plants grown under the treatment generally had lower fresh and dry weights than those grown under the control film (Table 6, Figure 28, Figure 29). However, with the variability of the data, there would be no statistical differences, although a statistical analysis was not completed. The effect of season and the increase of natural light from Experiment 1 to Experiment 4 for the 625 nm QD film was clearly indicated by the increase of fresh and dry mass growth during a 14-day period. This was not the case for the 650 nm QD film experiments 1-3 because of the summer season when they were tested. The appearances of the seedlings grown under the treatment and

control films were not visually different, although no data was collected regarding their color during this experiment.

Table 6. Average and Standard Deviation of Lettuce Seedlings Fresh and Dry Weight at Transplant (14 days from seeding) under the 625 nm QD Films, (g)

	Control		625 nm film		% Change from Control	
	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh	Dry
Experiment 1 Avg	0.084	0.006	0.072	0.005	-14.3%	-16.7%
Std Deviation	0.013	0.001	0.009	0.001		
Experiment 2 Avg	0.127	0.011	0.101	0.008	-20.4%	-27.3%
Std Deviation	0.011	0.002	0.019	0.001		
Experiment 3 Avg	0.231	0.020	0.206	0.018	-10.8%	-10.0%
Std Deviation	0.018	0.002	0.019	0.001		
Experiment 4 Avg	0.325	0.029	0.255	0.026	-21.5%	-10.3%
Std Deviation	0.050	0.005	0.058	0.006		

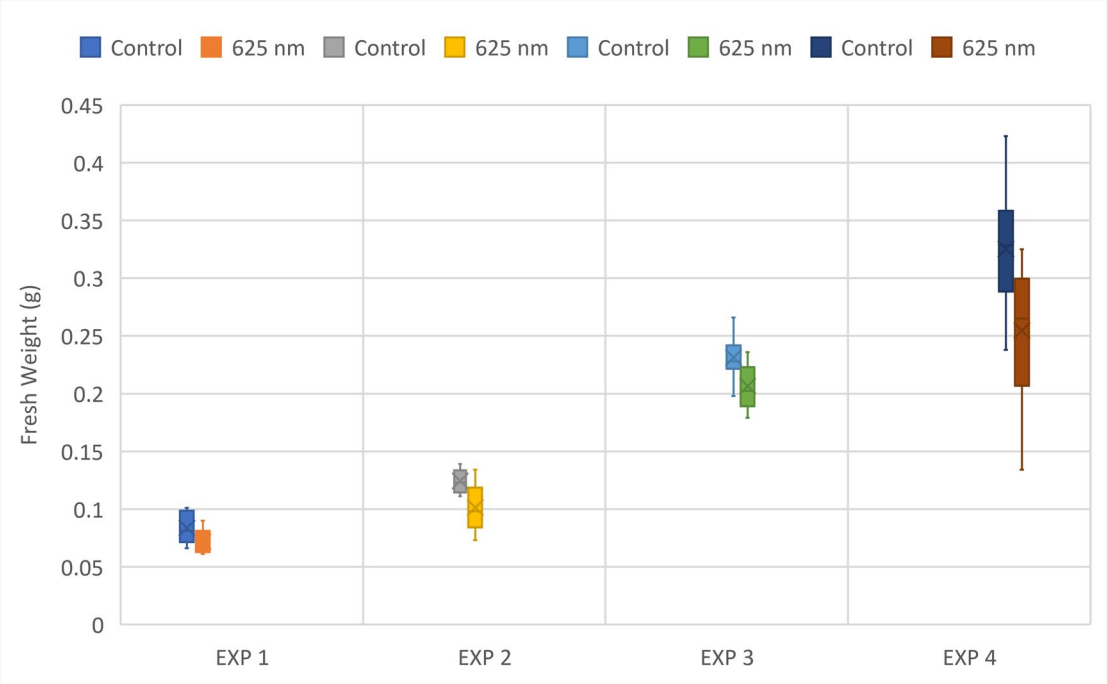


Figure 28. Average and Standard Deviation Fresh Weight (g) of Lettuce Seedlings at Transplant (14 days from seeding) under the Control and 625 nm QD Films.

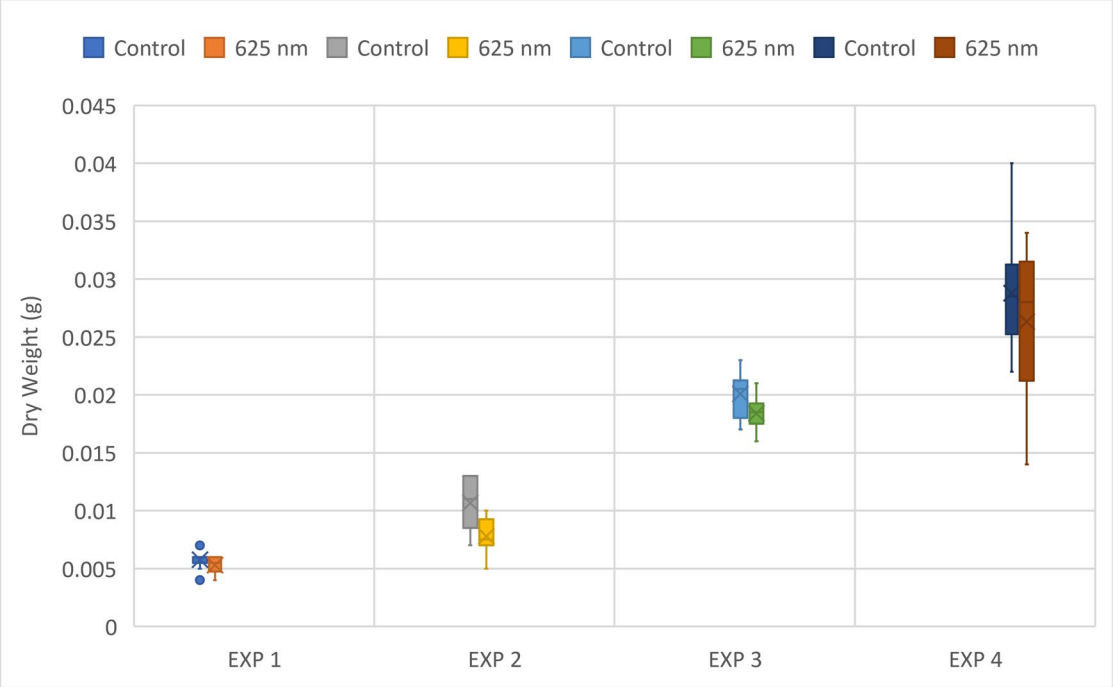


Figure 29. Average and Standard Deviation Dry Weight (g) of Lettuce Seedlings at Transplant (14 days from seeding) under the Control and 625 nm QD Films.

Under the 650 nm QD film and the control, the results at transplant were more varied than for the 625 nm QD film. At transplant during the first and second experiments, the lettuce seedlings grown under the treatment film had lower fresh weights than those grown under the control, but had higher dry weights. During the third experiment, the seedlings grown under the treatment film had a larger fresh weight than those grown under the control, and there was no difference in dry weight (Table 7, Figure 30, Figure 31).

Table 7. Average and Standard Deviation Fresh and Dry Weight (g) of Lettuce Seedlings at Transplant (14 days from seeding) for Control and 650 nm QD Films.

	Control		650 nm		% Change from Control	
	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh	Dry
Experiment 1 Avg	0.342	0.021	0.320	0.023	-6.4%	+9.5%
Std Deviation	0.060	0.004	0.066	0.003		
Experiment 2 Avg	0.272	0.018	0.238	0.019	-12.5%	+5.6%
Std Deviation	0.050	0.003	0.039	0.003		
Experiment 3 Avg	0.289	0.023	0.307	0.023	+6.2%	+0.0%
Std Deviation	0.031	0.003	0.042	0.003		

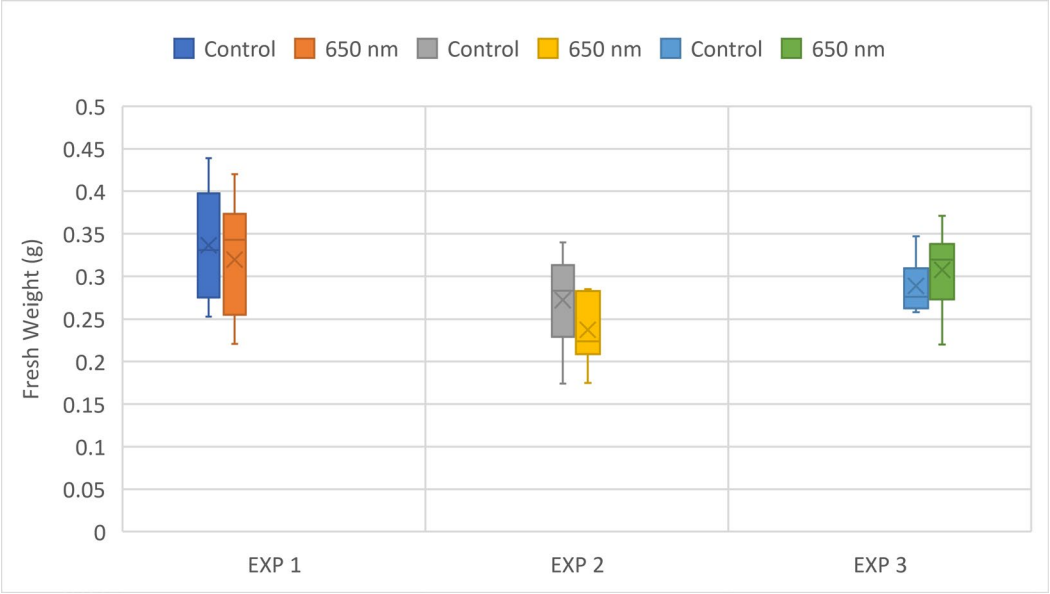


Figure 30. Average and Standard Deviation Fresh Weight (g) of Lettuce Seedlings at Transplant (14 days from seeding) under the Control and 650 nm QD Films.

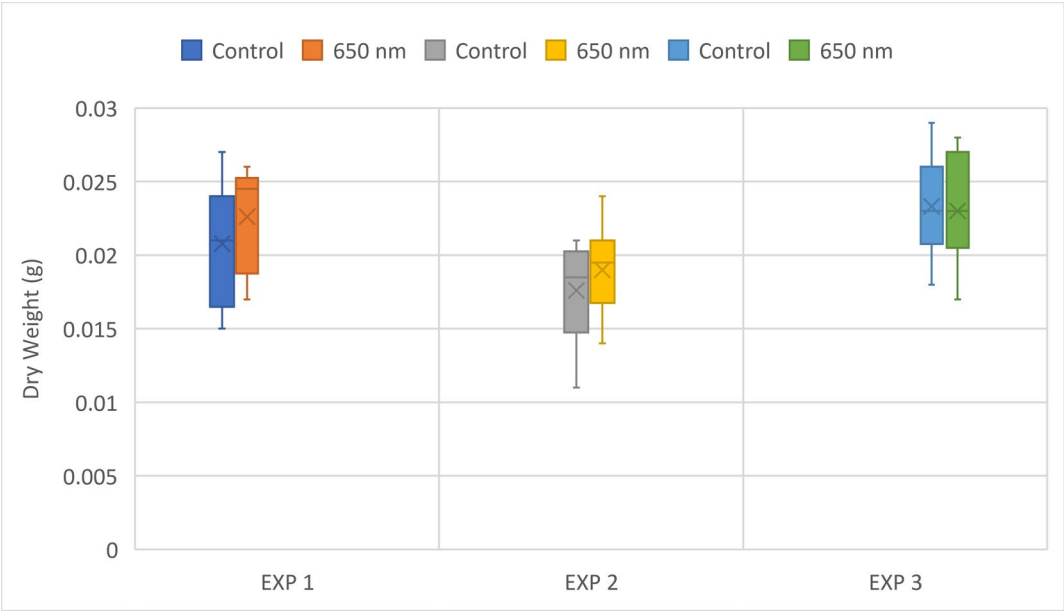


Figure 31. Average and Standard Deviation Dry Weight (g) of Lettuce Seedlings at Transplant (14 days from seeding) under the Control and 650 nm QD Films.

