Reaching New Heights: Using Complex Lighting Regimes to Improve the Efficiency of Vertical Farming

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Abstract

By 2050 over two thirds of the global population will be living in urban areas. Vertical farms offer the opportunity to produce nutritious foods in cities whilst reducing transport costs. However, vertical farms have significant costs and carbon emissions associated with lighting and temperature control. These costs can be mitigated by using complex lighting regimes which alter light quality and intensity in a way that reduces energy input but does not significantly alter crop growth. I have subjected microgreens which are commonly grown in vertical farms to complex lighting regimes to investigate their potential application in indoor farming. In chapter one I have analysed atmospheric data to create a lighting regime which changes light quality and intensity to realistically mimic a sunrise in York during the spring. I report that there were no significant impacts to the biomass of kale, but the biomass of radish was significantly decreased. This demonstrates that lighting regimes which mimic sunrises could reduce energy costs for some species. I have also subjected microgreens to transitioning photoperiods and measured their hypocotyl lengths. In microgreens, the order of photoperiod exposure influenced hypocotyl length for some species, but not others. Finally, I have developed an experimental design for efficiently optimising light conditions in small vertical farms, by taking advantage of the heterogeneity of light intensity that occurs at the plant level in a vertical farm. After measuring light intensity and biomass variation I have created a mixed effects model which suggests at an overall light intensity range of 300-500 μmol of light, red light intensity negatively impacts microgreen biomass. These findings suggest ways in which researchers can use complex lighting regimes to improve the efficiency of vertical farms.

Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for a degree or other qualification at this University or elsewhere. All sources are acknowledged as references.

Acknowledgments

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Introduction

The global population is rising and will reach approximately 9 billion by the year 2050. Global food production will need to be increased to accommodate an approximate 50% increase in the demand of food (1). It is estimated that by 2050 more than 6 billion people will be living in urban areas (2). This will require more food to be transported from farmland to densely populated areas. Such an increase will produce more CO_2 emissions, contributing further to climate change and potentially weakening global food security, which will be further exacerbated by increases in catastrophic weather events and pathogenesis of crops (3). A form of mitigation for this issue is using controlled environment agriculture (CEA) which enables crop growers to protect their crops against harsh environmental conditions, and therefore enable crops to be grown all year round. The conditions in vertical farms can also be tailored to the crops being grown to maximise yield. A form of CEA which can be implemented into urban areas to grow and supply crops locally is indoor vertical farming. Vertical farms are usually comprised of beds that utilise aeroponic or hydroponic growing systems, LED lighting and air conditioning units (4). Beds are stacked on each other vertically for better use of space. Aeroponic and hydroponic systems are beneficial because they have a higher water use efficiency relative to traditional agriculture (5). Some LED lighting systems can output different light qualities and intensities, enabling growers to make specific lighting regimes which aid crop growth. Typically, vertical farming does not require the use of pesticides and produces no runoff into nearby ecosystems. Pesticide runoff produced from traditional agriculture has been shown to harm ecosystems (6).

A type of crop that is grown frequently within vertical farms is microgreens, which are salad greens that are grown until the first pair of true leaves are developed (7). Microgreens are nutrient dense and have high concentrations of antioxidants (8) However, many microgreens have a shorter shelf life than mature vegetables (9). Vertical farms enable these nutritious crops to be grown in urban centres, reducing transport and storage requirements. Additionally, their short stature and small root system are particularly well-suited for stacked aeroponic and hydroponic set-ups. Microgreens such as Micro-Radish (*Raphanus raphanistrum*) Micro-Kale (*Brassica oleracea*) and Sunflower-Shoots (*Helianthus annus*) are regularly grown in vertical farms and have been used in my experiments due to their fast-growing time. As members of the *Brassicaceae* family, kale and radish are closely related to the model plant *Arabidopsis thaliana* (10) which makes them good model crops to investigate how processes that occur in *A. thaliana* can be applied to a CEA setting. The Brassicas are an important group of agricultural crops (11), and performing experiments using radish and kale may highlight beneficial farming practices that can also be applied to other members of the Brassica family, such as oilseed rape and cabbage.

Despite the energy savings associated with reduced transport costs, life cycle analysis has found that vertical farms may be more harmful to the environment across their life span because of the energy required to operate them (12). Less greenhouse gas emissions are produced when the electricity used was obtained from renewable energy sources, but this can be expensive (11). Up to 85% of operational energy consumption is associated with lighting and cooling (13), so these two areas are important to optimise to reduce the carbon footprint and improve the economic viability of vertical farms. In this thesis, we focus on finding energy-efficient lighting regimes, but having more energy efficient lighting regimes may also have a knock-on effect of reducing cooling requirements, as the lights produce a large amount of heat.

Other studies have investigated alternative strategies for optimising lighting regimes to improve yields and reduce energy requirements. These studies have primarily focussed on comparing the effect of a small number of light recipes, such as photoperiod lengths (14,15). One study aimed to find the growing conditions that produced the lowest CO_2 emissions for growing micro kale. This case study took place at a vertical farm which used industrial CO_2 to enhance the growth of crops. It was found that the optimal conditions that minimised emissions and maximised yield were 4000ppm of CO_2, a photoperiod of 24 hours and a temperature of 20°C (16). However, it's important to note that not all vertical farms use CO_2 to grow crops. Other studies have examined how light intensity and quality can impact the physiology of crops (17). A study conducted in two duckweed species examined how altering light intensity and the ratio of red and blue light impacted growth rate, crude protein content, relative protein yield and chlorophyll a content. It was found that varying the spectrum had no significant impact on any aspect of physiology examined. Increasing light intensity linearly increased growth rate and protein yields. Increasing light intensity did not impact crude protein content. Chlorophyll a content decreased as light intensity increased (18).

There have been a number of studies with slightly more complex light recipes that change the light quality over time. Kong et al., examined how changing the ratio of red and blue light 21 days into the growing period impacted growth phenotypes and nutrient concentrations in red and green romaine lettuce. Overall, in green romaine lettuce, a low proportion of blue light for the entire growth period results in the highest biomass. However, when the proportion of blue light was transitioned from low to high there was no significant changes in biomass. Additionally, a significant increase in beta-carotene content was observed. The study concluded that transitioning blue light proportion from low to high towards the end of the growing period is the optimal light treatment (19). Additionally, changes to monochromatic light at certain times during the photoperiod have been shown to impact physiology. Two studies have grown tomato and lettuce respectively under constant light. During the first 12 hours of the photoperiod plants were subjected to red light and in the final twelve hours switched to blue light. It was found that in both cases lettuce and tomato growth was enhanced when compared with plants grown using light/dark cycles (20,21). This data shows that changes in monochromatic light quality over time can alter growth and photosynthetic activity in a positive way. Some research groups study how different photoperiods impact the biomass of some crops. A study in cannabis grew plants in either 10,12, or 14-hour photoperiods before transitioning them to one of the alternative photoperiod lengths. It was reported that plants had significantly higher biomass when grown in 14-hour photoperiods compared to 12-hour photoperiods. Interestingly, one species exhibited significantly greater biomass when switched from 14h to 10h photoperiods and an even more significant increase was seen when transitioning from 14h to 12h (22). If this behaviour under transitioning photoperiods is conserved in other crops such as microgreens, then implementing photoperiod shifts into vertical farms could improve crop yields and lower energy costs.

These studies indicate that by adding a layer of complexity to lighting regimes in the form of varying intensity or quality over time can potentially impact yield and nutrient content. However, the benefit of incorporating complex lighting regimes in the production of microgreens is unclear as there are few studies that explore this.

Research on the model organism Arabidopsis suggests several additional complex light regimes that may have applications in vertical farming, including gradual sunrises and photoperiod shifts. One study has examined how lighting that simulated sunlight by increasing intensity in a sinusoidal way with a static light quality which mimicked sunlight at midday impacted the growth rate and metabolism of Arabidopsis. It was found that there were no significant differences in growth rate between plants grown under artificial or natural conditions. Sinusoidal lighting regimes may require less total energy for lighting and cooling and so may be a mechanism for making vertical farms more energy efficient. However, plants grown under natural conditions accumulated less starch and sucrose than those grown under sinusoidal conditions, demonstrating that gradual changes to lighting impact metabolism and not physiology (23). This study did not change light quality, which in nature would vary massively throughout a 24-hour period, as the colours of the sunrise and sunset vary over time. The impact of gradual changes to light quality and intensity on plant growth remains uncharacterised and may have applications in vertical farms. Of particular interest is how gradual changes in light quality and intensity impact microgreen growth. This is because of the waves of transcriptionally activity linked to the regulation of photomorphogenesis that occur in the early morning (24). Additionally, Arabidopsis plants can detect very subtle differences in ambient temperature and light quality (25,26). Redmond et al has characterised developmental asynchrony in near isogenic lines of Arabidopsis (27). It is unclear the extent to which miniscule irregularities in the microenvironment contributed to this heterogeneity and whether heterogeneous conditions in commercial vertical farms also contribute to phenotypic variability, which is an undesired characteristic of a crop.

Plant growth can be impacted by several factors. Different species of plants have different optimum temperatures that can enhance growth. However increasing or decreasing temperature away from the optimum temperature will decrease crop growth (28). Relative humidity is another factor that impacts plant growth by impacting transpiration rates of the leaves. If relative humidity is too high then transpiration rates become too low, hindering growth. Alternatively, when relative humidity is too low then transpiration increases lowering photosynthetic rate. Implying that like temperature, different plant species have different optimal relative humidity percentages for growth (29). Similarly plant growth can be enhanced by growing plants in an environment which has an optimal light intensity (30). Plants also behave favourably towards combinations of different light qualities. Most growers use combinations of blue, red and far red to maximise crop growth and nutrient content (31). Plant growth favours certain environmental conditions. Theoretically, the optimal conditions for individual plant species could be replicated in a vertical farm setting to maximise crop growth. However, the ideal growth conditions for microgreens remains unknown. This is because there are no studies that individually examine all the environmental factors within a vertical farm and how they impact growth.

The aim of the thesis is to translate the work in Arabidopsis around sensitivity to sunrises, photoperiod shifts and heterogeneous development to improve the lighting regimes of vertical farmed microgreens. The work presented in this thesis aims to improve the efficiency of vertical farms by studying how complex lighting impact plant growth. The ideal scenario would be to create lighting regimes which positively improve or maintain agricultural traits like yield, nutritional quality or aesthetic quality, but reduce energy inputs.

In chapter one I examined how changes to gradual light quality and intensity at the beginning of the photoperiod impact fresh biomass in microgreens. Using atmospheric data and tuneable LED lighting, plants were exposed to sunrise like lighting for the first two hours of the photoperiod. It was found that gradual changes to light quality in the morning did not impact biomass of kale. However, they did significantly decrease the biomass of radish. This shows that some microgreens are sensitive to sunrise conditions and not others. This also shows that sunrises could potentially be used to grow certain microgreens like kale at a reduced energy cost in vertical farms. For other microgreens like radish, it highlights that they could be grown using less energy at the cost of decreased biomass.

Chapter two investigated how transitioning between long and short-day conditions impacted the hypocotyl length of microgreens. Previous observations (in prep) showed wild type ecotypes of Arabidopsis that were transitioned from long days (LD; 16 hours light:8 hours dark) to short days (SD; 8 hours light:16 hours dark) grew taller than those transitioned from SD to LD. I subjected microgreens to similar changes in photoperiod and observed that hypocotyls transitioned from SD to LD grew taller than those that underwent the opposite transition. This could potentially be utilised in a vertical farm to produce microgreens that are aesthetically and nutritionally viable at a reduced energy cost.

The final research chapter of this thesis aimed to characterise the ideal growing conditions for microgreens in a vertical farm. I suggest an innovative experimental design that exploits the existing heterogeneity within the commercial farm to investigate the impact of light quality on production. I examined light quality and intensity heterogeneity in a vertical farm in York and found that both aspects varied at the plant level within trays used to grow plants and across beds. I then grew plants in these varied conditions, harvested them and produced a mixed effects model to see if there were significant predictors of fresh biomass. I found that the amount of red light and bed that the plants were grown in impact fresh biomass. This suggests that red light and bay are key inputs that have a significant impact on the amount of fresh mass of microgreens.

These results provide novel ways in which microgreens can be grown at a lower energy cost. These methods could be employed by other research groups to lower the carbon footprint of other vertical farms.

Chapter one: The impact of gradual changes to light intensity and quality on microgreen growth

Introduction

Early morning light in natural settings is not a binary change, it is a series of gradual changes to both the intensity and the quality of light. Other studies conducted in *A. thaliana* that examine the transcriptome of mature plants under sinusoidal light show that plants alter their gene expression differently than under constant light (32). Additionally, observations in a preprint suggests that plant physiology and circadian gene expression is impacted by the length of twilight (33). This mounting evidence suggests that plants can detect and mount physiological responses to gradual changes in light frequency distribution and intensity.

Most vertical farms and growth chambers have binary changes to light intensity and quality. The Ezer lab discovered an early morning gene network that had HY5 and BBX genes as hub genes (24). This network was discovered in plants that had been grown in controlled growth cabinets. These genes have also been implicated in the stress response to light intensity (34). Because of this we hypothesised that the sudden drastic change a plant experience when the lights are switched from off to on may cause light stress in controlled growing conditions such as vertical farms and growth cabinets.

Light stress can have various negative impacts on microgreens. It has been shown that the carotenoid content of *Brassicacea* microgreens can be reduced under high light intensities, reducing their nutritional value (35). Moreover, high intensities of certain light qualities can also negatively impact the nutritional content of microgreens. It has been demonstrated that Vitamin E levels are negatively impacted by higher proportions of blue light in microgreens (36). Higher light intensity also negatively impacts other crops besides microgreens. A study conducted in lettuce and spinach found that increased light intensities can cause aesthetic damages which contributes to crop losses (37).