3.1.2.3 Lettuce Harvest Data Statistical Analysis Summary

An overview of the results from the ANOVA analysis for the 625 nm film treatment has been summarized in Table 8 and for the 650 nm QD film in Table 9. Highlighted cells indicate statistical significance at a 95% confidence interval. Many measured plant parameters were

statistically significant for both QD films, with some differences. Fresh and dry biomass and leaf area were not statistically significant for the 625 nm QD film, but were significant for the 650 nm QD film for the initial harvest. Whereas all parameters except leaf length and LUE for the final harvest of the 650 nm QD film were statistically significant for DLI for both QD films, the interaction with DLI was only significant during the lower light conditions of the winter and spring for the initial harvest.

Table 8. Summary of Statistical Significance (Based on P-value >0.05) for 2-Factor ANOVA Test of 625 nm Film and DLI for Each of the Plant Parameters for Initial Harvest (23 Days from Transplant) and Final Harvest (30 Days from Transplant) for Experiments 1 – 4 Combined. Highlighted Cells Indicate Statistical Significance at 95% Confidence Interval.

	2-Factor Factorial Test p-values for 625 nm Film			
	Variable	625 nm Film	DLI	Interaction (DLI, Film)
At Initial Harvest	Fresh Weight	0.2618	<0.0001	<0.0001
	Dry Weight	0.0821	<0.0001	<0.0001
	Leaf Count	<0.0001	<0.0001	0.0002
	Leaf Length	<0.0001	<0.0001	<0.0001
	Leaf Area	0.2296	<0.0001	<0.0001
	Relative LUE	0.0083	<0.0001	0.0022
At Final Harvest	Fresh Weight	<0.0001	<0.0001	0.0002
	Dry Weight	0.0305	<0.0001	0.2669
	Leaf Count	<0.0001	<0.0001	0.0835
	Leaf Length	0.1895	<0.0001	<0.0001
	Relative LUE	<0.0001	<0.0001	0.0974

Table 9. Summary of Statistical Significance (Based on P-value >0.05) for 2-Factor ANOVA Test of 650 nm Film and DLI for Each of the Plant Parameters for Initial Harvest (23 Days from Transplant) and Final Harvest (30 Days from Transplant) for Experiments 1 – 3 Combined. Highlighted Cells Indicate Statistical Significance at 95% Confidence Interval.

	2-Factor Factorial Test p-values for 650 nm Film			
	Variable	650 nm Film	DLI	Interaction (DLI, Film)
At Initial Harvest	Fresh Weight	<0.0001	<0.0001	0.0921
	Dry Weight	<0.0001	<0.0001	0.872
	Leaf Count	<0.0001	<0.0001	0.6736
	Leaf Length	<0.0001	<0.0001	0.3114
	Leaf Area	<0.0001	<0.0001	0.0567
	Relative LUE	<0.0001	<0.0001	0.6252
At Final Harvest	Fresh Weight	0.0001	<0.0001	0.7937
	Dry Weight	0.0066	<0.0001	0.703
	Leaf Count	0.0002	<0.0001	0.038
	Leaf Length	0.0625	0.9897	0.0567
	Relative LUE	<0.0001	0.5279	0.2807

3.1.2.4 Lettuce Data at Harvest

For the 625 nm QD film experiments, a summary table is included for fresh weight, dry weight, and relative LUE (Table 10). The percent change from the control data group to the QD film group is included in each table and on each plot.

During the seasonal limited sunlight conditions in Experiments 1 and 2, the 625 nm QD film seedlings at transplant had significantly lower fresh and dry weight than the control, but by harvest they were equivalent to the control. However, the 625 nm QD film seedlings grew to a significantly larger fresh and dry weight at transplant and at harvest when sunlight conditions increased in Experiments 3 and 4.

For Experiment 1 and Experiment 2, the DLI was ~10% lower for the treatment plants than for the control plants. During this time, the fresh and dry weights of the initial harvests were lower under the 625 nm QD film than under the control, although only the Experiment results

were statistically significant. For the final harvests of Experiments 1 and 2, the difference in fresh weight and dry weight was minimal and not statistically significant, even with the lower DLI.

For 625 nm QD film Experiments 3 and 4, the fresh and dry weight were consistently greater than the control, ranging in yield differences of 18 – 41% for fresh weight and 15 – 31% for dry weight. These differences were shown to be statistically significant at both harvests, with the only exceptions of dry weights at Experiments 2 and 4 for both harvests, and Experiment 1 for the final harvest. In some cases, like the second harvest of Experiment 2, the fresh weight improved while the dry weight did not significantly change. In these cases, more water was held by the leaf, while the same amount of dry weight was accumulated.

The DLI did shift in favor of the QD film over the control during the greater light and warmer days, with an average DLI increase of +4.4% (Experiment 3) and +4.2% (Experiment 4) for the treatment side compared to the control. The relative LUE was observed to be greater for the 625 nm QD film seedlings for nearly all experiments.

Table 10. Average Initial and Final Harvest Fresh Weight (g), Dry Weight (g), Relative LUE (g/mol), and Percent Differences for Experiments 1 – 4 under Control and 625 nm QD Film Treatments.

			Fresh Weight (g)			Dry Weight (g)			Relative LUE (g/mol)		
	Avg DLI	Harvest	Control	625 nm	% diff	Control	625 nm	% diff	Control	625 nm	% diff
E1	8.7	Initial	28.1	22.9	-19%	1.6	1.3	-19%	0.034	0.032	-6%
	9.0	Final	74.7	72.5	-3%	3.9	4.0	3%	0.070	0.078	11%
E2	11.7	Initial	65.1	60.0	-8%	2.7	2.6	-4%	0.048	0.049	2%
	14.3	Final	130.2	136.3	5%	5.4	5.4	0%	0.080	0.092	15%
E3	21.2	Initial	101.7	121.2	19%	4.4	5.7	30%	0.047	0.054	14%
	21.6	Final	141.2	198.9	41%	6.9	8.1	17%	0.065	0.087	34%
E4	12.9	Initial	118.0	143.2	21%	3.7	4.3	16%	0.058	0.066	22%
	13.1	Final	172.5	203.5	18%	7.7	8.8	14%	0.088	0.095	9%

The lettuce grown under the 650 nm QD film generally had increased fresh weight, dry weight, leaf count, and relative LUE than the lettuce grown under the 625 nm film. This was true for the initial and final harvest, and, unlike the 625 nm QD film experiments, this remained consistent among the experiments. This may be due to the relatively similar light conditions among the 650 nm QD film experiments, since these experiments were all conducted during the summer with nearly equal DLI. The relative LUE of the lettuce per experiment, like the fresh and dry weight, showed consistent improvement under the 650 nm film compared with the control film.

Table 11. Average Fresh Weight (g), Dry Weight (g), and Relative LUE (g/mol) for Experiments 1 – 3 under 650 nm Film.

			Fresh Weight (g)			Dry Weight (g)			Relative LUE (g/mol)		
	Avg DLI	Harvest	Control	650 nm	% diff	Control	650 nm	% diff	Control	650 nm	% diff
E1	19.9	Initial	125.3	141.9	13%	4.6	5.6	23%	0.066	0.087	31%
	19.4	Final	150.6	177.8	18%	6.5	7.7	19%	0.082	0.095	16%
E2	17.6	Initial	92.4	115.1	25%	3.8	5.0	30%	0.054	0.066	22%
	17.5	Final	148.5	173.7	17%	6.1	7.2	18%	0.085	0.099	17%
E3	12.9	Initial	68.1	80.8	19%	2.6	3.1	20%	0.046	0.064	39%
	13.1	Final	115.7	133.5	15%	4.7	5.4	14%	0.081	0.107	33%

Plant response data have been combined with graphs for all experiments and film treatments to allow comparison of the absolute and statistical differences among treatments and the seasonal effects of DLI and VPD (Figure 32 through Figure 37).

The 625 nm film was a significant factor in the fresh weights of the initial harvest, where statistical significance is defined as a p-value less than 0.05. However, both the DLI and the interaction between the 625 nm film and the DLI were statistically significant, implying that there is potential for the film to be more effective at increased DLIs.

With respect to the three experiments under the 650 nm film, both the film and the DLI are significant factors impacting the fresh weight at the initial harvest, but the interaction between the film and the DLI was not significant. At the final harvests, the 650 nm QD film and the DLI are both statistically significant factors; again, however, the interaction between the treatment and the DLI was not significant.

At the final harvest, the 625 nm film, along with the DLI and the interaction between the DLI and the 625 nm film, had a significant effect on the fresh mass. This implied that the QD film had a statistically significant effect on fresh weight, but that the effect of the film was affected by the amount of DLI received during the growth period. The lettuce grown under the

625 nm film was increasingly bigger, on average, than that grown under the control film. As expected, higher light levels grew larger plants, but also, more interestingly, the film was more effective for growing larger plants during higher light periods.

Figure 32. (A) Fresh Weight (g) at Initial Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. (B) Fresh Weight (g) at Final Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. Average Daily VPD and DLI Indicated for Each Experiment. (*Indicates significant differences of control and treatment means within each experiment).

The results of the ANOVA test showed that only the DLI and the interaction between the 625 nm QD film and the DLI were each statistically significant factors affecting dry weight at the first harvest. While DLI changed the dry weight significantly, the plants under the 625 nm film frequently had greater dry masses than those under the control film. At the final harvest, the

625 nm QD film and the DLI were both statistically significant factors impacting dry weight, although their interaction was not.

At both the initial and final harvests, the 650 nm QD film was a statistically significant factor impacting dry weight. Plants grown under the 650 QD film had a larger dry weight than those grown under the control film. This was consistent throughout the 650 nm QD film experiments. DLI was also a statistically significant factor, but the interaction between the treatment and DLI was not significant, indicating that while higher DLIs caused an increase in dry weight, the film was equally effective under a higher light environment.

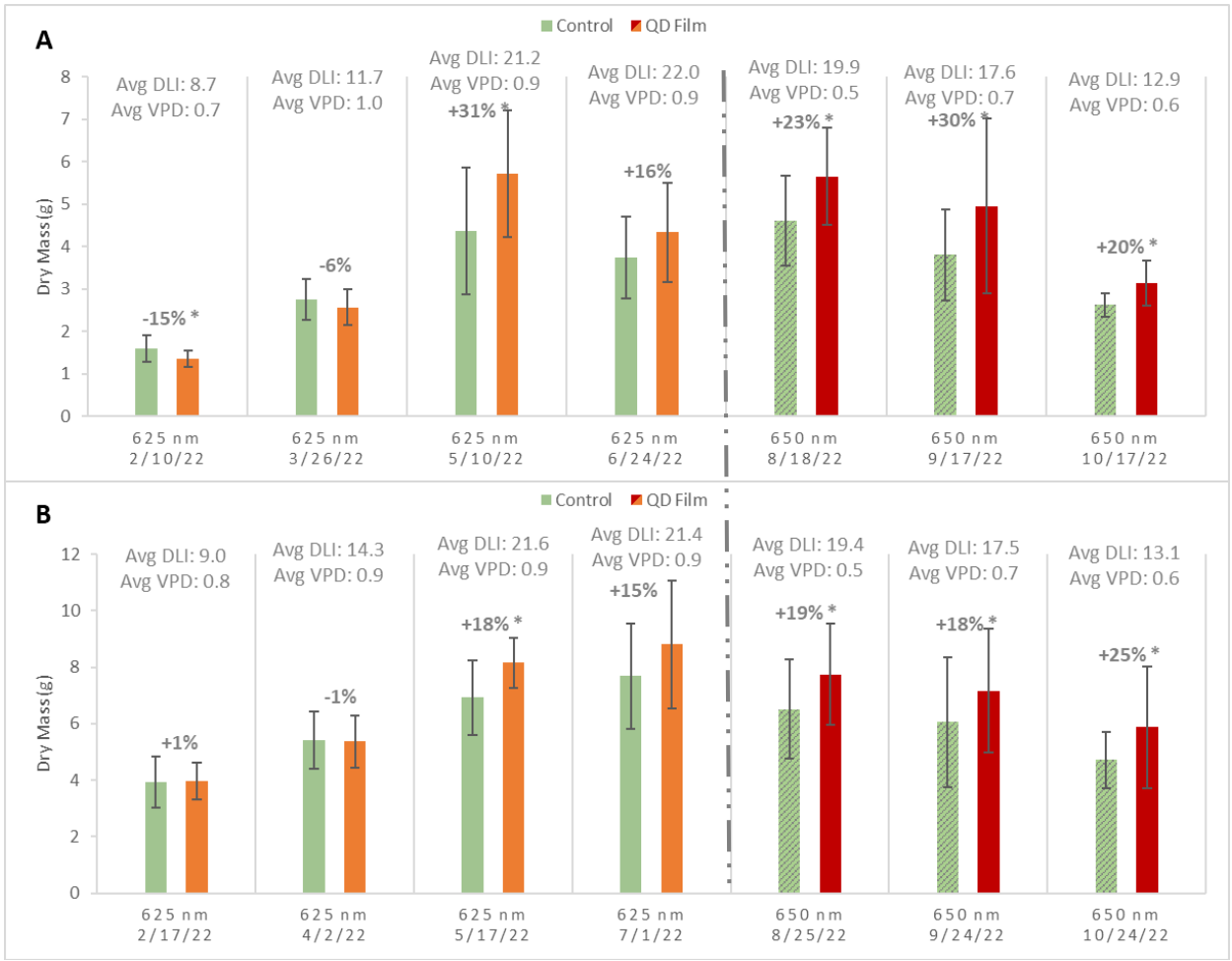


Figure 33. (A) Dry Weight (g) at Initial Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. (B) Dry Weight (g) at Final Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. Average Daily VPD and DLI

Indicated for Each Experiment. (*Indicates significant differences of control and treatment means within each experiment).

Leaf count and leaf length data was not collected at the final harvest for the second experiment under the 650 nm QD film, since the high DLI and temperature caused significant elongation and subsequent wilting after the initial harvest, so that data was not usable.

The seasonal effect can also be seen in the morphological data. The leaf count increased under each film during all trials as a result of increased DLI, although the increase was not statistically significant for 625 nm Experiments 1 or 2. The 625 nm QD film and the DLI each had a statistically significant impact on the leaf count at each harvest. The crops grown under the 625 nm QD film consistently had higher leaf counts, as did crops grown in higher light conditions. At the initial harvest, the interaction between the 625 nm QD film and the DLI was significant, indicating that the film was more effective in a higher light environment. At the final harvest, the interaction was not a statistically significant factor, signifying that the film was not more or less effective in a higher light environment.

The impact on leaf count of the 650 nm quantum dot film and the DLI are both statistically significant factors increasing the leaf count at each harvest. Again, increased light conditions and the treatment film each contributed to a greater leaf count. There was no statistically significant interaction between the DLI and the treatment film at the initial harvest, but there was a significant interaction between them at the final harvest. Therefore, when the plants were harvested earlier, the amount of light did not impact the effectiveness of the film at increasing leaf count, but when they were harvested seven days later, the film created more leaves at higher DLIs.

Figure 34. (A) Leaf Count at Initial Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. (B) Leaf Count at Final Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. Average Daily VPD and DLI Indicated for Each Experiment. (*Indicates significant differences of control and treatment means within each experiment).

For leaf length at the initial harvests, the effects 625 nm QD film, the DLI, and the interaction between them were statistically significant. However, in this case, the control film produced a greater leaf length than the 625 nm film, regardless of DLI. At the final harvests, while the 625 nm QD film did not have a significant effect on leaf length, the DLI and the interaction between the 625 QD film and the DLI did have significant effects.

At the initial harvests during the 650 nm QD film experiments, the QD film and the DLI were both significant factors affecting the length of the longest leaf, but the interaction between them

was not. By the final harvest, none of these factors had a statistically significant impact on the length of the longest leaf. One possible explanation is that the sample size for the 650 nm QD film experiments at the final harvests was smaller than others, since leaf length data was not collected for 650 nm QD film Experiment 2.

Figure 35. (A) Longest Leaf Length (mm) at Initial Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. (B) Longest Leaf Length (mm) at Final Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. Significant Differences Indicated with an Asterisk (*). Average Daily VPD and DLI Indicated for Each Experiment. (*Indicates significant differences of control and treatment means within each experiment).

Leaf area calculations were performed for the initial harvests only. The effect on leaf area of the 625 nm treatment film was not statistically significant. The effect of the DLI and the interaction effect between the film and the DLI were statistically significant. Both the 650 nm

QD film and the DLI had a statistically significant effect on leaf area, but the interaction effect between the film and the DLI was not significant.

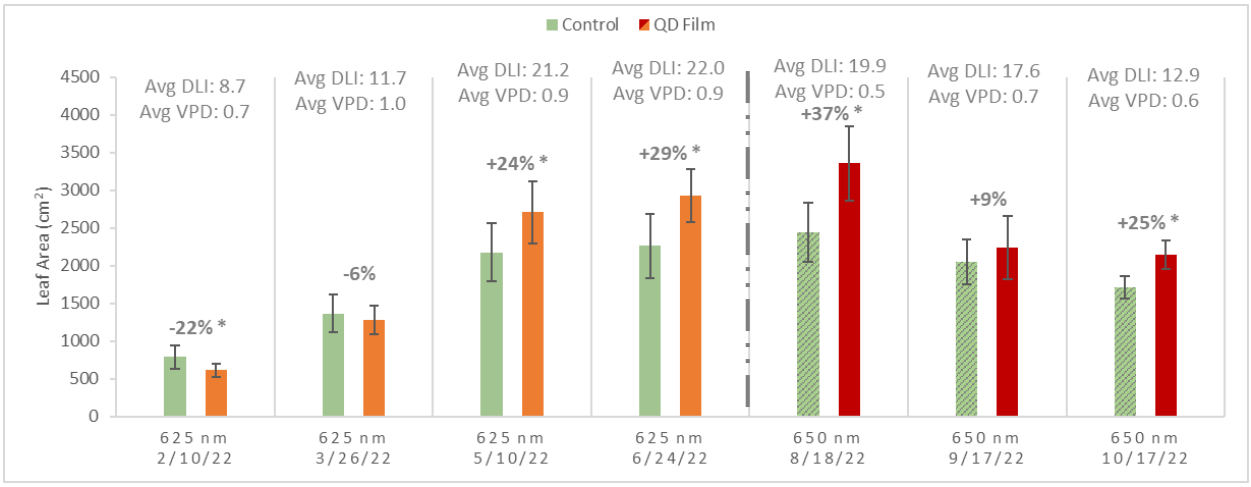


Figure 36. Leaf Area (cm^2) of Lettuce Plants at Initial Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. Average Daily VPD and DLI Indicated for Each Experiment. (*Indicates significant differences of control and treatment means within each experiment).

The relative fresh mass LUE was statistically significantly different for the initial harvest in the 625 nm QD film experiments compared to the control for the treatment film, the DLI, and the interaction between the film and the DLI. The 625 nm QD film provided greater LUE overall, and the LUE resulting from combinations of the 625 nm QD film and increased DLI were generally greater than those produced by the reference film and/or lower DLIs.

Both the 625 nm film and the DLI were significant factors for increasing the LUE at the final harvest. However, their interaction was not statistically significant.

The 650 nm QD film caused a statistically significant increase of the relative fresh mass LUE at both the initial and final harvests. The increase caused by the DLI was statistically significant at the initial harvest, but not at the final. The interaction between the 650 nm QD film was not statistically significant for either harvest.

At both the initial and final harvests, both the 625 nm and the 650 nm QD films caused statistically significant increases in the relative LUE of lettuce plants compared with the control films.

Figure 37. (A) Relative LUE (g/mol) at Initial Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. (B) Relative LUE (g/mol) at Final Harvest of 625 nm QD Film Experiments 1 – 4 and 650 nm QD Film Experiments 1 – 3. Average Daily VPD and DLI Indicated for Each Experiment. (*Indicates significant differences of control and treatment means within each experiment).

3.1.2.5 Combined 625 nm and 650 nm QD Film Analysis

Because the 625 nm and 650 nm QD film experiments were not run simultaneously, they did not experience the same cumulative DLI, and therefore could not be analyzed using the same ANOVA process as was used previously. Therefore, an ANCOVA test was conducted, using the

model indicated by Equation 3. This produced the covariance plot in Figure 38 for fresh weight at the initial harvest (23 days from transplant). This allowed for extrapolation of the plant response data at the DLIs that were tested to the potential results that may have been observed at other higher or lower DLI.

From Figure 38 and Figure 39, the relative efficacies of the two treatment films and the control film may be compared, along with interactions of each film with the control film. They show the fresh weight of lettuce grown at each film and each DLI, and project the fresh weight to higher and lower DLIs than were tested.

By the initial harvests, the plants grown under the 650 nm QD film, were always bigger than those grown under the 625 nm QD film at the tested DLI values, and are projected to still be larger at higher and lower DLIs than tested. Although plants grown under each of the QD films were larger than those grown under the control film at increased light conditions, the plants grown under the control film had greater fresh weights than those grown under either QD film at much lesser DLIs. This interaction effect, indicated where the lines in Figure 38 intersect, occurs at a lower DLI under the 650 nm QD film than the 625 QD nm film, indicating that the 650 nm QD film may be more effective under lower light conditions than the 625 nm QD film. This prediction would have to be validated by further testing.