Plants that are subject to light intensity that exceeds the necessary amount for photosynthesis undergo a process known as photoinhibition. Photoinhibition is a reduction in photosynthesis as a result of lower CO_2 metabolism under high light conditions (38). Photoinhibition occurs, when the rate at which light damages a plant exceeds the rate in which plant can repair itself. To stop photoinhibition plants must employ a wide range of hormonal photosynthetic and transcriptional changes (39). Mitigating photoinhibition under high light stress may result in plants not utilising light as effectively under less intense conditions. If plants grown in controlled environments are experiencing light stress during the beginning of the photoperiod then they may not be efficiently transducing light energy. This highlights a novel potential for gradual changes to light quality and intensity in the morning as a means of saving energy and ensuring plants are able to efficiently utilise light energy in vertical farms.

Using available atmospheric data, we have analysed how the intensity and frequency distribution change over a morning in the spring of 2021 in the North of England. Using specialised lighting equipment (Heliospectra DYNA), we have developed a lighting regime which mirrors the rate of change of intensity and frequency distribution of a morning. We then subjected three species of microgreens to these conditions before harvesting them and recording their biomass. We show that gradual changes to light intensity and frequency distribution do not impact germination, nor do they impact the biomass of two varieties of kale. The biomass of radish is significantly decreased under gradually changing conditions, highlighting that radish may be sensitive to gradually changing early morning light and that morning light sensitivity may vary on a species-by-species basis

Methods

Designing a Sunrise

Sunrises were designed using an online tropospheric ultraviolet and visible light (TUV) calculator (40). The TUV calculator receives inputs for longitude, latitude, time and date. These inputs are used to calculate spectral irradiance values for specified wavelengths of light. I collected spectral irradiance data from the 14th of April 2021 along the longitude and latitude of York City Centre between 05:50 (Just before sunrise) and 13:30 (after noon) in 10-minute increments. A spectral irradiance value was obtained for all wavelengths between 400nm and 761nm. Our growing cabinets had eight channels of LED lights which could output light corresponding to 409, 423, 449, 519, 630, 660 ,737, 448nm meaning I had to select spectral irradiance values from my data that corresponded to these wavelengths only. We then decided to focus on the LED channels that were easiest to programme and output light frequencies near the peak absorbance of cryptochromes and phytochromes (449, 660, 737nm). These channels correspond output blue, red and far-red light respectively. These LEDs could not output light as intense as would occur naturally in York therefore the values for spectral irradiance were scaled to the maximum output in watts for each LED. Information on sunrise and noon times on this day was obtained from the TimeandDate.com (41) .April 14th was chosen because all crops can be sown in this month for traditional outdoor agriculture purposes in the North of England.

Growing Microgreens

Seeds were sterilised by being soaked in pure ethanol and a mix of 1 % bleach and 20% triton respectively for two minutes before multiple washes in sterile water before being suspended in 0.1% agar water. Once sterile, individual seeds were placed on to 4mL of Murashige – Skoog media with no sucrose (MS0) that had been poured inside falcon tubes. Tube openings were covered in a transparent plastic film to enable light to pass through and prevent contamination, holes were pierced into the film to enable transpiration. There were 49 plants per species and each species was placed in a box which was inlayed with a 7 x 7 grid which ensured the tubes could stand upright without touching each other. The seedlings were then wrapped in foil for three days to germinate. After the germination period plants were exposed to the designed sunrise for the first two hours of the photoperiod. Once the sunrise had reached its final light quality and intensity the lights were kept at this intensity and quality for an additional twelve hours. This was followed by 10 hours of darkness. Plants were grown until the first hypocotyl of each species began to touch the plastic film and were then harvested. Percentage of plants that had germinated was recorded daily throughout this experiment. As a control experiment, microgreens were grown under days with 14 hours of light and 10 hours of dark. The light period matched the light quality and intensity at the end of the sunrise condition.

Harvesting Microgreens

Plants were carefully removed from the media using tweezers and had their roots cut from the stem with scissors. The mass of the whole shoot system was weighed. This method of harvesting was employed because it is similar to how microgreens are harvested in vertical farm settings. Once the whole mass was recorded, the leaves were ripped at the base of the petiole and the leaf mass was recorded.

Results

Light intensity increases during the morning

To grow plants under realistic sunrise conditions which gradually change in both light intensity and light quality I had to analyse existing sunrise data and examine the qualities of a sunrise. Analysing the raw data outputted by the TUV calculator (Figure 1) shows that as the morning progresses spectral irradiance increases across the entire waveband. Figure 2 shows how spectral irradiance changes across wavelengths of light that can be programmed in our plant growth chambers. Data generated from the TUV calculator begins just before the actual sunrise on this day (06:05) and ends shortly after noon (13:04). Across all three wavelengths spectral irradiance increases steadily until it plateaus at approximately 12:00. From this time point until the end of the timeseries spectral irradiance begins to decrease. Due to the limitations on the intensity that the lights in our growth chambers could output the irradiance values were scaled down to a range that could be outputted in a controlled setting. The plants in this experiment were not exposed to a sunrise that is representative of a natural sunrise with respect to light intensity (Figure 2). However, the shape of the three curves is preserved completely, meaning that the plants in these experiments did receive a sunrise that reflects the rate of changes in light frequency distribution and intensity.

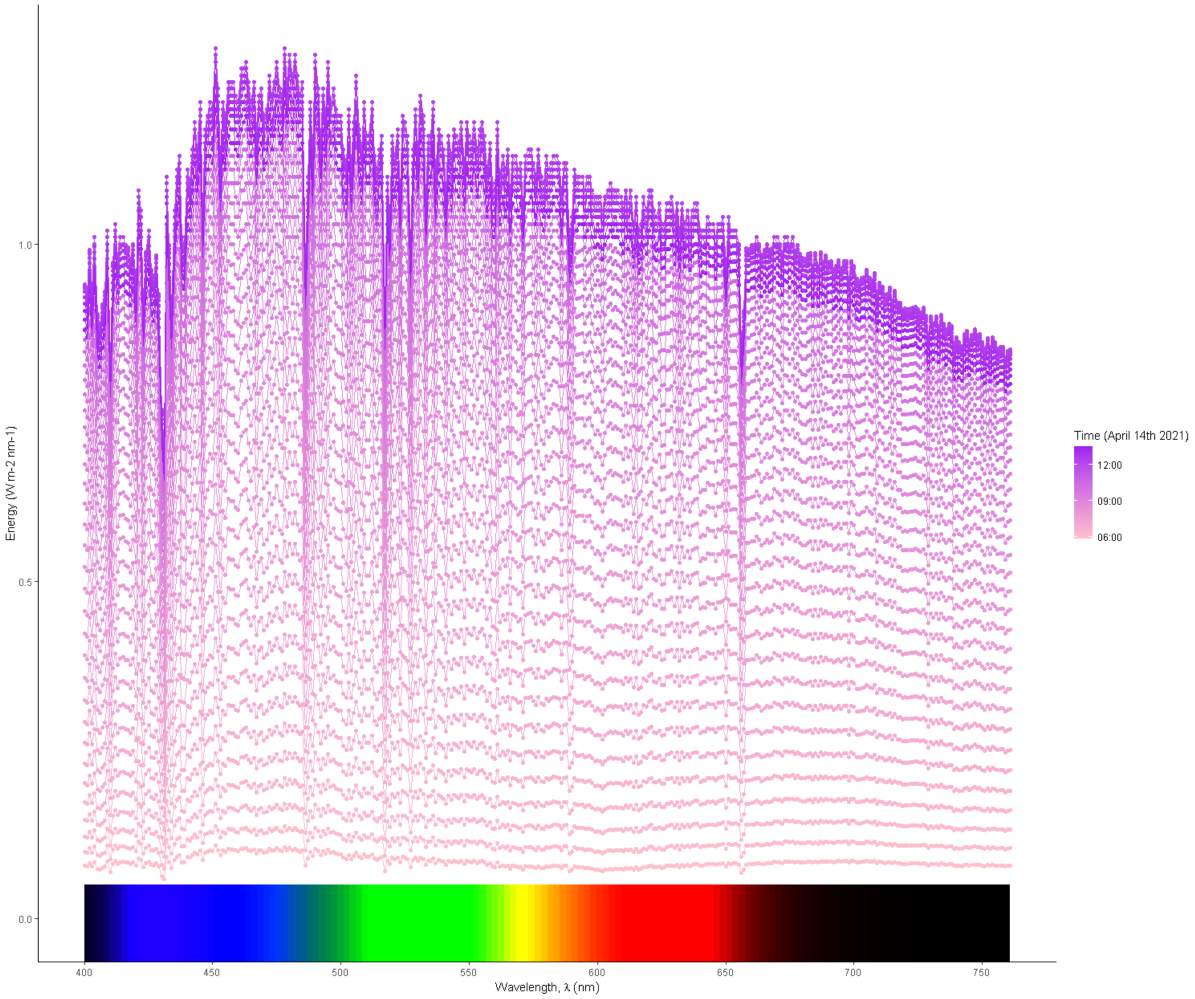

Figure 1.1 Spectral irradiance between 400 and 761 nm between 05:50 and 13:30 on April 14th 2021 in York. Data was collected using the TUV calculator. Each time point is represented by a single colour. Dawn is shown at the bottom of the figure. As time passes energy increases. Irradiance is given in W m^{-2} nm^{-1} which indicates the energy received by the equipment in watts per metre for every wavelength in the visible light spectrum. Sager et al have published formulae to convert between absolute photons and energy. (42)

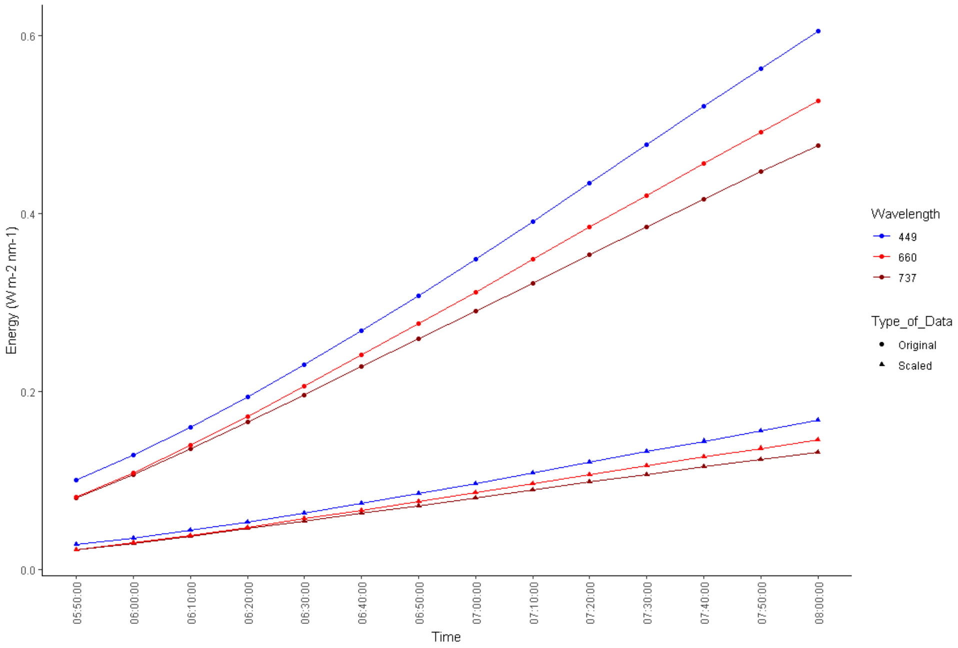

Figure 1.2 Spectral irradiance emitted from red, far-red and blue wavelengths of light on the morning of April 14th 2021. Raw data from the TUV calculator and scaled irradiance values are shown. Due to limitations with the lighting system, only the first 2 hours of the sunrise were programmed.

Sunrise conditions reduce radish biomass but not kale.

Plants were grown under the conditions shown in figure 2 and harvested so that the impact of sunrises on the growth of microgreens could be analysed. To analyse the impacts of sunrises on biomass I performed analysis of variance and Tukey HSD tests. My analysis suggests that sunrises significantly decreased the biomass of radish plants (figure 3). Whereas, in both varieties of kale there is no significant change to biomass between control and sunrise conditions. These results were also mirrored in leaf mass (figure 4). Though the decrease in radish leaf mass is not as significant as the decrease in whole mass. Sunflower plants were removed from the experiments due to mould forming in the tubes.

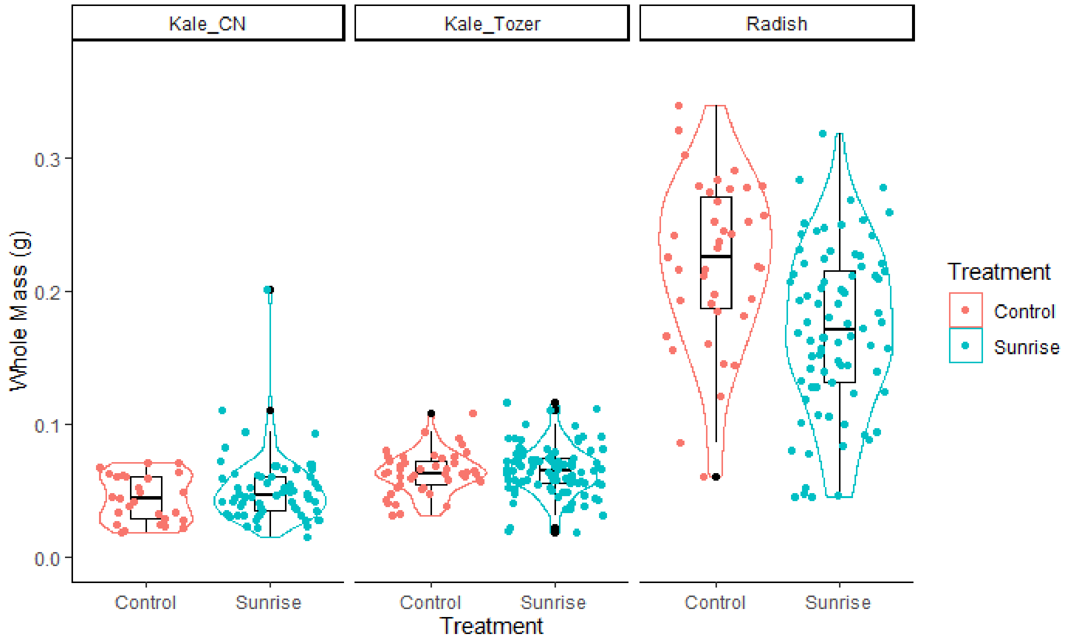

Figure 1.3 Biomass of harvested microgreens across control (n=~30) and sunrise conditions (n=~80). One way ANOVA was performed followed by Tukey HSD and Shapiro-Wilks test were performed. Radish exposed to sunrises had biomass significantly decreased (p = 4.68e-05).