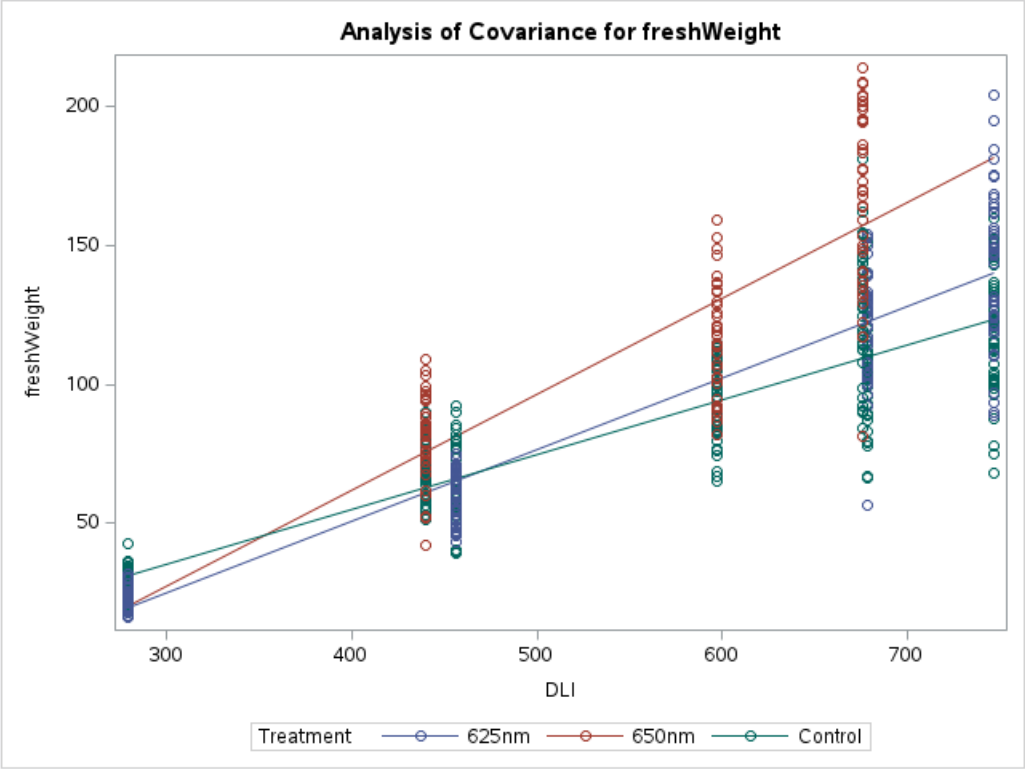


Figure 38. Projected Fresh Weight Trends by an Analysis of Covariance for Initial Harvests of Lettuce Plants Grown at Various DLI Under 625 nm and 650 nm QD Films and Control Film.

By the final harvests, a slightly different pattern can be observed (Figure 39). The 650 nm QD film was predicted to not have an interaction effect with the control film, meaning that plants grown under the 650 nm QD film were predicted to always have a greater fresh weight than those grown under the control film. The 625 nm QD film has been demonstrated to produce smaller plants than the control film at very low DLIs, but the ANCOVA analysis extrapolates that at very high cumulative DLIs, plants grown under the 625 nm QD film will be larger than those grown under the 650 nm QD film. This prediction would have to be validated by further testing.

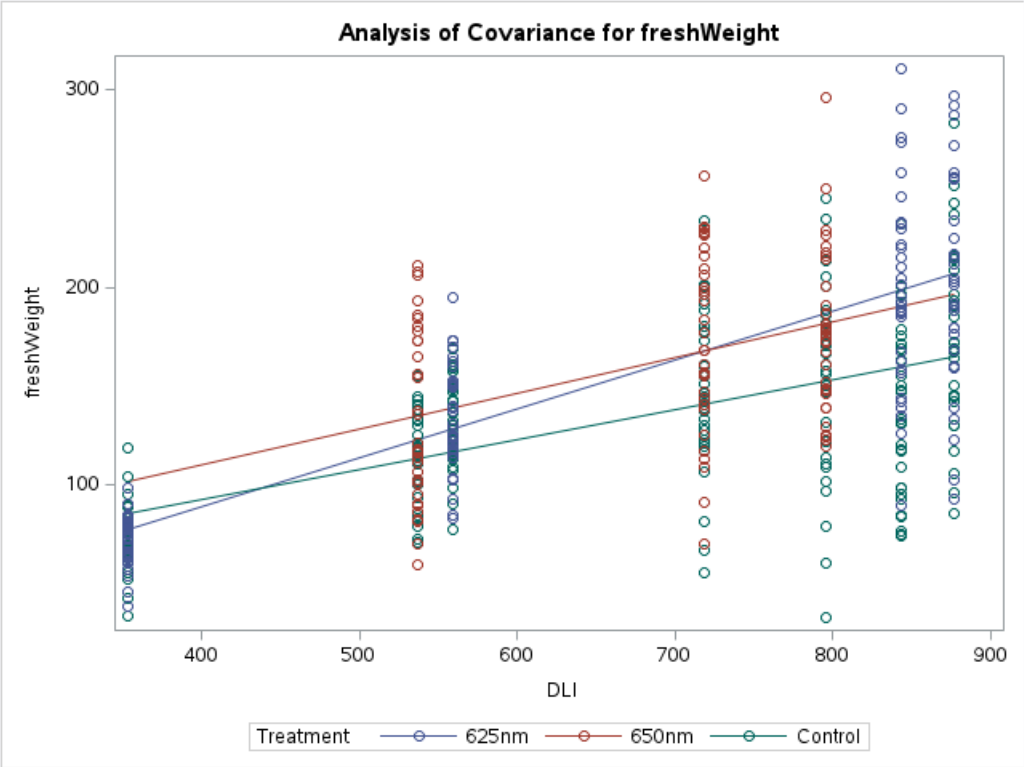


Figure 39. Projected Fresh Weight Trends by an Analysis of Covariance for Final Harvests of Lettuce Plants Grown at Various DLI Under 625 nm and 650 nm QD Films and Control Film.

4 Discussion

4.1 Discussion of Lettuce Results

4.1.1 625 nm QD Film Discussion

The plant response differences between the 650 nm QD film and the control film were analyzed for each response variable (fresh weight, dry weight, leaf area, leaf count, longest leaf length) by harvest for significance at a 95% confidence interval.

Then, the differences were analyzed for each response variable across all experiments with respect to treatment film and DLI, as shown in the statistical analysis section, by using a 2-factor factorial ANOVA test to obtain the effects of the treatment film and DLI on each response variable, as well as interaction effects between the treatment film and the DLI. The analysis was completed for each response variable for each of two harvests. A statistically significant interaction would indicate that the effectivity of the treatment film was influenced by the DLI experienced by the plants during growth under the film. This interaction effect of plant growth responses indicates that the film was more effective at growing larger plants when the DLI was higher and less effective when the DLI was lower, or vice versa.

The results of the plant biomass analysis indicated a general improvement of the lettuce under the 625 nm treatment film compared to the control film, as did initial analyses of the fresh mass under the 650 nm film. Several plant response factors, including the fresh weight and leaf area (final harvest), the dry weight at each harvest, and the leaf count at each harvest for the lettuce grown under the 625 nm film exhibited statistically significant increases compared to the plants grown under the control film. The leaf length under the 625 nm film compared with the control film indicated no change in leaf length at the first harvests and an overall decrease in leaf length at the final harvests.

Given the statistical significance of the interaction between the plant response from the 625 nm QD film and the DLI associated with each experiment, it can be determined that the 625 nm QD film was less effective in producing a plant response in lower DLI environments and more effective at higher DLI environments.

The interaction between the 625 nm film and the DLI was significant, even though there was no statistically significant difference in plant responses between the control film and the 625 nm film at the initial harvests. As shown in Figure 31, the plants grown under the 625 nm film were smaller than those grown under the control film under lower DLIs, and larger than those grown under the control film under higher DLIs.

Since early experiments with the quantum dot films in indoor growth chambers, performed by Charles Parrish (2020) and Michael Blum (In Review) were performed under an ideal, set DLI, the results likely did not change between the experiments, but remained consistent throughout all replications.

The films within the indoor laboratory testing showed greater statistical impact on the response variables than within the greenhouse trials of this study. The results from this were less consistent and dramatic than those in the indoor trials because of the significantly increased variability of the greenhouse environment. Whereas the DLI in the indoor plant growth test chamber (PGTC) was set to 17 mol m^{-2} d^{-1}, the average DLIs throughout the Tucson, AZ experiment ranged from 9 to 15 mol m^{-2} d^{-1} in the winter season to 21 to 30 mol m^{-2} d^{-1} for the summer season, and included significant variation in greenhouse air temperature throughout the seasons.

It is also worth noting that the air temperature and, more importantly, the vapor pressure deficit (VPD) changed throughout the experiments as air temperature and relatively humidity increased moving into the summer months with Tucson's monsoon season.

The significance of the growth period length is further suggested by the fact that the 625 nm film was statistically significant at the second harvest, but not at the first. This variation in temperature and DLI during the experiments under the 625 nm film is a unique and interesting result of this project, in that it elucidates the seasonal effectivity of the film under different light and environmental conditions. The ability to perform two harvests per experiment may provide valuable insight into the effect of the film under different growing periods and potential interactions between the 625 nm film and the growth period length of lettuce.

There may have been additional variation given the separate deep water culture tanks (two per treatment per experiment) and their relative positions in the greenhouse, which is expected within a greenhouse production setup, whereas the water tanks of the laboratory trials were shared across all treatments and were continuously monitored and kept to consistent air temparture and water nutrient quality. This provided more possibility for variation among the DWC tanks. Although they were treated the same and regularly monitored, the deep water culture tanks provided for more variation for growing conditions than occurred in the laboratory experiments. The root zone environments (nutrient and water availability, temperature, dissolved oxygen) were relatively consistent among the DWC tanks, but in some cases, there were differences, such as when the dissolved oxygen (DO) content in tank C2 near the end of 625 nm Experiment 3, the DO dropped toward the end of the experiment when the air stone failed.

Other factors affecting the results of these experiments were the changes of seasonal sun angle changes during the experiments and the impact of the growing period on plants at different

times of year, since plants reached maturity more quickly in the summer. An equal growing period was chosen for consistency among all experiments and to match the length of growing period in the laboratory experiments. In the summer months, this produced large plants at the first harvest and elongated plants by the second harvest, especially with the increasing DLI. The fresh weights and other measured factors were still useful from a research perspective, but the elongation was undesirable for a commercial lettuce crop. It is likely that a cultivar of lettuce with a different growing pattern (i.e., one that grew into a tighter head) would make for an easier experiment in the future, especially with the relatively large DLIs and set growth period. Another solution would be to set the length of the growing period to be ideal for a time later in the year; in this case, it was set when the DLI was very low and plants were small, so that by the final experiments, the growing period was long enough to cause crowding and elongation among the plants.

4.1.2 650 nm QD Film Discussion

The same process for data analysis, including determination of interaction effects, was followed for the 650 nm QD film results as described in the 625 nm QD film discussion section. The increase in fresh weight per experiment, for the 650 nm QD film, ranged from +13.3 % to +24.6%, and the increases were statistically significant during each experiment and at each harvest. For the 650 nm QD film, the overall increase in fresh weight was +27% at the initial harvest and +17% at the final harvest. In each case, the increase due to the film was found to be statistically significant, and there was found to be no significant interaction between the QD film effect and the DLI effect.

The plant response due to the 650 nm film and the DLI were both consistently statistically significant. In every instance except leaf length at the final harvest, the fresh weight,

dry weight, leaf area, leaf count, leaf length, and LUE of the lettuce plants were all significantly increased by the 650 nm film and by higher DLIs. Additionally, with only one exception (leaf count), all of the interaction effects between the QD treatment film and the DLI of the experiment were not significant.

The experiments performed under the 650 nm quantum dot film provided more consistent results than did the experiments grown under the 625 nm quantum dot film. The results were generally the same at both harvests during the 650 nm film experiment (i.e., if a response was statistically significant at the initial harvest, it was also significant at the final harvest, and vice versa).

This is likely due to the comparatively smaller seasonal variation experienced by the lettuce crops under the 650 nm quantum dot film, which occurred from July to October, while the 625 nm QD film experiments were completed between January and July. Therefore, the lettuce grown under the 650 nm film never experienced the very low DLI of by the lettuce under the 625 nm film. It is possible that the statistical interaction effect would have been significant under both films if they had each been tested in similarly low light seasons. It may be that quantum dot films were more effective than the control film only if not used in very low light settings, like those seen in January in Tucson. The lack of interaction effects for almost all response variables made the data more consistent and easier to interpret, but also removed the ability to observe the impact of low DLI scenarios.

4.1.3 Combined Lettuce Discussion

The plant responses under each QD film were discussed separately because the experiments were not performed simultaneously, and so the films could not be compared to each other using ANOVA analysis. However, by using ANCOVA analysis, it was possible to

extrapolate what the results may have been if the two films and the control film had been tested simultaneously at the same DLI as each other. Therefore, an additional discussion is possible comparing each of the treatment films to each other and to the control at each harvest under each tested DLI.

The hypothesis of the study was that the lettuce grown under each of the quantum dot films would be larger in terms of fresh and dry weight, and have a higher LUE compared with the lettuce grown under the control film. This was the case under high light conditions for both of the quantum dot films, but the lettuce grown under the 625 nm QD film did not exhibit a statistically significant increase in biomass compared with the plants grown under the control film. Light use efficiency, however, was significantly increased under each of the quantum dot films compared with the control films.

The fresh weight results align with the leaf area results, and they shared similar statistical significance. The increased percentage of blue light in the control (2 – 4%), especially at higher light intensities, inhibited leaf expansion, resulting in a lower biomass production in terms of fresh and dry weight. This is supported by previous studies where cell expansion and resulting leaf area was suppressed by higher proportions of blue light (Dougher and Bugbee, 2004, Karimi et al., 2022). Thus, the plants grown under the decreased blue light and increased red light and under the quantum dot films respond with an improved leaf size and biomass production.

Each quantum dot film provided an increase in fresh weight, dry weight, LUE, and other morphological characteristics over the control film at each harvest, as long as the harvest took place after March (at a DLI greater than 11.7 mol m^{-2} d^{-1}). Although the DLI and other environmental factors influenced in the effectiveness of the QD films, the general improvements

can be attributed to the increase in red to blue light ratio in the light environment of the plants under the quantum dot film (Matysiak et al., 2021). The finding that light environments with increased red proportions improves the output of lettuce plants has been supported in previous greenhouse studies, including at the University of Arizona's Controlled Environment Agriculture Center (CEAC) that used a different red glazing technique (Whalen, 2014). They also encountered seasonal environmental changes that, once accounted for, determined that the red glazing did increase lettuce biomass production compared to a control film.

It may be concluded by the ANCOVA analysis of these studies that at most cumulative DLI greater than 475 mol m^{-2} that both quantum dot films will produce greater biomass accumulation in lettuce plants than the control film at the initial harvest length. It may also be concluded that the 650 nm film produced a greater increase in biomass production than the 625 nm film. Additionally, at the initial harvest, the control film produced larger plants than either film at low DLI, but the improvement of the 650 nm QD film over the control film can be seen to down a lower DLI (approximately 350 mol m^{-2}) compared with the 625 nm QD film, which only produces an improvement over the control film down to approximately 475 mol m^{-2}.

ANCOVA analysis of the fresh weight data for the final harvest, in Figure 39, shows that for each film, the statistical analysis is similar to that of the initial, with a few exceptions. First, the 650 nm QD film is projected to always produce larger plants than the control film by the final harvest, with no point at which the plants grown under the control film are larger than those grown under the 650 nm film. Second, with a longer growth period, the 625 nm QD film can be projected to produce larger plants than the 650 nm film at very high DLI values.

Throughout all experiments, the light use efficiency was consistently greater under the quantum dot treatment films than the control films. Light use efficiency (grams of fresh weight

per mol of light received) accounts for any inconsistencies in lighting for plants grown on different sides of the greenhouse, because it factors in how much fresh weight was accumulated given the amount of light received, rather than looking only at the fresh weight. It also quantifies how well the plants used the light they received. There were periods where either the treatment side or the control side received a higher DLI than the other. The fact that the LUE was consistently higher under the treatment films shows that the plants more efficiently used the light under the quantum dot films than under the control films.

4.2 Conclusions

The increase in production under each QD film demonstrated its potential value to passively improve crop production without additional energy requirements. The consistent and significant increase in light use efficiency (LUE) demonstrated the potential of the QD films to provide a better quality of light to enhance plant growth regardless of light conditions.

These films may be used by growers in greenhouses to maximize their lettuce production without increasing their energy use. They may also be used to more efficiently produce food in space, where UV light is plentiful.

4.3 Improvements and Future Work

If further experimentation is performed using these films in a greenhouse setting, the ability to simultaneously utilize three greenhouse spaces, covered with the control film and with each of the treatment films, would provide a more straightforward comparison of their effect on plant responses. There would be less influence of the changing sun angle throughout the day as the central dividing wall would be eliminated in the greenhouse layout for this experiment. This could be more challenging with keeping consistency in environmental conditions such as

temperature and RH, but it would likely help mitigate some challenges with interpreting PPFD and DLI.

Additionally, whether future experiments are performed in one greenhouse or in multiple simultaneously as suggested, placement of the DWC tanks should be further away from the evaporative cooling pad wall, so that they are not shaded by the pad wall and the heater, as they were to some extent during this study.

Finally, locating a quantum sensor at each of the lettuce canopies (or, at least, placing one under each film at the height of the lettuce canopies) would have allowed the LUE to be more effectively and accurately calculated for the lettuce within each experiment, especially in the variable environment of a greenhouse setting.

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Appendix A – Hort Technology Manuscript Draft

Greenhouse Application of Luminescent Greenhouse Films Embedded with Quantum Dots to Improve Growth of Red Romaine Lettuce

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KEYWORDS. photoselective films, greenhouse covering films, light use efficiency

ABSTRACT. Many industries in arid environments, especially agriculture, are facing water and energy resource challenges, such as drought, increasing costs, and worsening pollution. Controlled environment agriculture (CEA) can increase water use efficiency and reduce environmental impact with closed loop hydroponic systems. However, energy is required to power irrigation pumps, electrical lighting, and other controls to achieve optimal plant growth and production in CEA. A new luminescent agriculture film technology presents a solution for improving crop production without requiring electricity. Quantum Dot (QD) technology embedded in the luminescent films and deployed in greenhouse covers can passively alter less photosynthetically efficient ultraviolet (UV) and blue wavelengths from the sun to red wavelengths inside a greenhouse, which are more efficient in promoting plant growth and increasing biomass. Two QD films with emission peaks in orange (625 nm) and red (650 nm) were selected for study in a greenhouse to determine the growth impacts of these films. Seven repeated experiments of red romaine lettuce (Lactuca sativa cv. 'Outredgeous') were grown in deep water culture (floating raft) tanks under the quantum dot films and simultaneously under a control film with the similar reflective and diffusive properties as the quantum dot films (four crops were grown under the 625 nm QD film and three under the 650 nm QD film). Each experiment was replicated under each film in two tanks of 56 plants each. The air (temperature, RH) and root zone (EC, pH) environments and the light received (PPFD) were monitored during growth, and upon harvest, the wet and dry biomass and morphological properties (leaf count and length) were measured. The light use efficiency (LUE), or the grams of biomass produced per mol of light received, and the daily light integral (DLI) were calculated. The lettuce plants grown under the quantum dot films increased in fresh mass (+10 to 27%), dry mass (+15 to 24%), and LUE (+7 to 35%) compared to the plants grown under the control film. The quantum dot films have demonstrated the potential to produce increased biomass in lettuce plants grown in greenhouses without increased energy cost, and have potential for numerous future applications.

Controlled environment agriculture (CEA) systems are a valuable tool to grow crops in intemperate locations or during seasons of the year not normally conducive to growth (McCartney and Lefsrud, 2018). Although these systems increase crop output and quality and reduce resource use, they require energy inputs for heating, cooling, and fertigation, at a minimum (McCartney and Lesfrud, 2018). It is valuable, therefore, to investigate methods to maximize crop output while minimizing energy input to the system.

One technique to improve resource use efficiency that has been broadly explored has been the tailoring of light quality to ensure the most efficient use of light by plants (Mickens et al., 2018; Matysiak et al., 2021).

LIGHT QUALITY. Each wavelength within the visible light spectrum, and some wavelengths beyond it, contributes to the quality and quantity of plant production and to the ability of growers to care for their crops (Ferreira et al., 2021), but the ratio of red to blue light can have a significant effect on biomass production and morphology (Mickens et al., 2018). The specific combinations selected rely both on the crop and on the desired output of the grower (Mickens et al., 2018; Alwadai and El-Bashir, 2022).

Blue light has been demonstrated to increase photosynthetic rates over other colors (Kang et al., 2016; Wang et al., 2016), but it also inhibits leaf expansion, slowing down the growth process (Dougher and Bugbee, 2004; Karimi et al., 2022). Red light, therefore, contributes more to biomass production and the increase of several morphological characteristics (McCree, 1972; Wang et al., 2016; Karimi et al., 2022). Blue and UV wavelengths, however, are still required for leafy green production, as they contribute to nutritional content and to leaf color in plants like red lettuce (Kang et al., 2016; Lin et al., 2018). Green light is also beneficial, because it can increase plant height and biomass (Orlando et al., 2022) and improves the ability of growers to interpret plant signals based on crop appearance (Kim et al., 2004). Therefore, while light quality improvement can increase crop growth, a more complete light spectrum than red and blue alone is beneficial in lettuce growth (Kim et al., 2004; Mickens et al., 2018; Ferreira et al., 2021).