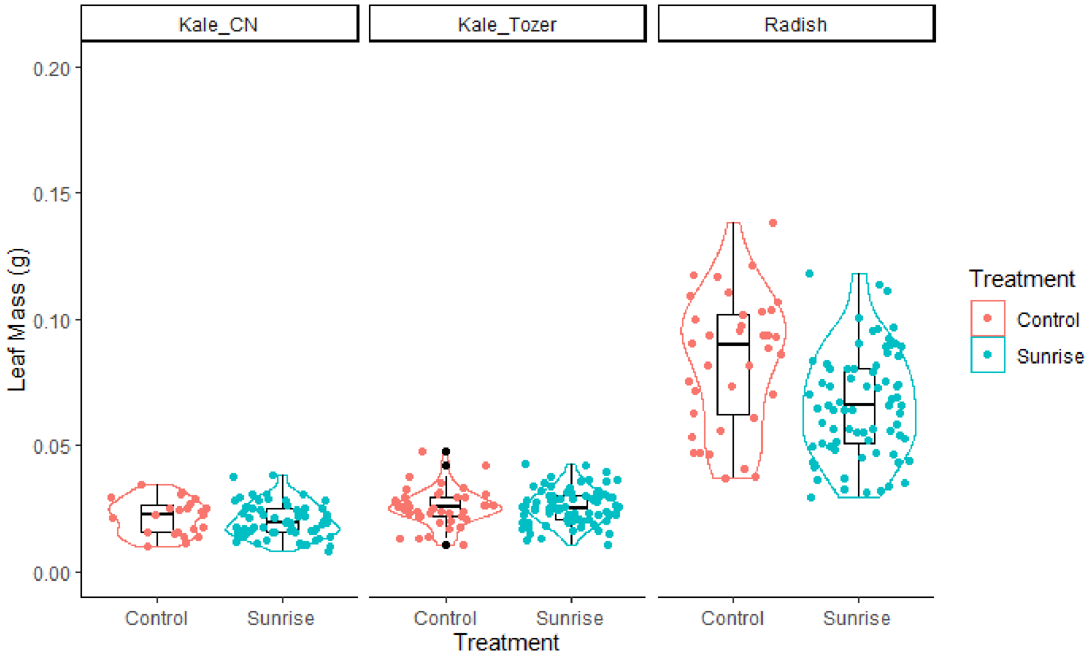

Figure 1.4 Leaf mass of microgreens grown under control (n=30) and sunrise conditions (n=80). One way ANOVA, Tukey HSD and Shapiro-Wilks tests were carried out. Radishes exposed to sunrises had significantly smaller leaf mass (p = 0.00566).

Sunrises do not impact germination in kale or radish

To examine if microgreen germination was impacted by the intensity of light at the beginning of the photoperiod, I counted how many seeds of each species grew each day. Microgreen germination was unaffected by sunrise conditions (Figure 5). This suggests that realistic sunrise conditions do not have an impact on germination rate.

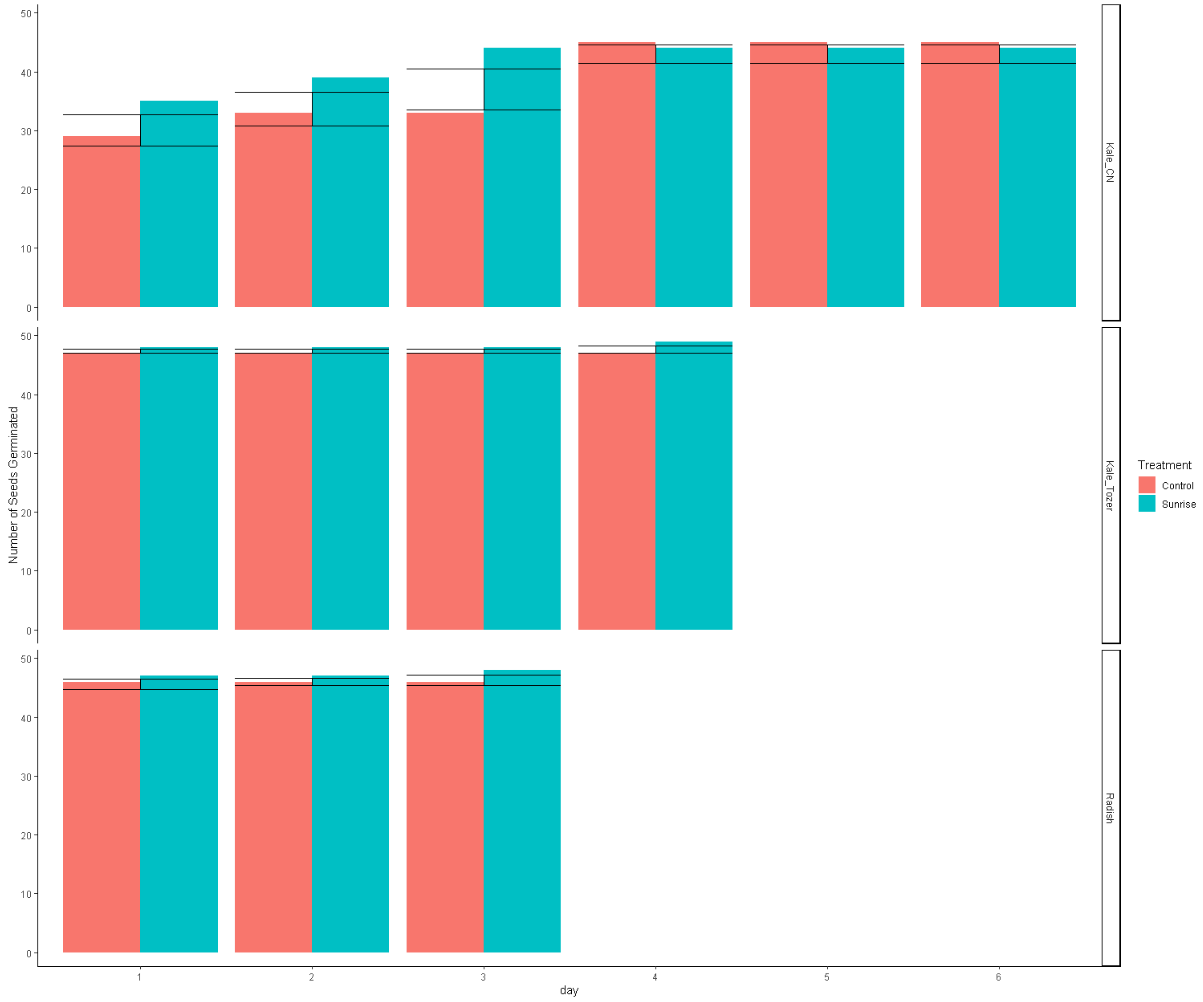

Figure 1.5 Germination of microgreens over the course of sunrise and control experiments (n=~49). Germination was moinitored daily.

The largest difference in germination rate was observed in the CN variety of kale. This could indicate that kale germination is sensitive to sunrises and may warrant further investigation. To achieve this I would repeat this experiment with a larger sample size and include different sunrises.

Discussion

Gradually changing light intensity and quality impacted the biomass of radish

My data shows that when subjected to gradual changes in both light intensity and frequency distribution radish plants have their biomass reduced. However, the biomass of kale was not significantly altered, highlighting that gradual early morning light may have an impact on the development of some species of plants but not others. This may suggest that Radish is either better able to utilise the early morning light for photosynthesis or its photomorphogenesis pathway is sensitive to light in the morning. It is intriguing that radish appears to be sensitive to early morning light and not kale, given that Arabidopsis is sensitive to early morning light and more closely related to kale than radish. Perhaps kale is sensitive to early morning light and experiences less obvious changes in physiology such as changes to photosynthetic pigment and mineral concentrations. To fully understand the mechanism that underlines the decrease in radish biomass the transcriptomic profile of microgreens that are experiencing changes in light quality and intensity needs to be examined. This could be done through the utilisation of RNA-seq approaches from samples that have been snap frozen during changing lighting conditions. Several samples should be harvested from each species at different times during the changing lighting conditions so that the dynamics of early morning gene expression can be examined. Expression may occur in waves like what was shown in the Arabidopsis early morning gene network (24). Future studies in radish should focus their transcriptomic analysis on how expression changes for genes that have GO terms associated with growth or auxin synthesis to see if early morning light directly regulates growth.

The lack of sensitivity exhibited by kale varieties towards gradually changing morning light conditions highlights the potential use of artificial sunrises in a vertical farm setting. Our data shows that there is no significant difference between the biomass of kale when grown either in fixed lighting conditions or gradually changing conditions. The appeal of this result to a vertical farm is that there is a reduced energy cost associated with the gradually changing lighting conditions because less light is required to grow the plants. Furthermore, the less light required to grow crops in a vertical farm causes less heat accumulation, further reducing the energy requirements for cooling the farm. Lighting and cooling account for up to 85% of operational energy expenditure on vertical farms (13). However, sensitivity towards gradually changing lighting conditions in the early morning exhibited by radish highlights how gradually changing light conditions should be evaluated on a species-by-species basis. Our data shows a decrease in biomass in response to gradually changing light conditions in the morning which highlights the relationship between biomass and light input / energy costs. Growers could use this data to make more informed choices about the crops they grow and the lighting regimes they use on those crops.

It is worth noting that the decreased biomass associated with sunrise regimes seen in radish could be because the sunrise regimes receive less light compared to the control conditions. However, this highlights that small changes to light regimes have the potential to save energy in vertical farms whilst only slightly reducing biomass. Similarly for the kale varieties sunrise regimes highlight how energy efficiency can be improved at no cost to biomass. To determine if these phenotypes are the result of sunrises or the result of a disparity in total light received these experiments should be repeated with a control that does not vary light quality or intensity and matches the total light outputted of the sunrise regime.

Future experiments should examine if plants are more sensitive to gradually changing intensities in the morning or gradually changing light qualities in the morning. This could be done by growing plants and subjecting them to gradual increases in light intensity that are a fixed light quality in the morning. To examine the impacts of light quality in the morning, plants should be grown under a single light intensity but a changing light quality. It may also be interesting to look at how sunrise lengths impact plant physiology given that observations that are yet to be subject to peer review indicate twilight lengths have a significant impact on physiology. Sunrise length may have a profound impact on plant physiology as it is an environmental factor that fluctuates massively over a period of 365 days. Understanding the underlying mechanism of the sensitivity exhibited by radish towards gradually changing light conditions in the morning may lead to a lighting regime that can positively exploit this mechanism and improve crop yields for a lesser energy cost.

The use of gradually changing lighting conditions in vertical farms

Our findings do suggest that gradual changes in light quality and intensity could reduce energy costs in a vertical farm. However, our data is limited by the species and developmental stage of plants that we used to perform our experiments. An actual vertical farm will grow a greater variety of crops and as our results show, gradual changes to lighting conditions in the morning can mediate different physiological responses in different species and perhaps even different varieties of the same species. Also, our study examined how seedlings respond to gradual changes in light quality and intensity, commercial vertical farms may not exclusively grow crops that are at this development stage. It may be that plants exhibit sensitivity to gradual changes in lighting differently at different developmental stages, similarly to how plants perceive photoperiods differently across developmental stages (43). Future studies should repeat our experiments but using different species to uncover how viable gradual changes to lighting conditions are as a holistic approach to making vertical farms more efficient. I also did not track the extent to which the reduced lighting impacted energy utilisation and costs, but this would be valuable information to quantify the impact of gradual lighting regimes on vertical farm efficiency.

Another factor which determines the potential of gradually changing lighting in vertical farms is the layout of the farm itself. In farms where there are multiple beds stacked alongside each other, light may from one bed may be cast on to others. This would alter the total light plants receive and may cause physiological differences than what has been observed in our experiments. Circumventing this issue may prove to be a challenge in most vertical farms as blocking lights from other beds would obstruct air flow and alter humidity. This would negatively impact growth, offsetting the potential benefits of gradually changing lighting regimes. However, whether or not light bleeding from one bed to another would negatively impact plant growth remains to be seen. Perhaps light bleeding from other beds could enhance growth and the layout of a vertical farm could be arranged so that light bleeding could be utilised to the benefit of the grower. Future studies should aim to grow crops in a vertical farm setting where there is light bleeding between beds and examine the heterogeneity of physiological traits. Then the practical implementation of gradually changing light conditions can be properly assessed.

Furthermore, plants experience different ratios of light at different times of the day. Lots of studies which aim to improve vertical farming practices examine how changes to the ratio of different wavelengths of light impacts plant performance (44–46). Perhaps plants are sensitive to how the ratio of blue and red-light changes diurnally. The plants in this experiment were subjected in darkness before a sunrise. However, this is not completely representative of what occurs in nature, before the sunrise plants will experience high proportion of blue light at low light intensities before the ratio of light changes. Perhaps plants would be more receptive to gradual changes in light quality and intensity if they experienced low intensity a low intensity of blue light before the artificial sunrise.

Chapter two: Photoperiod shifts can be used to reduce energy costs of vertical farming

Introduction

Due to the earth rotating on its axis and around the sun plants experience diurnal and seasonal changes in light, temperature, and humidity. Using this information plants can determine the length of the light phase in a day, known as a photoperiod, and entrain their circadian clock (43). Photoperiod entrainment synchronises the phase of the circadian clock with the light phase of their environment enabling the plant to time physiological processes such as photosynthesis and growth in line with changes in its environment (47).

Moreover, plants use photoperiod sensing to determine seasonal changes. Detecting changes in season is advantageous because it enables flowering at the most optimal time, improving the odds of successful reproduction (48). There are two coincidence models postulated for the detection of photoperiod. The external coincidence model assumes that there is a quantitative threshold that a circadian regulated molecule must overcome. When this threshold is surpassed and light is still being perceived by the plant, a photoperiodic response is mediated (49). The internal coincidence model states that a photoperiodic response is mediated when more than two rhythms are in phase with each other during the same photoperiod. Each rhythm would have different responses to photoperiod entrainment (50).

Once the length of the photoperiod is detected physiological responses are mounted. An example of this is seem in wild-type *A. thaliana* long day conditions (LD; 16hr light) produce shorter hypocotyls than those grown under short day conditions (SD; 8 hr light)   (51). These phenotypes are regulated by two transcription factors, *PHYTOCHROME INTERACTING FACTOR 4* (*PIF4*) and *PHYTOCHROME INTERACTING FACTOR 5* (*PIF5*) (52). *PIF4/5* are both downstream targets of Phytochrome B (PhyB) in the light signalling pathway. In the presence of light PhyB degrades *PIF4/5* (53). In SD conditions, where there is an absence of light, *PIF4/5* are not degraded by PhyB and reach high concentrations (54). This results in elongated hypocotyls because in darkness *PIF4/5* regulate genes involved in the production of Auxin (53). *PIF4/5* are reported to upregulate *CYCLING DOF FACTOR 5* (*CDF5*) which upregulates *YUCCA8*, a gene involved in auxin synthesis (51,55).

Most literature focuses on how a single photoperiod impacts plant performance (6,11,12). However, as seasons change so do the photoperiods which plants are subjected to. For instance, during the transition between summer and autumn the photoperiod decreases. How plants respond to these changes remains unclear. One area of interest is how plants integrate information about past photoperiods to make developmental decisions. Ronald et. al (In prep) demonstrated that in many ecotypes of *A. thaliana,* hypocotyl elongation was impacted by the order of two different photoperiods that seedlings were exposed to, for example two SDs followed by two LDs or vice versa. A trend in the ecotypes used in this study was that their phenotypes were determined by the last photoperiod they were exposed to. However, Ws-2 ecotypes did not follow this trend suggesting that sensitivity to transitioning photoperiods can vary across ecotypes of a species.

Ronald et al's findings highlight a potentially exploitable phenomenon to improve the efficiency of indoor vertical farming. Longer photoperiods are associated with increased nutrient content (56) and shorter photoperiods are associated with increased hypocotyl length (51). We hypothesized that photoperiod shifts would strike a balance between the high nutrient and aesthetic phenotypes associated with LD and the improved hypocotyl lengths of SD grown plants. This would present a novel lighting regime produces commercially viable microgreens that reduces energy.

We investigated if transitioning between different photoperiods would have a significant impact on hypocotyl length in microgreens which are used in a vertical farm setting. If the transition from LD to SD or SD to LD produced microgreens that phenocopied the LD plants, then there is the possibility that they could be implemented into commercial vertical farms to reduce energy costs for microgreens. To test if photoperiod shift transitions could be used in vertical farms, Kale, (*Brassica oleracea*) Radish (*Raphanus raphanistrum*) and Sunflower (*Helianthus annuus*) were grown in either SD or LD and then switched to the alternate condition. We determined that CN kale, radish and sunflower hypocotyls phenocopied the first photoperiod exposure– the opposite of what Ronald et al observed– suggesting that these microgreens are less sensitive to photoperiod shifts than Arabidopsis.

Methodology

Sowing Microgreens

Microgreens (Table 1) were sown into trays containing a compost mix comprised of peat, sand, vermiculite, coir and Osmocote fertiliser. Seeds were sown approximately 1cm below the surface of the soil. Each species of microgreen was sown into four trays which would be subjected to one of four different experimental conditions.

Microgreen	Species	Variety
Sunflower Shoots	*Helianthus annuus*	Black
Kale (Tozer Seeds)	*Brassica oleracea*	Dwarf Blue
Kale (CN Seeds)	*Brassica oleracea*	Dwarf Blue
Radish	*Raphanus raphanistrum*	Sangrin

Table 1. Species and variety of the microgreens used for photoperiod experiments.

Growing Microgreens

Sixteen trays were placed into one of two growth cabinets. Both cabinets were set to 19°C, 60% humidity and 70 µmols of light. The short day (SD) cabinet had lights on for 8 hours a day and the long day (LD) cabinet had lights on for 16 hours a day. Trays were inspected daily to see if hypocotyls had emerged from the soil and were visible. Once at least 50% of the microgreens in the soil were visible the date of this observation was noted. Three days after this date the tray would either be moved to the alternate cabinet, or it would stay in its original cabinet as a control. In either case the tray would be in its original or alternate cabinet for a further three days until it was taken out to have the microgreens imaged. Imaging and switching took place at ZT5. This protocol ensured that most of the plants that underwent photoperiod shifts would have had equal exposure to each photoperiod treatment, although the order of photoperiod exposure varied between the groups. This experiment was carried out sequentially three times to produce three biological replicates, with 40 Individual plants per photoperiod treatment per experiment.

Imaging Microgreens

Hypocotyl lengths were measured in this experiment so that the results could be compared with those from Ronald et al. To image the microgreen hypocotyls, all leaves were removed, this made orientating and flattening the microgreens easier. Once the leaves were removed the microgreens were removed from the soil and were laid flat in an Epson scanner. The scanner would then take a high-resolution scan which was used to determine the length of each individual microgreen across all conditions.

Analysing Microgreen Images

Microgreens were measured using ImageJ and a stylus. The stylus was used to draw a line over the microgreens from the shoot apical meristem to the base of the stem. Using the measure function in ImageJ, outputted a length in pixels which could be converted to a length in mm using the following equation, Length in pixels * (25.4 / DPI). This was validated using a scan of a steel ruler. This data was then incorporated into RStudio, followed by an analysis of variance, Shapiro-Wilk tests and Tukey HSD.

Results

The impact of photoperiod shifts on microgreens.

Following the removal of microgreens from the soil and subsequent scanning, hypocotyl lengths for each species were analysed. Hypocotyls grown under LD were the shortest across all species, this is consistent with observations made by other groups studying *A.thaliana* (51). In radish there was no significant difference between the hypocotyl lengths of plants grown in SD conditions and those that were transitioned between SD to LD. Hypocotyls grown under LD to SD were significantly shorter than those grown under SD to LD and SD. The Tozer Seeds variety of kale showed a significant increase in hypocotyl length between hypocotyls grown under SD to LD relative to those grown under LD to SD. Hypocotyls grown under SD conditions were significantly larger than those grown under any other condition. The CN variety of Kale showed no significant difference between hypocotyls grown under SD and those grown under SD to LD. Hypocotyls grown under both SD and SD to LD were significantly larger than those grown under LD and LD to SD. The only significant difference observed in hypocotyl length of sunflowers was a significant increase between hypocotyls grown in SD and hypocotyls grown in all other conditions respectively.

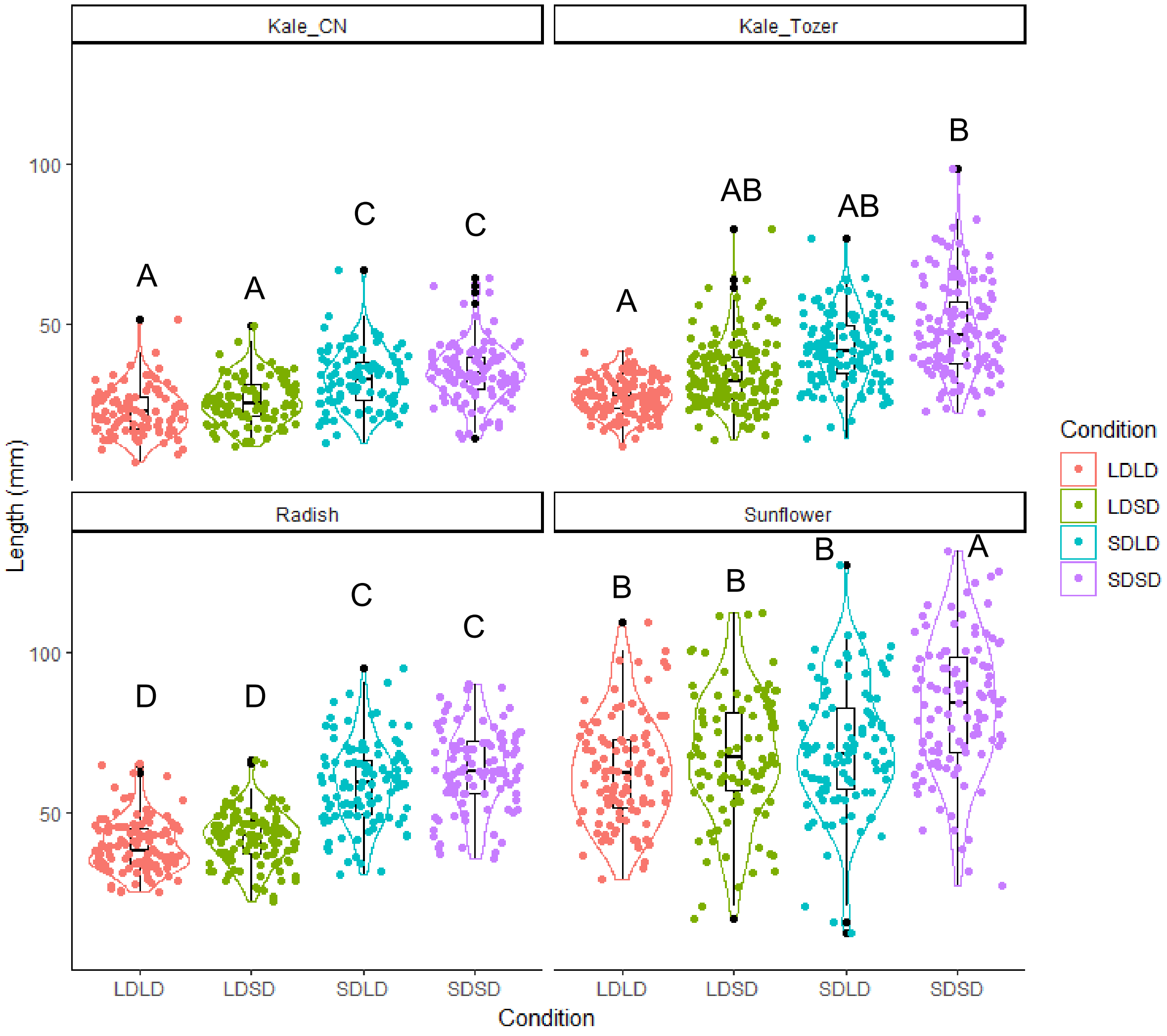

Figure 2.1 Hypocotyl lengths of microgreens grown in different photoperiodic conditions. ANOVA and Tukey HSD tests we carried out to assess the difference in hypocotyl length between plants grown in different photoperiodic conditions. A = Plants grown under this photoperiodic condition exhibited significantly different hypocotyl lengths compared to plants from the same species that were grown under different photoperiodic conditions. B = Plants grown under this photoperiod condition have significantly different hypocotyl lengths than plants of the same species that were grown under SD conditions. C = Plants grown under this condition have significantly different hypocotyl lengths compared to plants of the same species that are grown under both LD and LD to SD. D = plants grown under this photoperiod have significantly different hypocotyl lengths compared to plants of the same species that are grown under SD and SD to LD.

Discussion

Transitioning from SD to LD increases hypocotyl length relative to those grown under LD to SD

Our results show hypocotyls that were transitioned from SD to LD are significantly longer than those transitioned between LD to SD except for *H. annuus*. This is contrasted by Ronald et al, who show that *A. thaliana* hypocotyls that were transitioned from LD to SD were significantly longer than those transitioned from SD to LD. Ronald et al grew their hypocotyls using Murashige – Skoog media. Studies have been conducted which show that growing mediums affect the growth of seedlings and therefore using different growing mediums would have impacted seedling growth (57). MS media does not have a soil microbiome present. The soil microbiome improves plant growth by improving nutrient uptake from the soil into the plant (58). Also, Ronald et al sterilised their seeds which then were cold stratified before being placed under light, whereas the microgreens were not sterilised and were placed into the soil. To penetrate the surface of the soil the microgreens will need to grow meaning that they are at a different stage of development by the time that they can register light information from their environment. The microgreen seedlings were also not exposed to the 4-degree cold stratification, as the microgreens are not stratified at the vertical farm. It is well established that plants use temperature and photoperiod to detect seasonality, and this may have impacted the physiology of the plants (59). Both *A. thaliana* and *H. annuus* do not develop longer hypocotyls when transitioned from SD to LD. This could be due to variation in the way that different species detect photoperiodic information, for instance I observed that cotyledons of *H. annuus* remain in the seed for longer than any of the other species. This could limit the quantity of photoperiodic information *H. annuus* receives, thus altering its response to transitioning photoperiod.