Much of the research has been conducted with respect to electrical lighting in growth chambers. Although use of electrical lighting can be beneficial, and is in some instances necessary, the energy required can be expensive, economically and environmentally (McCartney and Lesfrud, 2018). Plant growth in a greenhouse allows for many of the resource saving opportunities of a growth chamber, but removes the requirement of electrical lighting, allowing crops to receive light solely from the sun. This clearly reduces cost and energy input and provides a more complete light spectrum than electrical lighting (Ferreira et al., 2021), but there are benefits to be gained by modifying light quality received by crops.

Photoselective greenhouse covering films, which passively modify the light entering into a greenhouse, have been studied (Hemming et al., 2006; Alwadai and El-Bashir, 2022). These films can be developed by adding organic fluorescent dyes, rare earth complexes, or quantum dots to plastics (Alwadai and El-Bashir, 2022). Many of these types of films reduce light intensity in the photosynthetically active radiation (PAR) region of the light spectrum such that plant response is significantly inhibited (Hemming et al., 2006), so new, effective photoselective films are being explored.

QUANTUM DOTS. Quantum dots (QDs) are colloidal, semiconductor nanocrystals that have the ability to change the wavelength of light as it passes through the dot. A plastic film containing a new type of non-toxic, copper-zinc QD that changes light in at blue and UV wavelengths to light at red and orange wavelengths at a quantum yield, or efficiency, greater than 85% has been developed. This film has been tested in growth chambers beneath broad spectrum electrical lighting and has been found to improve the biomass production of red romaine lettuce in these systems (Parrish et al., 2021). The improvement of light quality gained from shifting wavelengths from UV and blue into the red and orange spectrum could be further applied in future extraterrestrial food production, where the maximization of resource use efficiency is crucial (Parrish et al., 2021).

Based on the findings from Parrish et al., 2021, two films were selected for study in a greenhouse to investigate their potential to improve crop growth at a larger scale in a greenhouse more representative of commercial growing scenarios. The first QD film tested in this study absorbs blue and UV photons and converts them to a band centered around an orange 625 nm wavelength, while the second converts them to a band centered around a red 650 nm wavelength. It was expected that each film would improve the biomass output and several morphological qualities of red romaine lettuce (*Lactuca sativa* cv. 'Outredgeous').

Materials and Methods

GREENHOUSE LOCATION AND DESIGN. Each experiment was conducted in a north-south aligned A-frame greenhouse with another gutter connected greenhouse on the west side of the experimental greenhouse and with the east side wall whitewashed to ensure a similar light environment on each side. It was 7.3 meters by 14.6 meters in area, and was 2.6 meters high at the gutter and 4.4 meters high at the gable. The side walls of the greenhouse were made of double-layer polycarbonate, and the it was covered with Ethylene Tetrafluoroethylene (ETFE) plastic, which allows 80-90% of UV light to pass through (Brownell, n.d.). Beneath the ETFE film, a QD film was suspended on the east half of the greenhouse and a clear polyethylene control film sharing reflective and diffusive qualities with the QD film was suspended on the west half of the greenhouse. An opaque plastic divider was suspended vertically between the two halves of the greenhouse to ensure that plants under each side of the experienced only the light environment of the desired treatment film.

EXPERIMENTAL DESIGN. Red romaine lettuce plants were grown in deep water culture (DWC) tanks at the south end of the greenhouse near the evaporative cooling pad. Two 1.33 x 1.33 x 0.3m tanks of 56 plants each (31.7 plants m^{-2}) were grown in the control and treatment side of the greenhouse for each experiment. Four 43-day experiments were conducted sequentially under the 625 nm QD treatment film and the control film between January and June 2022. Three experiments were subsequently conducted under the 650 nm QD treatment film and the control film between July and October 2022. The air and root zone were monitored throughout each experiment, and the plant biomass and morphology were measured at transplant and harvest.

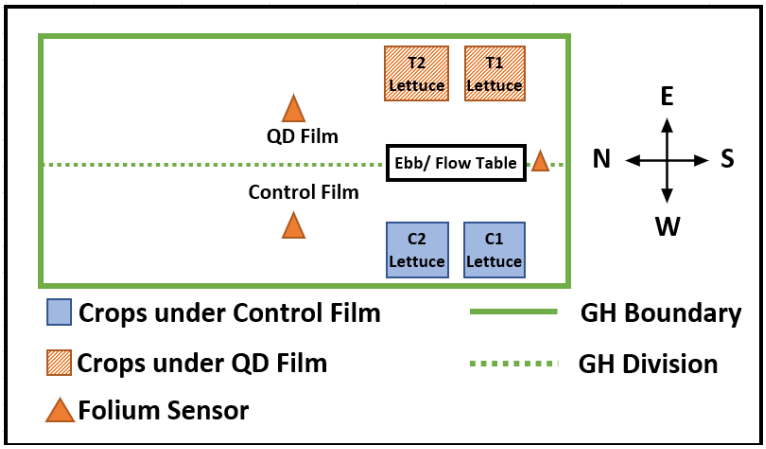

Figure A 1. Greenhouse Layout.

ENVIRONMENTAL MONITORING AND CONTROL. The greenhouse was ventilated using an evaporative cooling pad at the south end and two exhaust fans at the north end. Air was circulated through the greenhouse using two horizontal airflow (HAF) fans in the attic space of the greenhouse, one each on the east and west sides. Heat was provided in the winter using a gas-fired hot air heater located at the south end of the greenhouse.

The heating and cooling setpoints were monitored and controlled using a Wadsworth EnviroStep Control system. The temperature was controlled throughout the experiment to 27.2 to 30 °C during the natural photoperiod (without supplemental lighting) and 16.7 to 23.8 °C during the night. Relative humidity was not controlled, and CO_2 was neither controlled nor monitored.

The aerial environment, including air temperature, relative humidity, and light intensity in the PAR region, was measured continuously using a wireless Folium sensor group from WayBeyond. Each sensor group included a temperature sensor, a relative humidity sensor, and a quantum sensor. One quantum sensor was mounted at the south end of the greenhouse between the treatment groups at approximately one meter above the lettuce canopy. The PAR data from the south quantum sensor was used to calculate the daily light integral (DLI) through each experiment. Two additional sensor groups were mounted in the greenhouse, one each under the QD and control treatment films. These were mounted north of the DWC lettuce tanks and above the lettuce canopies. Although these sensors were not mounted directly at the lettuce canopies, the PAR data from the quantum sensors was used throughout this report to describe the relative difference in light intensity under each treatment.

The root zone environment in each DWC tank was measured weekly throughout each experiment for pH, electrical conductivity (EC), temperature, and depth. An air stone with associated pump was placed in each lettuce tank to maintain dissolved oxygen content of the nutrient solution at about 8 ppm.

LETTUCE GROWTH AND PROCEDURES. For each experiment, 224 plus 15% extra one-inch rockwool cubes were double-seeded and placed into trays on a flood irrigation tray table, where they were covered with an opaque film until all seeds had germinated, within approximately 96 hours. At 8 days from seeding, the plants were thinned to one seedling per rockwool cube. At 14 days from seeding, the seedlings were transplanted into floating rafts in DWC tanks. At 36 days after seeding (23 days from transplant), when the plants were mature and had reached canopy closure, 20 data plants were harvested from each tank.

LETTUCE DATA COLLECTION. For each experiment, the harvested data plants were weighed for fresh mass and leaves over one centimeter long were counted. For 5 randomly selected plants per DWC tank, each leaf was laid on a white posterboard under a clear plastic sheet, and an image was taken from a fixed point above the board. From these pictures, the leaf area was calculated for each selected plant using a custom leaf area analysis program as described by Parrish et al. (2021). Each of 20 randomly selected plants per treatment group was placed in a paper bag into a drying oven at 45 °C for 168 hours until leaves were dry, and each plant was weighed again for dry mass. The fraction of dry mass was compared to fresh mass and percent dry mass was calculated.

The calculated DLI, determined from the summation of the PPFD measured from the quantum sensors on the east and west sides of the greenhouse, was used along with the fresh mass, the length of the growing period, and the planting density, to determine the light use efficiency (LUE) of the plants under each film. LUE is a measure of how many grams of fresh biomass were produced per mole of light during each experiment. LUE was calculated using Equation (1).

$$(1)\quad LUE = \frac{FW \times (Plant\ Density/\#plants)}{Avg\ DLI \times Days\ after\ Treatment}$$

STATISTICAL MODELING. Outlier checking and removal was performed on each dataset for each experiment using a Z-score test with a threshold of ±3 standard deviations. All data from outliers was removed from the analysis. The measured variables include the fresh mass, dry mass, leaf area, and leaf count of the lettuce plants.

Each measured variable was analyzed to determine the impact of the QD film compared to the control film, as well as the daily light integral (DLI) for each experiment, and the interaction between the QD film and the DLI. A two-factor factorial model with a confidence interval of 95% ($\alpha = 0.05$) was used (Equation (2)) with online statistical analysis software (SAS ver. 3.81; SAS Institute Inc., Cary, NC, USA).

$$(2)\quad measured\ variable = treatment + DLI + (treatment \times DLI)$$

Results

BIOMASS. For the first two experiments under the 625 nm QD film, the DLI was ~10% lower over the treatment plants than over the control plants. During this time, the fresh and dry masses of the lettuce plants at harvest were smaller under the 625 nm QD film than under the control, although only the first experiment's results were statistically significant (Figure A 2A, Figure A 2B). For 625 nm QD film Experiments 3 and 4, the fresh and dry mass was consistently higher than the control, ranging in yield differences of 18-41% for fresh mass and 15-31% for dry mass (Figure A 2A, Figure A 2B). These differences were statistically significant at both harvests, with the only exception of dry masses at Experiment 4. The DLI increased on the treatment side as the season progressed into spring, with an average DLI increase of +4.4% (Experiment 3) and +4.2% (Experiment 4) for the treatment side compared to the control.

Overall, the 625 nm film was not a significant factor contributing to higher fresh mass or dry mass. However, both the DLI and the interaction between the DLI and the 625 nm QD film were significant, indicating that there is potential for the film to be more effective at increased DLIs. The 650 nm film was found by statistical analysis to significantly improve fresh and dry mass compared with the control film. The DLI was also a significant factor, but the interaction was not,

indicating that the light level did not influence the effectiveness of the film to promote plant growth.

The lettuce grown under the 650 nm QD consistently grew larger than the control with respect to fresh and dry mass than the lettuce grown under the 625 nm film compared to the control. This may be due to the relatively similar light conditions among the experiments with the 650 nm QD

Figure A 2. Lettuce Data at Harvest for Control and QD Films during 625 (Orange) and 650 (Red) nm QD Film Experiments. Average daily DLI (mol m^{-2} day) and Harvest Date Included per Experiment. (A) Fresh Mass (g) under Control and QD Films; (B) Dry Mass (g) under Control and QD films; (C) Light Use Efficiency (LUE) under Control and QD Films. Values with '*' were statistically significant at the 5% confidence level.

film, since they were all conducted during the summer, while the 625 nm QD film experiments were conducted between January and July.