If the total amount of light a plant receives determines its hypocotyl length, then it would be assumed that there would be no significant difference between plants transitioned from SD to LD and those transitioned between LD to SD. This idea is not supported by my results, instead it appears that the order of the photoperiod a plant is exposed to is a determinant of hypocotyl length. Understanding what causes plants grown under SD to LD to have hypocotyls longer than those grown under LD to SD would be instrumental to exploiting this phenomenon to improve agricultural practices. To better understand why this occurs, future studies should study how varying the number of photoperiods and order of photoperiod impacts physiological traits.

The differences seen between species could be the result of unique hypocotyl expansion patterns of each species. It is possible that sunflower hypoctyl expansion occurs later than that of other species. This is unlikely to be the case for *Arabidopsis* because Ronald et al observed hypocotyl expansion occurring after 5 days of growth and our experiments spanned 6 days of growth in total. This is also unlikely to be the case for sunflower because other groups have measured hypocotyls of sunflower under different light qualities and report that hypocotyl elongation occurs between 2 – 4 days of growth under different types of LED lighting (60).

Can varying photoperiods be used to improve the efficiency of vertical farms?

Studies have attempted to find an optimal photoperiod length which improves yields, nutritional content and increases the energy efficiency of vertical farms. One study examined how constant light can be used to improve these qualities in *Brassicacea* microgreens. It was found that constant light improved yield and nutritional value (61). This is consistent with other studies that also suggest constant light improves the energy efficiency of vertical farms whilst maintaining crop quality (62). However we are unaware of any research which examines how photoperiod shifts can be used to improve the efficiency of vertical farms.

My data shows that some varieties of kale and radish can be grown to the same length as those under SD conditions for lower energy requirements. Shorter microgreens grown under longer photoperiods have many properties that are beneficial to vertical farmers, such as darker colouration and higher nutrient density (11). Future research should investigate the nutritional quality of LD to SD grown microgreens to see whether we were able to achieve agriculturally relevant traits in a more energy-efficient manner. In the other variety of Kale (Tozer) shifting photoperiods in either direction resulted in significantly shorter hypocotyls than those grown under SD conditions still highlights how microgreens can be grown to be tall whilst also reducing costs associated with lighting. Changing photoperiods appeared to have no impact on the physiology of sunflower plants which could be due to the seed covering, as discussed above. To understand how to implement photoperiod shift changes in to vertical farms to improve their efficiency this experiment should be repeated using the growing methodologies that are common place in vertical farms. Vertical farms typically use aeroponic or hydroponic systems and grow crops using various mediums such as recycled carpet matts. Ronald et al's., results were obtained using plants grown on media and my results were obtained using plants grown in soil. This could suggest that the medium used to grow plants does not impact plant growth. Yet this does need to be investigated by performing photoperiod shift experiments on media, carpet matts, in soil, hydroponically and aeroponically on a species-by-species basis.

Regardless of the impact of growing medium on hypocotyl length it is essential that the impact of shifting photoperiods on nutrient content and taste be investigated. As mentioned in the introduction SD conditions are associated with elongated hypocotyls and LD conditions are associated with nutrient rich plants. It remains unclear if shifting between SD and LD can produce nutrient rich microgreens which are taller than those typically associated with LD whilst being closer to SD grown plants with respect to energy costs. This must be investigated by repeating this experiment in a vertical farm setting and carrying out elemental analysis on harvested plants. This would allow clear comparisons to be made between SD to LD, LD and SD conditions. Similar analysis needs to be performed but with compounds associated with taste to examine how taste is impacted by photoperiod and if better tasting microgreens can be attained by altering photoperiodic conditions.

Plants also use temperature as well as photoperiod to determine seasonality (59). Given that these two envoronmetal factors interact and alter plant physiology in nature it would be interesting to explore this phenomena in a vertical farm setting (63) One of the largest costs associated with vertical farms is the cost to cool the farms. In this experiment temperature was kept constant between the different photoperiods. Therefore, the outcome on the plant of transitioning between both photoperiod and temperature remains uncharacterised. This should be studied by altering temperature while photoperiod changes and examining how this impacts physiology. If changing temperature has a significant impact on physiology, then understanding the relationship will be crucial in forming novel growing methodologies to improve the efficiency of vertical farms. Finally future experiments should test a wider selection of both plant species and varieties. My data suggests that there is a range of sensitivity to photoperiod changes across varieties of kale and between sunflower and all other species included in the experiment. Repeating these experiments in a vertical farm setting with a greater variety of microgreens will highlight which microgreens are the most sensitive to shifting photoperiods. This will allow growers to select the most energy efficient and economically favourable crops to grow.

Chapter three: Exploiting heterogeneity in a vertical farm to produce novel approaches to optimise growing conditions

Introduction

Vertical farms allow growers to customise the growing conditions of crops. Plants are sensitive to minor changes in environmental conditions, such as light, temperature and humidity. For instance, Arabidopsis is sensitive to as little as a 2°C change in temperature (25). It is likely that there are optimal levels of these conditions which maximise yield, which will be specific to each species (64,65). Minor environmental differences may also impact agriculturally important qualities other than yield, such as aesthetic qualities, nutrient concentration and taste compounds (66–68). Farmers need to balance these requirements when designing their optimised vertical farm conditions.

To optimise vertical farm conditions, researchers will employ an experimental design in which they measure agricultural traits in several different conditions, a strategy that has been successfully deployed in many studies to optimise light, temperature and humidity separately. For instance, this kind of experimental design was deployed to determine that changing the ratio of red and blue light has distinct impacts on lettuce physiology. Increasing the proportion of blue light decreases lettuce leaf area and shoot fresh mass (69). Another study conducted using lettuce showed that, increasing light intensity can increase crop growth and antioxidant capacity to an extent. However, too great of an increase can reduce crop growth and antioxidant capacity. A similar behaviour is seen in basil but no reduction in growth is observed, it is just maintained and antioxidant capacity is unchanged regardless of light intensity (67). Increasing the temperature of the air that basil is grown in has been shown to increase its fresh mass and height to an extent. Models based on experiments which grew basil at various temperatures show that at approximately 30°C these qualities stop increasing and begin to plateau when this temperature is exceeded (70). Finally, increasing relative humidity up to 85% increases the growth of lettuce (29).

However, these studies do not consider how changing one growth condition may impact another. Other researchers have combinatorically assessed the impact of several environmental variables concurrently. An example of this is shown in a report where lettuce was grown under different light intensities, different air temperatures and different root level temperatures. It was shown that, growing lettuce at an air temperature of 24°C had caused the largest increase to fresh and dry mass. Interestingly, this increase was enhanced when lettuce was grown at both 24°C and 750μmol $m^{-2}s^{-1}$. Fresh and dry mass obtained approximately tripled under this treatment compared to when grown at 24°C and 200 μmol $m^{-2}s^{-1}$ (71). Statistical methods have also been deployed to select a smaller number of informative experiments to optimise several conditions at once in a vertical farm. One study has broken down a typical vertical farm environment into 6 individual components, day temperature, night temperature, electrical conductivity of nutrient solutions, CO_2 concentration, relative humidity and LED lighting. Each component was assigned 3 different levels which altered its intensity / concentration. In the case of LED treatment light quality and or intensity were altered between levels. Twenty-seven experiments were conducted where each of the components was set to a different level and after approximately 45 days the fresh biomass, dry weight, nutrient concentration, leaf area and number of leaves were measured. From these results the optimal growth conditions were determined for basil and lettuce respectively (66). Determining the optimal growth conditions for crops in vertical farms is a complex task and the reports discussed are greatly informative. However, these studies require the presence of many isolated spaces within a vertical farm or time-intensive sequential experiments. They also assume that the environments in which the plants grow is homogenous, when in fact light, temperature and humidity may vary within the physical space due to the layout of lights and the air flow, among other factors(72).

Although the physiological impact of heterogenous conditions in vertical farms has not been fully investigated, it has been widely established that in traditional field-based agriculture, the conditions are not uniform within a field, resulting in heterogeneity in crops. Soil topography can vary across and within fields, which has shown to impact how soil can accumulate water and by proxy impact yield (73). It has also been shown that increasing the distance from the edge of a field that a crop is grown in increases yield. Moreover, crop yields can also be impacted by the landscape that surrounds a field and the amount of shading that a crop receives (74). Within field variation a crop's environment is detrimental to both food security and to profits made by farmers. This is because within field variation impacts how a crop is shaped, its size, colour and weight. All factors that can lead to crops failing quality checks and being disposed of (75). Vertical farming removes sources of heterogeneity such as soil topography and landscape. However, there are still sources of heterogeneity that occur within vertical farms as I show here.

In this chapter I have taken advantage of the heterogeneity of light intensity and quality that exists in a vertical farm to suggest an innovative experimental design for condition optimisation. I have done so by measuring the position-specific light intensity and quality across 256 different positions in a vertical farm and modelling how these factors impact biomass in microgreens. Red light quality appears to negatively regulate plant biomass. We suggest that exploiting existing heterogeneity in vertical farms will enable us to optimise treatments using a less space-intensive set-up than traditional environmental optimisation experiments, allowing researchers to test hundreds of different combinations of conditions in a single experiment.

Method

Growth Conditions

Plants were grown on recycled carpet matting from Growfelt (76). Each matt was divided into 20 regions using waterproof tape. There were 16 equal regions in the centre of each matt (figure 1). These regions were used to grow microgreens which would have their biomass measured. The remaining regions comprised the area on the edge of the carpet matt and had microgreens grown on them, but their biomass was not measured. These regions experienced different conditions from the middle 16 because they were in proximity to the walls of the trays which the carpet matts were inside of. These conditions may have had an impact on physiology and were left uncollected at the end of the experiment. Once the matts were divided, seeds were sown on to the matts. To mimic realistic vertical farm conditions, I sowed microgreens seeds in the densities that are normally used for commercial purposes in this farm. One species of microgreen was grown per carpet matt. The seed density was divided across all 20 regions of matting and sown proportionally according to the area of the region. For instance, the regions on the edge of the matt were larger than those in the centre of the matt and received more seed.

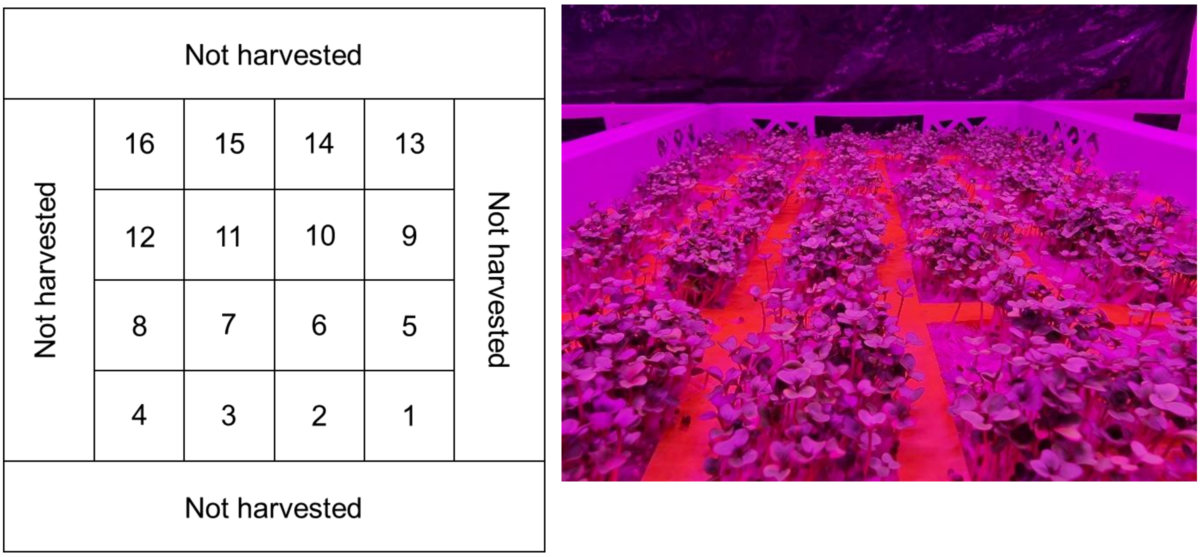

Figure 3.1 Diagram of a tray used to grow microgreens in my experiment and an image of the microgreens as they were growing in the experiment. Each tray was divided into 20 regions as seen in the left diagram. The four larger regions had microgreens grown in them but were not harvested. This was to prevent edge effects occurring within the 16 central regions. The regions were separated via tape seen in the right diagram.

Once sown on to the matting seeds were gently covered in water and stored at room temperature in the dark for 3-4 days to stimulate germination. Once removed from the dark, plants were placed under light conditions and grown for 5-7 days depending on the crop. Crops were grown under 12h light and 12h of dark. To accurately mimic vertical farm conditions, the light regime ordinarily used by the vertical farm was utilised. The software used by the farm does not provide units of intensity for the lighting but percentages of light intensity with 100% being the maximum each channel could output. There were four channels, blue was at 50%, red which was at 80%, far red and white which were both at 100%. Crops were grown using a hydroponic system where the roots of the crops were misted with nutrients dissolved in water every 5 minutes for 1 minute. Nutrients were provided using HydroMax Grow A and B. The electrical conductivity (EC) was set to 1.7, and pH was set to 5.9.

Lighting Conditions

Due to a gradient of light occurring across the growing beds, light intensity and quality was measured in each of the regions of the carpet matt. Light intensity and quality were measured respectively using a PAR special from Skye Instruments and an OceanView Flame from Ocean Insight. Due to human error and time constraints not allowing me to repeat the measurements, the output of the PAR values for the specific light qualities was obtained in an arbitrary unit called counts. However, measuring in counts does still produce a spectrum of light for each region which is the same shape as it would be with the correct units and it does show how light intensity of differing qualities changes. Plants were grown across a vertical stack of 4 beds (figure 2). Each bed had four bays, each bay had the capacity for one tray. Each tray had the capacity for one matt, which had 16 regions where microgreens were grown and had their biomass measured. This meant that there were 256 regions where biomass was measured, each had their light quality and intensity measured.

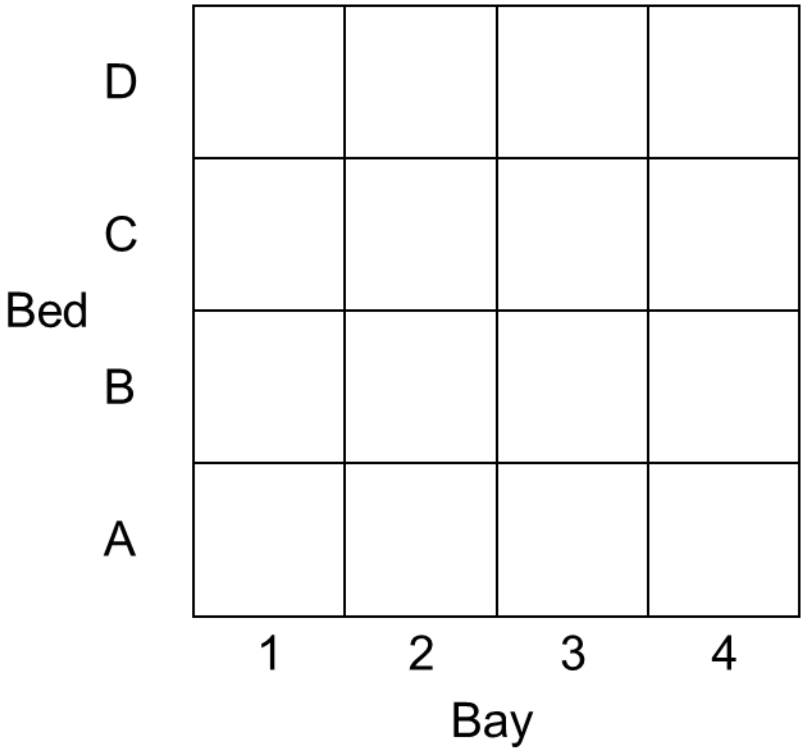

Figure 3.2 Vertical farms are arranged into vertical beds and horizontal bays. In one bed, there are 4 bays. In four beds there are 16 bays. Each bay has 16 regions where microgreens were grown and weighed (Figure 3.1). There were 256 regions where microgreens were grown and weighed.

Harvesting Microgreens

Microgreens were harvested one region of carpet matt at a time using scissors. Cuts were made approximately 0.5-1 cm above the base of the stem to ensure that no carpet mat was included during weighing and to ensure that harvesting during these experiments was an accurate representation of harvest techniques for this growth medium. Once all the plant material in one region was harvested, the fresh mass was recorded. This was repeated for all regions. Note that this is a similar harvesting strategy to that employed in the commercial part of the vertical farm and fresh mass is used as their primary indicator of yield.

Statistical analysis

I created a mixed effects model to determine if biomass could be predicted by light quality or intensity, using the lmer package in R. I took into account random effects such as position in tray (numerical value in figure 1), position type (Corner, edge, centre), bay, bed, and species. I also accounted for linear fixed effects like peak red light, peak blue light, peak far red and overall light intensity. The peak irradiance for red, far-red and blue light were determined by adding all the irradiance values obtained from 653-668nm for red light, 445-456nm for blue and 725-735nm. Peak irradiance values were obtained across wavebands for each light quality to account for species variation in the peak absorbances of photoreceptors and to differing accounts of the peak absorbances of photoreceptors in the literature (77–79).

Results

Total, red, far-red and blue light intensity vary within the vertical farm

Vertical farms will often space the lights used to grow crops in a way that prevents light reflecting off the white walls and back on to the beds. In the vertical farm where my experiments were performed, part of the wall was black. This resulted in less light being available to the crops that were closer to the wall (figure 3)

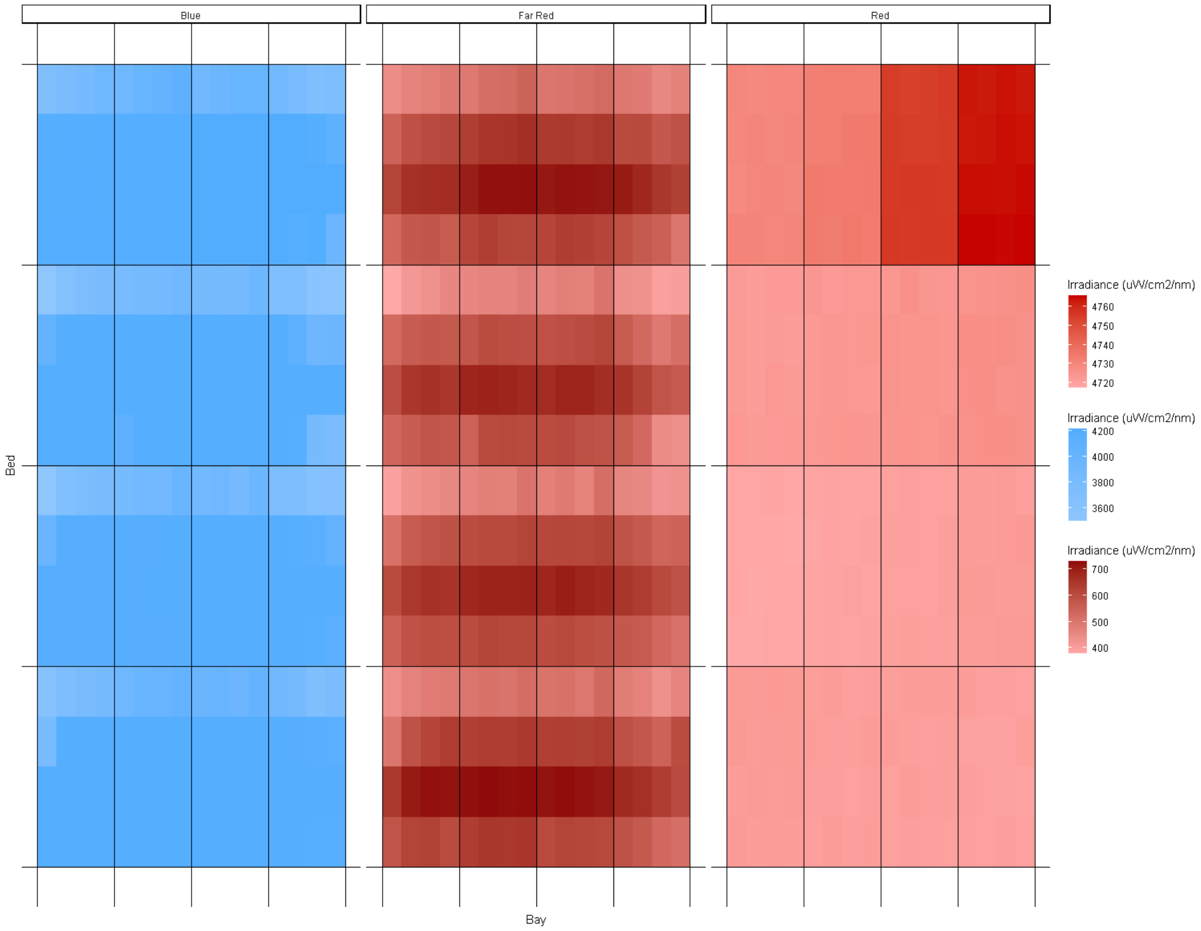

Figure 3.3 Heat maps displaying overall light intensity, peak far-red intensity, blue light intensity and red-light intensity at the plant level across beds used to grow microgreens. Total intensity was measured in µmols and the other light intensities were measured in counts. Each 4 x 4 square on the heatmaps represents a single tray as it is positioned in the vertical farm.

Figure 3 shows that for all trays there is less overall light at the plant level at the back of the tray closest to the wall. Light intensity varies at the plant level within each tray. A similar variation occurs for peak far-red and peak blue light intensity. However, there is little peak red-light irradiance variation in each tray but there is lots of variation between beds. It is important to note that plants sense the ratio of red to far-red light to illicit a shade response, which led us to the hypothesis that the bottom two shelves may experience a shade avoidance response, leading to increased hypocotyl elongation (80).

Differences in red light output between beds

To investigate the source of the observed variation of red light intensity across beds, I viewed the spectrum of a region in a tray from the top and bottom beds (Figure 4). The peak wavelengths of the LED lights do not directly align with the peak absorption bands of cryptochromes and phytochromes.

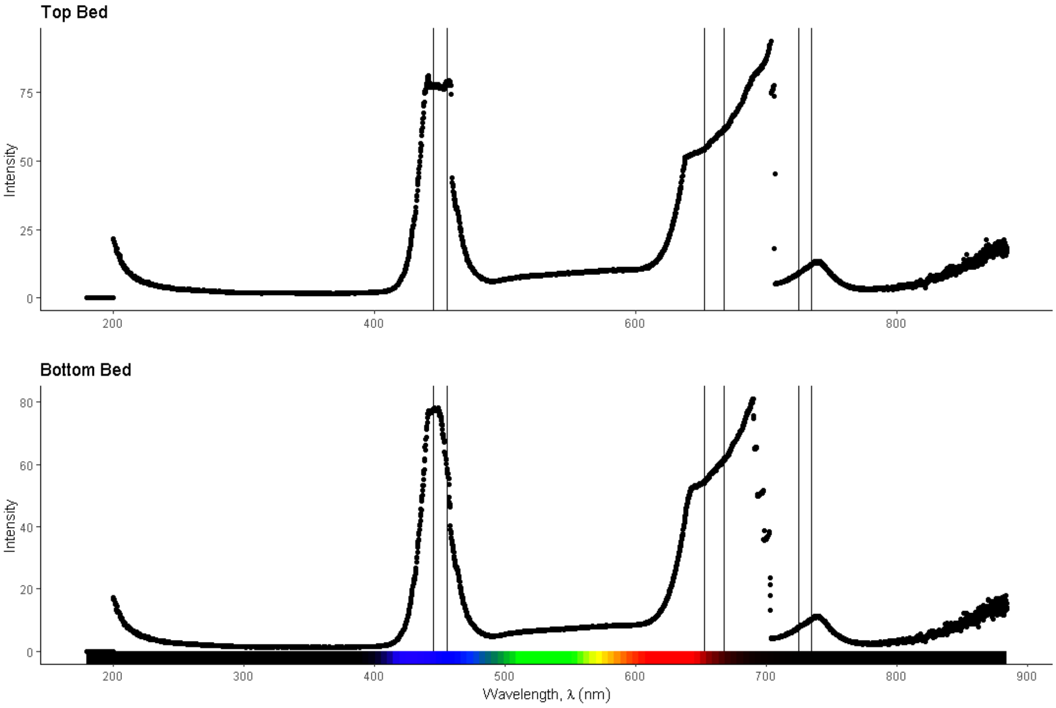

Figure 3.4 The spectrum of a region on the top and bottom beds of the vertical farm where my experiments took place. The vertical lines indicate the wavebands which I am associating with red, blue and far-red regions of the spectrum. Blue light has the highest intensity, followed by red light and far-red light has the lowest intensity. This figure shows that the spectrum outputted by the two different lights is different.

Figure 4 shows that there are clear differences in the slopes of the wavebands I have focused on. Given that red light has the largest contrast in intensity between beds I carried out a linear regression for every region's wavelength and irradiance within the peak red's waveband. I then extracted the slope of every regression and plotted them (figure 5).

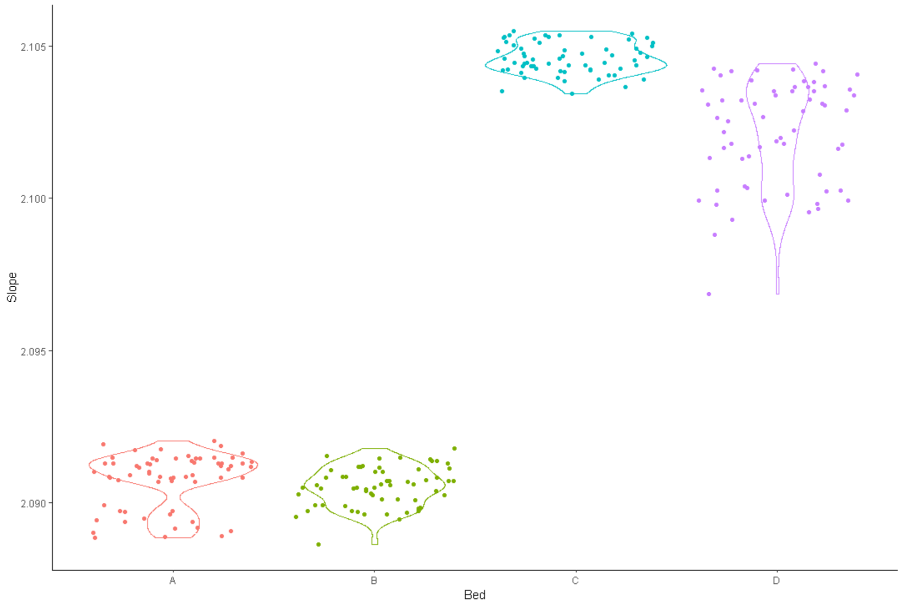

Figure 3.5 A violin plot depicting how the slope value of the red-light waveband changes across beds. This indicates that the light distribution seen in figure 3.3 is not the result of human error and that the lights in the top beds are outputting more red light than the the bottom two beds. The slope indicates how the spectrum of each light changes. The most dramatic shift is apparent between the lights on C and D and A and B.

Figure 5 clearly shows that the value of the slope of the red-light waveband increases for the top 2 beds and validates the observations made in figure 3. Information about the distribution of red light will be provided to the light manufacturers, as this may inform future engineering decisions.

Biomass varies differently to light intensity across beds

Biomass does not vary in a similar way to light intensity (Figure 4). Interestingly beds A and B appear to have higher biomass values for each species. These beds also receive lower intensities of peak red light.