MORPHOLOGY. The seasonal effect of the changing DLI can also be seen in the morphological data. The leaf count was higher under each film during all trials, although the increase was not statistically significant for 625 nm Experiments 1 or 2 (Figure A 3A). Statistical analysis showed that both QD films caused a statistically significant increase in leaf count under both the 625 and

650 nm QD films. The DLI had a significant effect under both films as well, but the interaction between the QD film and the DLI was only significant for the 625 nm QD film experiments.

The leaf area (Figure A 3B) followed the same seasonal trend as the fresh weight. During the first two 625 nm experiments, the leaf area was larger under the control film than under the 625 nm QD film, although the difference was only statistically significant for the first. The remaining experiments under each film showed an increase in total leaf area under the quantum dot films compared with the QD films. The 625 nm film did not cause a statistically significant increase in leaf area, although the effect of DLI and the interaction between DLI and the film were significant, indicating that the film may have been more effective in higher light conditions. The 650 nm QD film did significantly increase the leaf area, and in this case, the interaction effect between the film and the DLI was not significant.

For the morphological characteristics, like the biomass results, the interaction effects were not statistically significant during the 650 nm QD film experiments. This could, again, be a result of the more consistent DLI range observed under the 650 nm QD film compared with that under the 625 nm QD film.

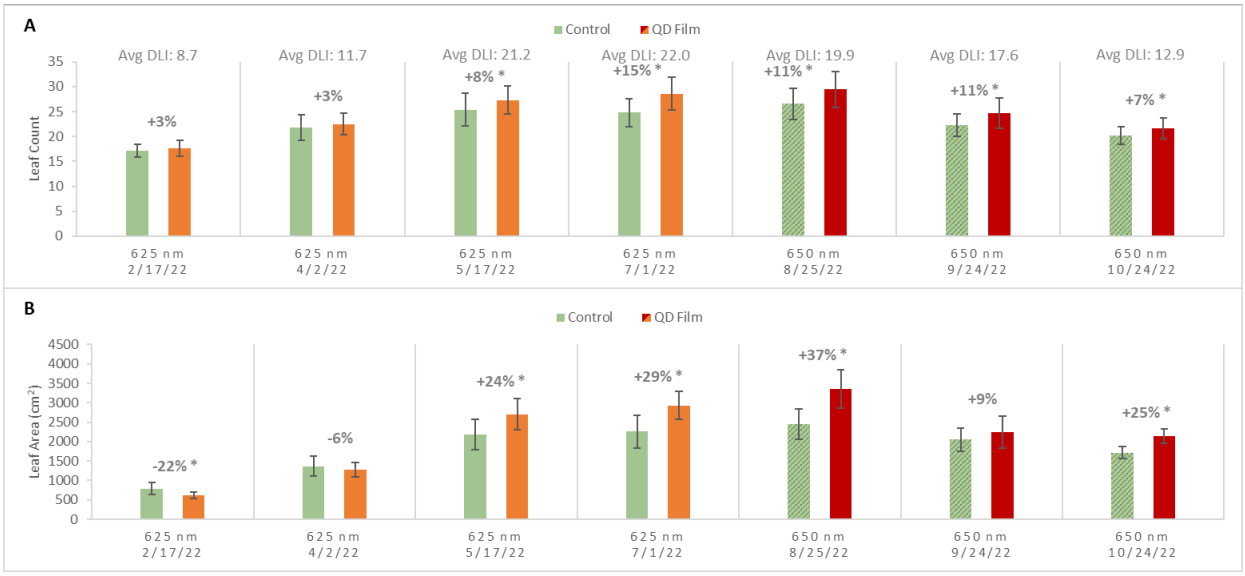

Figure A 3. Lettuce Data at Harvest for Control and QD Films during 625 (Orange) and 650 (Red) nm QD Film Experiments. Average daily DLI (mol m^{-2} day) and Harvest Date Included per Experiment. (A) Leaf Count under Control and QD Films; (B) Leaf Area (cm^2) under Control and QD Films. Values with '*' were statistically significant at the 5% confidence level.

LIGHT USE EFFICIENCY.The seasonal effect of the changing DLI was more muted with respect to light use efficiency (LUE), although DLI did have a statistically significant effect on the LUE under both films (Figure A 2C). The 625 nm QD film showed an increase in LUE during every experiment except Experiment 1, and the differences in the first two experiments were not statistically significant.

Under both the 625 nm and the 650 nm QD films, the QD film caused a statistically significant improvement in LUE compared with the control film. The DLI did have a significant impact on the LUE under each film; higher LUEs were seen under higher DLIs. The interaction between the 625 nm QD film and the DLI was significant. The 625 nm QD film provided higher LUEs overall, and the LUEs resulting from combinations of the 625 QD nm film and higher DLIs were generally higher than those produced by the reference film and/or lower DLIs. the interaction effect between the treatment and the DLI was not statistically significant. The LUEs under the 650 nm QD film was always significantly greater than those under the control film.

Discussion

Each QD film provided an increase in LUE and other morphological characteristics over the control film.

While the lettuce grown under the 625 nm QD film in the summer was larger than that grown under the control, the overall analysis did not show an improvement in fresh and dry mass over the lettuce grown under the control film. The lettuce grown under the 650 nm QD film, however, was significantly larger in terms of fresh and dry mass than that grown under the control film.

Although the DLI and other environmental factors influenced the effectiveness of the film, the general improvements can be attributed to the increase in red to blue light ratio in the light environment available to plants in under the quantum dot film (Matysiak et al., 2021). The finding that light environments with increased red proportions improves the output of lettuce plants has been supported in previous studies, where another type of red glazing technique encountered seasonal changes that needed to be accounted for, but found that the red glazing did increase lettuce biomass production (Whalen, 2014).

The fresh mass results are directly proportional to with the leaf area results, with their statistical significance mirroring each other for all experiments. The increased level of blue light in the control side, especially at higher light intensities, inhibited leaf expansion, resulting in a lower biomass production in terms of fresh and dry mass, which is supported by previous studies showing that cell expansion and resulting leaf area is suppressed by higher proportions of blue light (Dougher and Bugbee, 2004, Karimi et al., 2022). Thus, the plants grown under the increased red light and decreased blue light under quantum dot films show improved leaf size and biomass production.

Consistently, throughout the experiments, the LUE was greater under the QD treatment films than under the control films. Light use efficiency is a valuable metric, in that it accounts for any inconsistencies in lighting between the sides of the greenhouse, and demonstrates how well the plants used the light they did receive. Since there were times where either the treatment side or the control side received a higher average DLI than the other, the fact that the LUE was consistently higher under the treatment films indicates that the plants were able to more efficiently use the light under the QD films than under the control films. This increase in biomass production and LUE under films embedded with quantum dots has potential to improve commercial crop production without additional energy use by the grower, which would lead to financial savings and increased sustainability in greenhouse operations.

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Appendix B – Supplemental Information

Lettuce Fertigation

The lettuce DWC tanks held approximately 100 gallons of water each. The nutrient solution was split into two parts, tank A and tank B, which were kept separate until they were mixed into the DWC tanks. Tank A and tank B were each 5 gallon buckets with the fertilizer at a 100:1 concentration. The tanks were composed of the quantities of macronutrients and micronutrients shown in Table B 1.

Table B 1. Lettuce Nutrient Solution at a 100:1 Concentration in 5 Gallon Tanks

Macroelements				
Tank A				
$Ca(NO_3)_2$	Calcium Nitrate		1,640	g
$CaCl_2 \cdot 6H_2O$	Calcium Chloride		360	g
		Total	2,000	g
Tank B				
KNO_3	Potassium Nitrate		680	g
KH_2PO_4	Potassium Phosphate		400	g
$MgSO_4 \cdot 7H_2O$	Magnesium Sulfide		760	g
		Total	1,840	g
Microelements				
Tank A				
Iron chelate EDTA		(13.2%)Fe	35.0	g
Tank B				
Manganese sulfate		**(32%)Mn**	3.20	g
Zinc sulfate		(36%)Zn	1.80	g
Copper sulfate		(25%)Cu	0.38	g
Solubor		(20.5%)B	3.20	g
Sodium molybdate		(40%)Mo	0.24	g
		Total	8.82	g

Canopy Closure Pictures

During each of the experiments, a photo was taken from above each of the four deep water culture tanks once per week between transplant and the final harvest. An example of these pictures from the 625 nm QD film experiments is shown below in Figures B 1 through B 6.

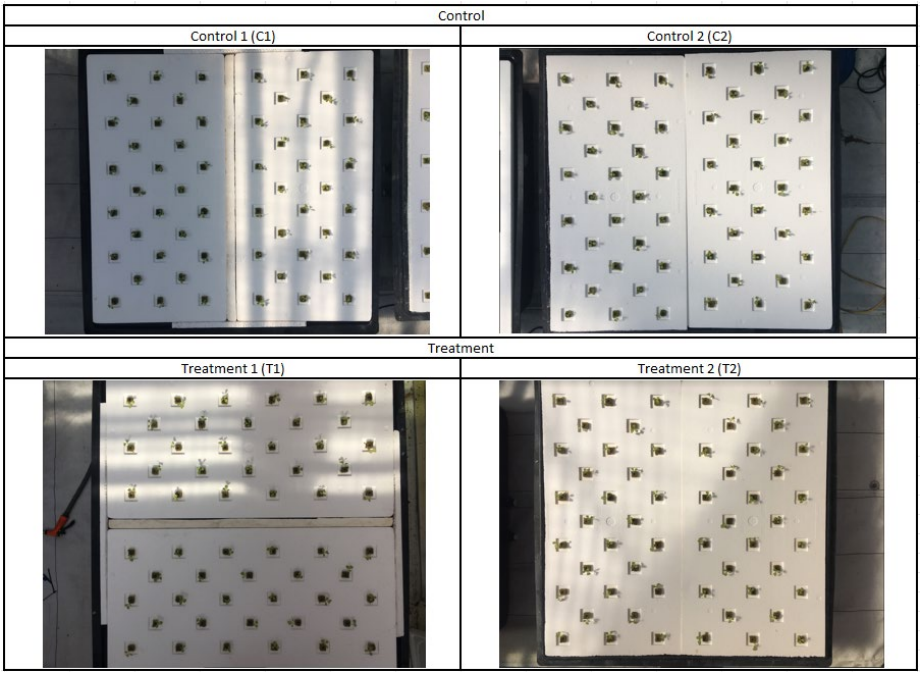

Figure B 1. Canopy Closure for 625 nm Film: Experiment 1 – 1/20/22.

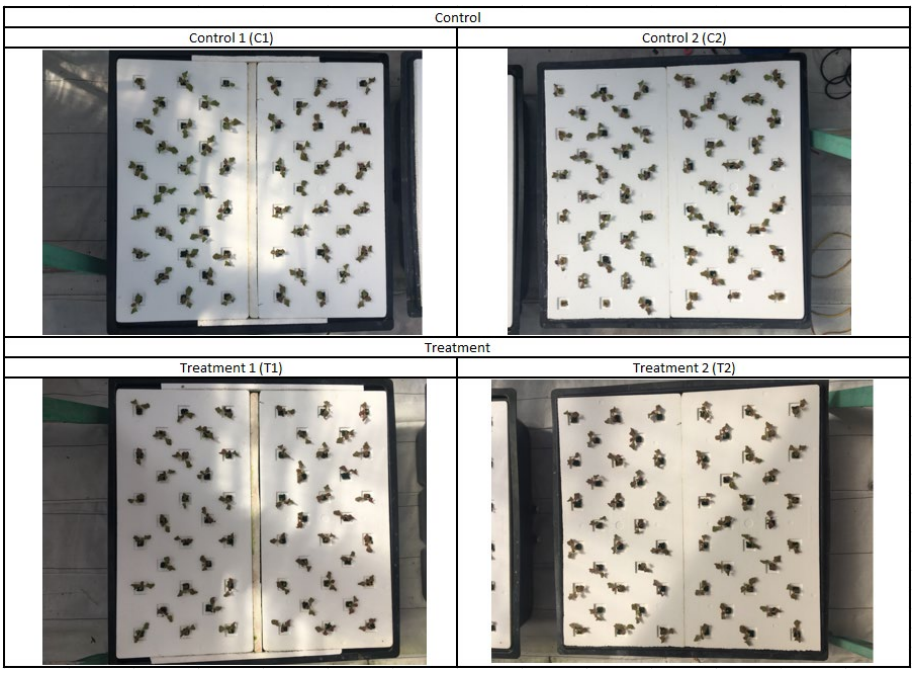

Figure B 2. Canopy Closure for 625 nm Film: Experiment 1 – 1/27/22.

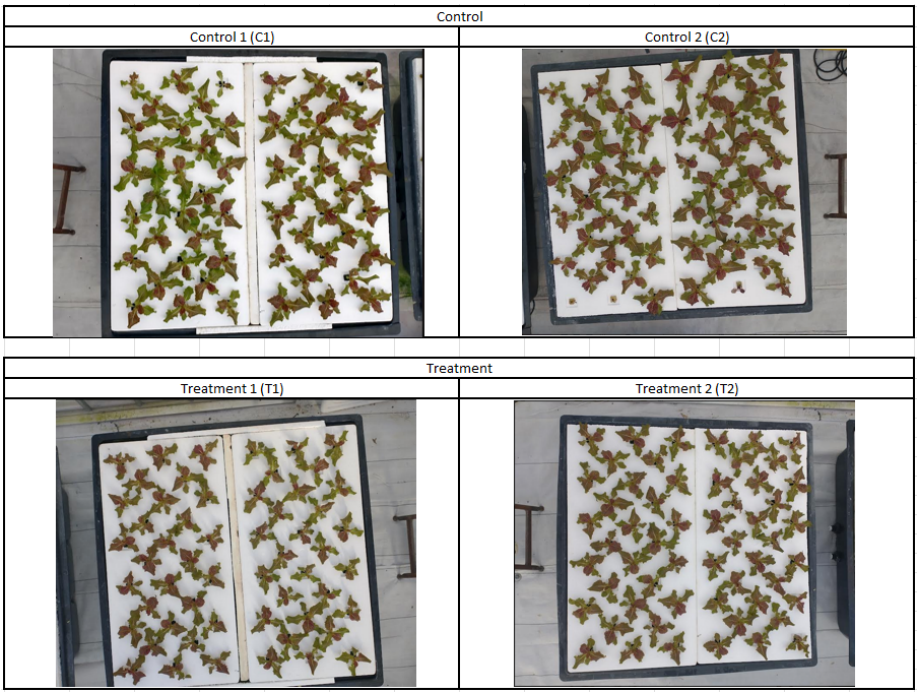

Figure B 3. Canopy Closure for 625 nm Film: Experiment 1 – 2/3/22.

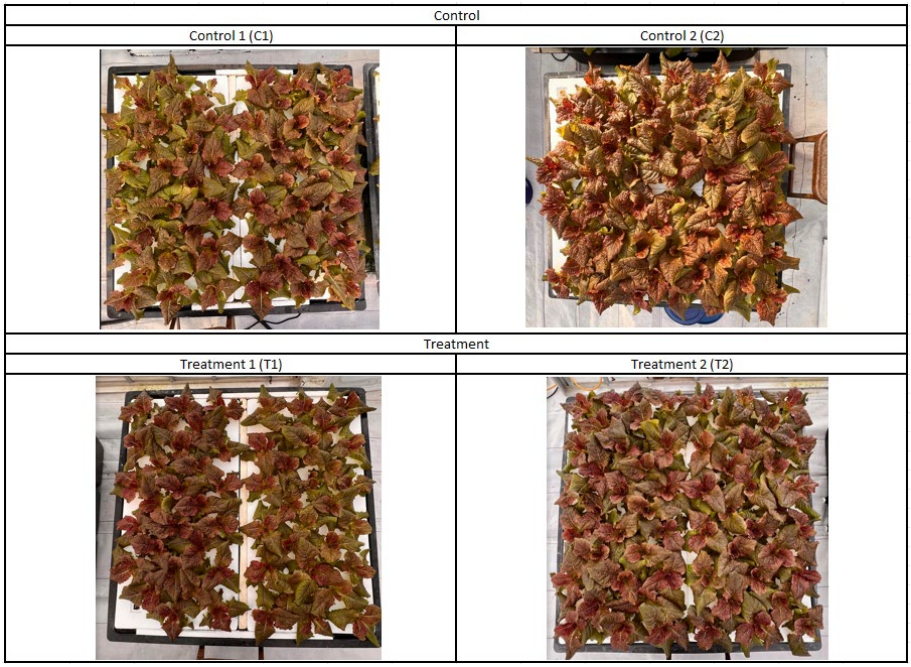

Figure B 4. Canopy Closure for 625 nm Film: Experiment 1 – 2/10 (Before Initial Harvest).

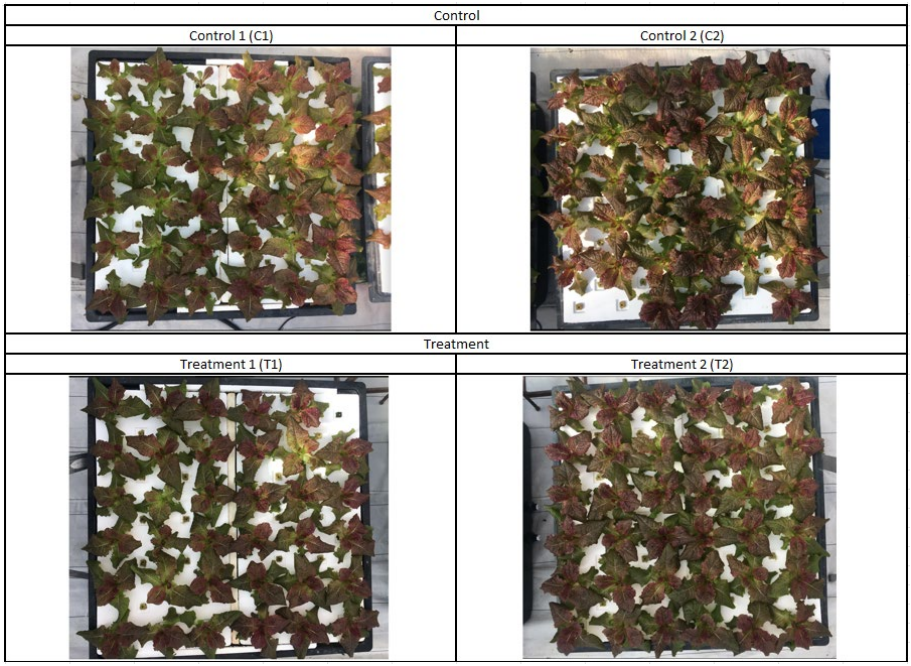

Figure B 5. Canopy Closure for 625 nm Film: Experiment 1 – 2/11/22 (After Initial Harvest).

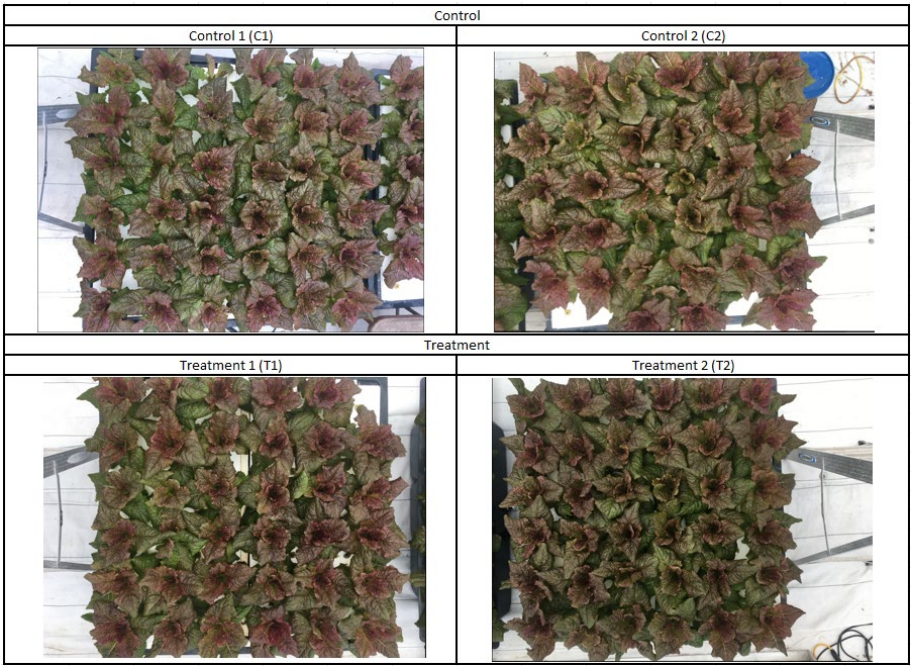

Figure B 6. Canopy Closure for 625 nm Film: Experiment 1 – 2/17/22 (Before Final Harvest).

Root Zone Average Values

The root zone environments were relatively similar between the four tanks during each experiment. The average root zone values were calculated for 625 nm QD Experiment 4 (Table B 2) and for 650 nm QD Experiment 1 (Table B 3), since these subsequent experiments both took place in the summer and therefore had reasonably similar environments. The tables below show that the root zone did not differ significantly between the four tanks or between the two treatments during the experiments.

Table B 2. Root Zone Environment During 625 nm QD Film Experiment 4 (Final Harvest 7/1/22).

	Tank			
	C1	**C2**	**T1**	**T2**
pH	6.1	6.2	6.3	6.2
EC (dS m^{-1})	1.9	1.8	1.8	1.8
Change in Water Depth (cm)	3.0	3.2	2.8	2.8
Water Temp (℃)	24.3	24.9	24.9	25.6
Dissolved Oxygen (mg L^{-1})	7.9	7.2	7.7	7.5

Table B 3. Root Zone Environment During 650 nm QD Film Experiment 1. (Final Harvest 8/25/22).

	Tank			
	C1	**C2**	**T1**	**T2**
pH	6.8	6.7	6.4	6.5
EC (dS m^{-1})	1.9	1.9	1.9	1.8
Change in Water Depth (cm)	2.8	3.4	3.2	2.9
Water Temp (℃)	24.8	25.2	25.2	25.5
Dissolved Oxygen (mg L^{-1})	6.9	6.2	6.9	6.6