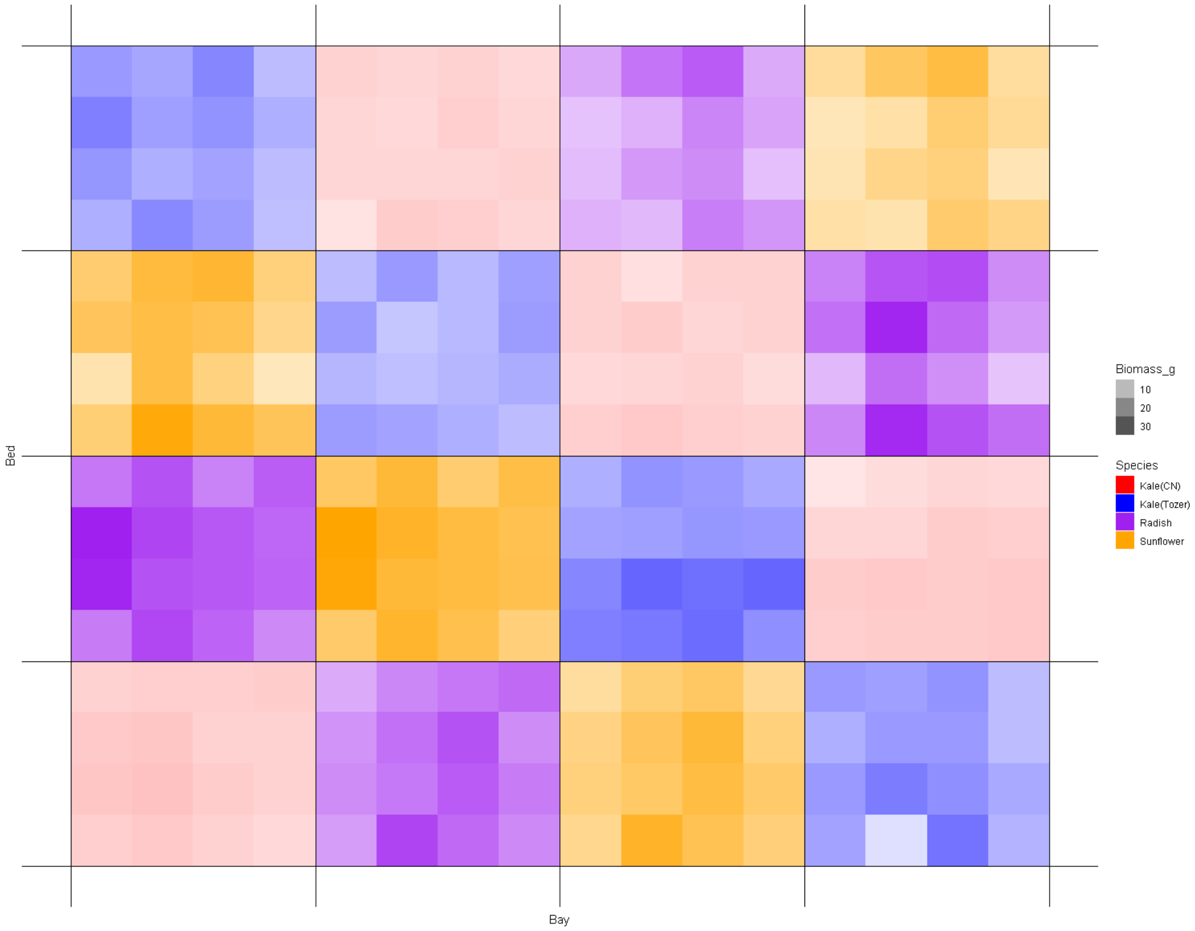

Figure 3.6 A heatmap showing the variation of fresh biomass across beds. Species plotted as they were arranged during the experiment. Each square where biomass was measured corresponds to the same square where light intensity was measured in figure 3.3.

Figures 3 and 6 suggest that increasing the intensity of red light, decreases biomass. To test this hypothesis, I performed further statistical analysis in the form of a linear mixed effects model.

Red light appears to negatively predict biomass

Because there were several random effects present whilst this experiment was being carried out e.g., species, bed, bay, position in tray, position type I created a mixed effects model. I first created a maximal model which had light intensity, peak red-light intensity, peak blue light intensity, peak far red intensity as fixed effects. The random effects listed above were included in the model. I then created a minimal model by employing a stepwise deletion algorithm, which involved removing the least significant fixed or random effect at a time and then performing an ANOVA test between the two models to assess their explanatory power of the variance in the data. This was repeated until the simplest model was obtained that had significant predictive power. The resulting minimal model had red light as the only linear effect and species, bed and position type as the random effects. Whilst this stepwise deletion approach has previously been used in the literature (81,82), it has no guarantee of producing the best model. A better alternative would have been to use a regularisation method, like lasso, to select the optimal set of variables to use in the model (glmmLasso package in R)(83), which would have guaranteed that the best set of variables were selected given a penalty for more model complexity, but this was not undertaken due to time constraints. Nevertheless, this step-wise deletion process provides us a useful first glimpse at how the environmental variables are associated with biomass.

To ensure that red light had a larger impact on plant biomass than other any light quality, models were created which were identical to the minimal model but substituted red light for the other light qualities. An analysis of variance was carried out between the linear mixed effect models that contained different linear effects and the p values of these tests are shown in figure 7.

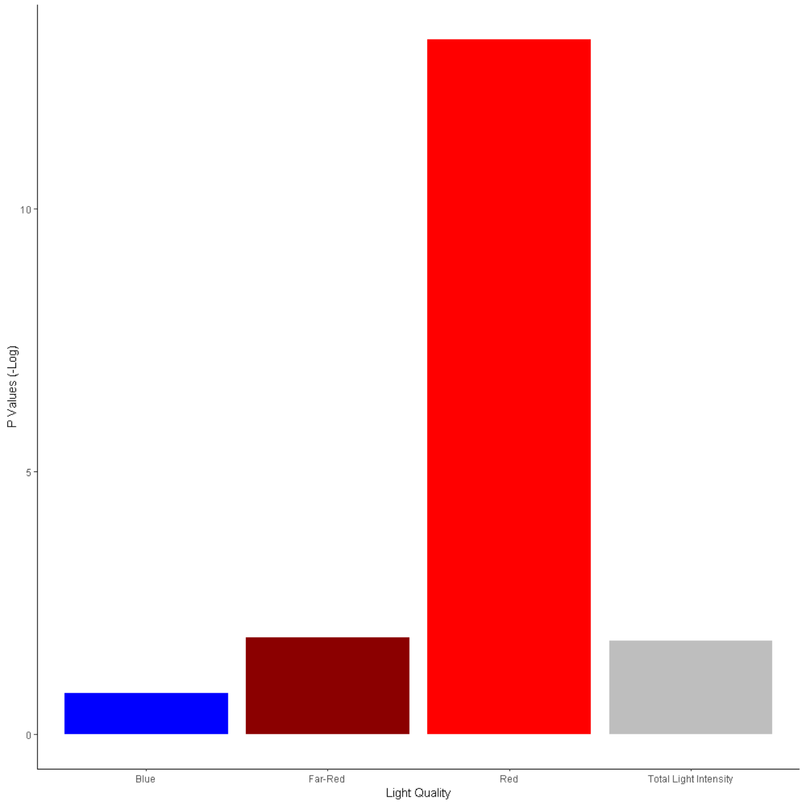

Figure 3.7 -log of the P values obtained from an ANOVA test between a maximal model and minimal models. Each minimal model contained one type of light quality.

Figure 7 shows that red light was the only light quality that significantly impacted biomass. To examine the size of the impact red light had on biomass I obtained the coefficients of each light quality from their respective models. The coefficients for each light quality from each model are shown in figure 8.

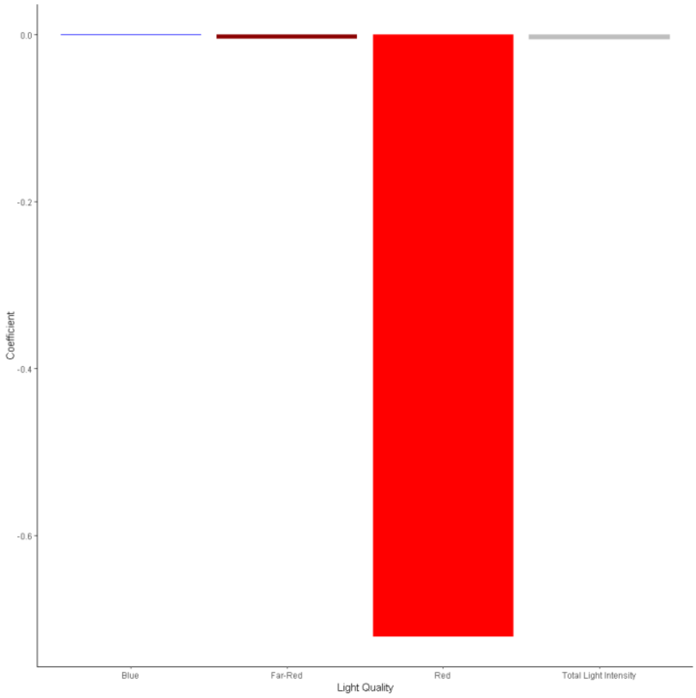

Figure 3.8 The coefficients for each light quality from each minimal model. This indicates the impact on plant fresh biomass that each light quality has per unit increase. It appears that at these conditions increasing the intensity of all light qualities will have a negative impact on plant biomass.

At the conditions measured, increasing each light quality per unit would negatively impact plant biomass. This may not reflect the relationship between plant biomass and these qualities of light under other conditions. However, red light has a significantly large negative impact on the biomass of microgreens.

Finally, to assess how well the model predicts biomass based on species, red light intensity, position type and bed I plotted the values the model predicts for biomass against the actual values for each species (Figure 9). Although our statistical analysis still suggests that red light is significantly associated with biomass, even when controlling for bay and species, Figure 9 indicates that bay and species have a more dominant effect on the final biomass. There may be unknown variables associated with bay, such as temperature and humidity, that may explain the bay-specific effects. Unfortunately, we did not have sufficient access to temperature/humidity sensors to monitor these factors throughout the experiment.

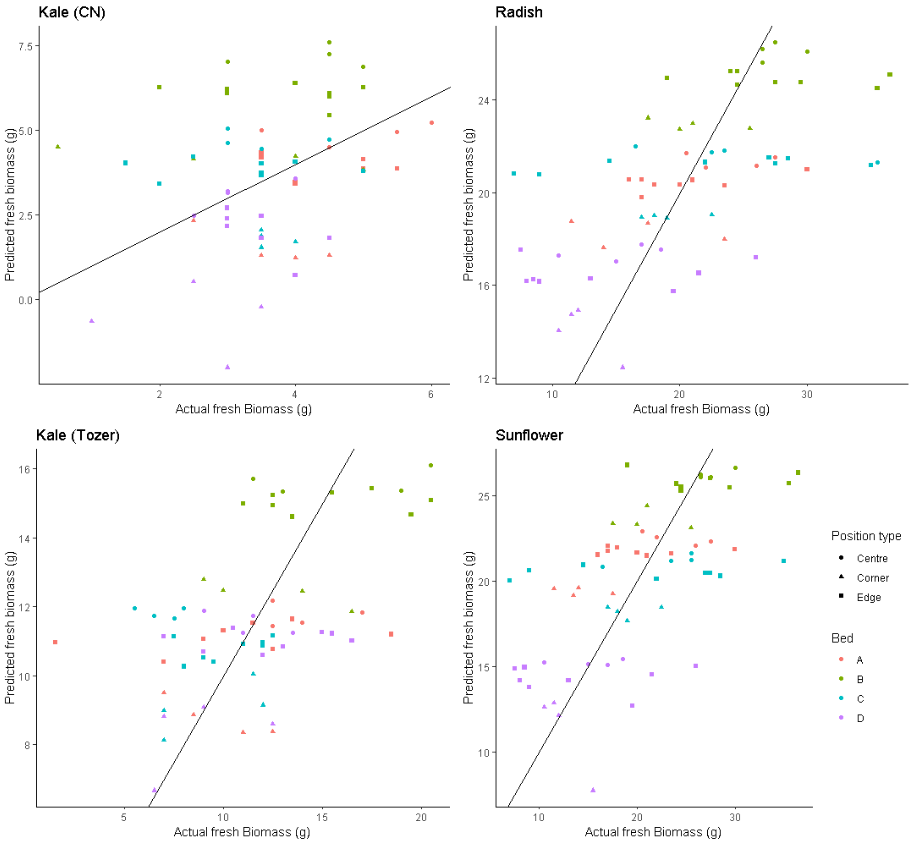

Figure 3.9 A scatter plot for the biomass of each species predicted by the model against the actual obtained values. Each model was a mixed effects model created using the glm package in RStudio. Model complexity was determined by removing effects and performing an ANOVA between the simpler and complex model. This was repeated until the simplest of each model was obtained. These models were then use to predict plant biomass values under the same conditions and were plotted above.

Discussion

Variance in red light intensity may be due to manufacturing

The distribution of red light in each bed was more uniform than the distribution of any other light quality. This could be because the vertical farm uses a higher proportion of red light in its light recipes, resulting in more red light being reflected on to the beds. The differences between red light intensity across the beds is likely due to the spectrum of the LEDs in the red channel being different, this could be due to discrepancies in the manufacturing process.as seen in figures 4 and 5. This result was unexpected because all beds had similar overall light intensities and shared the same light recipe and therefore should have been outputting the same intensity as each other. This result highlights that when growing crops in a vertical farm it is essential to check that the intended light intensities are the intensities that the crop actually receives. This should be an essential step when reporting any findings or recommending growing methodologies to ensure that changes to yield or any beneficial characteristic are a result of the intended treatment and not the result of variance.

Overall light intensity does not predict biomass

Overall light intensity does not predict biomass. This suggests that vertical farmers may not need to worry about the scale of light intensity heterogeneity observed in a vertical farm. Other studies that investigate the response to light intensity from Brassica microgreens show that the optimal light intensity for biomass and nutrient content across a 14-hour photoperiod is

70µmol (64). Other groups studying lettuce also report that whilst yield does increase as light intensity increases, there is visible damage to the crop at approximately 300µmol of light (37). It is likely that light intensity is a significant predictor for microgreen growth but the light intensity range used in this experiment is too small to observe any changes to fresh biomass. Figure 3 shows the range of overall light intensity in this experiment approximately ranged between 300 and 500µmol. To confirm if light intensity is a significant predictor of plant biomass, this experiment needs to be repeated between a light intensity range of 50 to 300µmol of light. This is a worthwhile experiment because if a lower range of light intensity can increase or maintain a similar biomass range to the one seen in this experiment, then it shows confirms that microgreens can also be grown with a lower energy cost.

Red light negatively predicts biomass

As shown in figures 7 and 8, increasing red light decreases biomass in microgreens. As stated previously and shown in figure 3, plants were receiving a higher intensity of red light than they were blue light. This is important because the literature shows that increasing the ratio of red to blue light can increase biomass relative to having a one to one ratio in some cultivars of basil. However it is shown that in other cultivars of basil a one to one ratio is preferable for increasing fresh biomass (84). It is also shown that when grown under a similar red to blue light ratio, both sweet pepper and lemon balm have increased biomass accumulation (44,45). The former study shows that increased proportions of red light can decrease biomass in some plant cultivars and not others. Therefore, increasing red light proportions when growing microgreens could potentially negatively impact their biomass. Given that pH and EC were kept at the same level during this experiment the other predictors of biomass are likely to be relative temperature and humidity. I hypothesis that there are also fluctuations in temperature and humidity across trays and beds. This is because heat is a waste product of the LED lights and the lights in this experiment were not evenly spaced out. There are also power units and electrical equipment on either side of the beds which is likely to obscure air flow and cause temperature differences. To better understand the relationship between light quality, intensity, temperature, humidity and biomass I would repeat this experiment with temperature and humidity loggers at the plant level. I would assess how temperature and relative humidity vary within trays, within beds and across beds and see if when modelled the predictors of biomass change. Carrying out this experiment would show how light quality, intensity, temperature and humidity interplay and could lead to studies which improve the efficiency of vertical farms using novel growth recipes.

Innovative experimental design

We have taken advantage of the heterogeneity of light that occurs in vertical farms to model the relationship of light available at the plant level and biomass of microgreens. We have shown that at a given range of overall light intensity red light negatively predicts biomass. This approach can be utilised by other researchers in smaller farm set ups to optimise growing conditions without the need to perform spatially and temporarily intensive experiments.

Discussion

Novel strategies for optimising light intensity and quality in vertical farms

To improve the energy efficiency and cost of vertical farms, lighting regimes which conserve the yield, nutritional values and aesthetic qualities of microgreens but also reduce the energy costs need to be found. In this Thesis I present data and recommendations of how to optimise vertical farms using complex lighting regimes. In chapters one and two I suggest that altering light quality, light intensity and photoperiod can reduce energy costs without having large phenotypic impacts on microgreens. In chapter three I present a novel protocol that has the potential to be used by growers and researchers to select the optimal conditions for growing microgreens. This is valuable because this protocol does not require large amounts of space and time and could be implemented easily in smaller vertical farms.

Understanding how natural environmental changes translate into controlled environments

Plants have evolved outside of controlled environments and likely use their resources in the most efficient manner when under conditions that reflect those in nature. For example, plants may not utilise light properly at the start of the photoperiod because in nature they do not experience a large change to light quality and intensity in a short time span at this time. There are several publications which study how implementing realistic field conditions in to controlled growth environments impacts plant performance (85–87). However, these studies do not attempt to implement these conditions into vertical farms for microgreens. One such study monitored, light quality, temperature, relative humidity, light intensity, precipitation and wind speeds of a field trial in various plant species. Using specialised growing equipment, the conditions recorded in the field trial were implemented into a growth chamber and the growing conditions experienced by different plant species were altered every 5 minutes to mirror the condition changes of the field trial. It was reported that in most areas of plant performance, plants grown under this condition outperformed sinusoidal and fixed on/off lighting conditions. However, one area of plant performance in which species grown under other lighting conditions performed better on average was plant dry mass (1).

In the first two chapters of this thesis, we employ two naturally occurring phenomena to microgreens, gradual changes to light quality and intensity, mimicking a sunrise. We also subject microgreens to different photoperiod lengths which occur in nature throughout changing seasons.

In chapter one, I have used available atmospheric data and highly programmable LED lighting to grow microgreens which experience sunrise like changes to light quality and intensity. I have demonstrated that gradual changes to light quality and intensity at the beginning of the photoperiod do not decrease the biomass of two varieties of kale. I also showed that when radish is subjected to the same light treatment there is a significant decrease in biomass. The result seen in the kale varieties could improve the efficiency of vertical farms because gradual changes to light intensity and quality do not require as much light as binary on/off changes to lighting. The result seen in radish will help vertical farmers choose their lighting recipes to strike a balance between gradual lighting changes that reduce energy costs and optimise yield.

The results that I have obtained from this experiment are consistent with Chiang et al., because I also observed that under realistic lighting conditions the performance of plant species varied relative to binary on/off conditions.

In chapter two I have shown that photoperiod shifts may be able to be implemented into vertical farms to reduce costs associated with lighting. I grew microgreens in soil and subjected them to SD conditions, LD conditions, SD to LD conditions and LD to SD conditions. I found that LD produced the shortest hypocotyls, SD produced the tallest, LD to SD the second shortest and SD to LD the second tallest in a variety of kale. In other varieties of kale and in radish I have shown there is no significant difference in hypocotyl length between SD to LD and SD grown plants. In sunflower all treatments produced the same average hypocotyl length, except for SD which increased hypocotyl length. This could reduce the energy costs of lighting in vertical farms significantly as SD to LD grown hypocotyls combine the optimal aesthetic qualities of LD grown hypocotyls and the taller hypocotyls associated with SD conditions for a lesser energy cost.

The results I have obtained in this experiment are like those obtained by Wheeler et al. who studied how potato plants respond to daylength changes. It was found that potato plants that are subjected to 12 hour days before being transitioned to constant light have increases in tuber dry mass compared to plants that are grown under constant light and then transitioned to 12 hour days (88). This is indicative that the order in which plants receive SD and LD conditions is a significant factor in different species of plant. My experiment adds to this area of scientific research because it shows there is species level variation in response to transitioning photoperiods and that the results seen in potato are not the result of a transition between SD to free running conditions. I have shown that some varieties of kale and radish perform as well as short day grown plants with respect to hypocotyl length when transitioned from SD to LD. In contrast, this is not observed in sunflower and other varieties of kale. It is my understanding that no other research groups have examined how transitioning the photoperiods that plants are grown under impacts crop growth in vertical farms.

Other studies have shown how unnatural conditions can enhance the efficiency of vertical farms and improve plant performance. Examples of this are the use of continuous low intensity light (62) and LED lights which output light at specific photosynthesis enhancing wavelengths. However, I present ways in which simulating natural environmental conditions can provide novel energy efficient growing methodologies which can improve the energy efficiency of vertical farms.

A novel experimental strategy to optimise the growing conditions in vertical farms

In chapters one and two I suggested how nature-like lighting regimes could be used to improve the efficiency of vertical farms. However, there is still the necessity to test a wide range of growing conditions on the crops grown in vertical farms to find the optimum conditions. The design of a strategy that would enable growers to view the impacts that different growth conditions have on yield would be invaluable to improve the efficiency of vertical farms. This would be particularly useful in smaller farms which cannot perform experiments involving large varieties of growing conditions.

In the third chapter of this thesis, I have tested an experimental strategy that exploits existing heterogeneity of lighting at the plant level in a vertical farm. I have examined the range of this heterogeneity by measuring the variance in the total light at the plant level and the peak red, blue and far-red light intensities 16 times within every tray that microgreens were grown in. I then grew microgreens in the same 16 positions in every tray and measured each position’s biomass, giving us the capacity to measure the impact of 64 lighting conditions per species.

Using this data, I created a mixed effects model to examine if any of the conditions in the vertical farm were significant predictor of biomass. The mixed effects model was reduced to its simplest form, bed, species and position type (corner of tray, edge, centre) were the random effects and red light was the only linear fixed effect. I report that red light is a negative predictor of plant biomass and that overall light intensity does not predict plant biomass.

Limitations of my experiments

Throughout this thesis I have specifically looked at how changing light quality and intensity in a manner that reflects nature impacts plant performance. However, in nature plants experience changes to other environmental conditions which may interact with each other and produce entirely different responses in plants.

A highlight of Chiang's et al's., study is that they show how changes to temperature and humidity in addition to changes in light quality and intensity are important to plant growth. This suggests that one of the limitations with my experiment in chapter one is that I consider changes in light intensity and quality exclusively. I did not implement temperature and humidity changes into my experiment and perhaps implementing other environmental factors such a temperature and relative humidity is the key to boosting plant performance in controlled conditions for a lesser energy cost. This idea is supported by studies discussed in chapter 3 which study the interplay between temperature and light in controlled environments. To test this hypothesis, I would collect temperature and relative humidity data from York across a 7-day period, specifically focusing on changes that occur during the sunrise. I would then combine this data with the atmospheric data available from York and grow plants in these conditions. I could then study biomass and examine if temperature and humidity changes enhance plant performance. I would then aim to implement these regimes into vertical farms.

Also, the experiment carried out in chapter two does not consider the fact that altering light intensity will impact photoperiod-dependant growth. There have been studies that showcase how having longer photoperiods or constant light can be viable in vertical farms because it is possible to maintain high yields with constant low light intensity. Lanoue et al., found that basil, collard greens and amaranth plants grown under 24 photoperiods had greater biomass than those grown under shorter photoperiods (5). Simillarly, Liu et al., found that the biomass of certain microgreens was maximised under particular light intensities and photoperiod lengths (64). Given these results. I hypothesise that transitioning the photoperiod that microgreens are grown under from short days (8-12 hours) to constant light would improve the growth of microgreens and further increase energy use efficiency. To test this hypothesis, I would grow a wide range of microgreens and expose them to short days and constant light in in a vertical farm in different orders at different light intensities. I would then measure their biomass, nutrient content, taste compounds and energy use efficiency to assess commercial viability.

Additionally, this thesis focuses on a narrow range of traits such as biomass and hypocotyl elongation. However, other traits are important to farmers and have been shown to be light dependent. Traits such as pigment concentration, mineral concentration and taste compounds are all impacted by light (62,64,87,89). Pigment concentration is an essential trait because it impacts the colour of a crop which has been shown to be an important factor for consumers when purchasing fruits and vegetables (90,91). Microgreens in particular are marketed for having a high mineral content and it is important that these concentrations are maintained so that consumers have access to a variety of healthy foods (92). Finally, taste compounds determine the taste of vegetables and it is important that the taste of microgreens are not negatively impacted by using more energy efficient lighting regimes (93). If I included the analysis of these qualities in microgreens under different lighting regimes then my analysis of their commercial viability and potential use in vertical farms would have been more robust.

Future experiments

The experiments in chapters one and two, need to be repeated in a vertical farm setting so that the impact of the conditions that cannot be recreated in controlled environments such as, growing mediums and heterogeneity of lighting conditions can be studied. These are crucial experiments which will determine if these approaches can be employed to vertical farms. However, assuming that these approaches can be carried out in vertical farms I have shown that gradual changes to light intensity, light quality and transitioning photoperiods have the potential to improve their efficiency. I believe future studies should combine these two approaches by carrying out experiments of a similar design to that of the one in chapter two with the addition of gradual changes to light intensity and quality at the beginning of the photoperiod. This would allow any interplay between gradual changes to lighting in the morning and photoperiod transitions to be examined. The possible interplay could be exploitable and further improve the energy efficiency potential of the individual approaches. I also believe that the impacts of the length of the period of gradual lighting changes should be assessed. I believe that this should be assessed because observations that are yet to be subjected to peer review suggest that increasing that the duration of twilight lengths can enhance plant performance (33). Sunrise and twilight are similar in that they both are periods in which a plant would experience gradual changes to light intensity and quality and perhaps the length of this period could alter the observations observed in chapter one. This could be tested by slowing the rate the of which the light intensity and quality change, increasing the total time of the period in which these changes occur. I also believe that future studies should test the impact of a wider variety of sunrise like conditions. Different sunrises will have different qualities and Chiang et al., show that plant performance changes when subjected to different realistic conditions from different seasons (87). This could be achieved by using available atmospheric data to programme vertical farm lighting to behave like sunrises from different seasons in the early morning, subjecting them to microgreens and comparing their biomasses. Combining these experiments with photoperiod transitions in a vertical farm will enable growers to determine the optimal way in which yield can be maximised at lower energy costs.

I also believe that the data from these experiments could be used to create an improved version of the model from chapter three. Moreover, I believe that this model could be enhanced by repeating both the future experiments discussed above and the experiment in chapter three in a vertical farm that has the capacity to test a greater range of environmental conditions. It has been documented that environmental conditions such as electrical conductivity, industrial CO_2 input, night temperature and day temperature all impact the growth of crops in vertical farms (66). Some of these effects may alter what I have observed in my model and may be larger predictors of plant biomass. This is an important experiment because building on the model created in chapter three will improve our understanding of the relationship between the environmental conditions in vertical farms and the biomass of crops. As discussed, this model would be particularly useful to smaller vertical farms which do not have the means to carry out these kinds of experiments.

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