The Effect of Supplemental Far-Red Light on *Arabidopsis thaliana* and *Lactuca sativa* 'Rex'

By

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Foreword

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Henry Imberti, Sr VP of Engineering

Introduction

Light-emitting diodes (LED) are becoming increasingly popular as an indoor lighting solution as high pressure sodium (HPS), incandescent, and fluorescent bulbs fade out in popularity. LED lighting is more efficient than other forms of lighting, leading to lower long-term costs compared to other alternatives. The spectrum produced by most LEDs is different than HPS or fluorescent bulbs and the spectra produced can have drastic impacts on plant growth. The ability of LED light fixtures to provide a narrower range of light can be utilized to create specific expression in plants related to plant size, growth rates, flowering time, and many other factors. LEDs can also be manipulated to produce light only in the photosynthetically active range (PAR) between 400-700nm. This report looks at the importance of far-red light (700-780 nm) in the spectrum of LED lights. Previous studies have shown increases in leaf expansion and biomass among a variety of crops under supplemental far-red lighting (Legendre, Lee, Zhang). Studies have also shown that flowering time is reduced under far-red lighting (Deppe). In this study, leaf length, leaf width, leaf length/width, flowering time, and fresh harvest weight were investigated under varying levels of far-red light in *Arabidopsis thaliana* 'Col0-WT' and *Lactuca sativa* 'Rex'.

Materials and Methods

Equipment

Two growth chambers were selected for this experiment which took place at the Percival Facility (Perry, Iowa). The first growth chamber was a PGC-20ALB that housed the SciWhite + Red (SWR) and SciWhite + Red + Far-red1:1 (SWRFR1:1) conditions. This chamber featured two doors, and a styrofoam board was placed between the two sides in order to create a matching environment and prevent light contamination from the opposite side. On the SWR side, a SciWhite + Red light fixture was suspended from the ceiling. On the SWRFR1:1 side, a SciBrite4 (SB4) tile containing adjustable white, blue, red, and far-red LEDs was suspended from the ceiling, which were adjusted as necessary. The second growth chamber was an E-41HOC8 chamber which housed the SciWhite + Red + Far-red 2:1 (SWRFR2:1) condition. This chamber contained a SciBrite7 (SB7) light fixture which allowed control over seven LEDs, these were adjusted as necessary to create the spectrum shown in Table 1 and Figure 1.

Environment

For all treatments, a 16-hour photoperiod was used with an 8-hour dark period. The daytime temperature was 22°C and the nighttime temperature was 20°C. The relative humidity was set at 60%.

Flats were placed into the chamber 18" below the light fixture. The light intensity was measured weekly using a LI-COR LI-80 Spectrometer at the center of the flats. The control group, SWR, was set at 150 µmol m^{-2} s^{-1} (±5%) PPFD. Both the SWRFR1:1 and SWRFR2:1 conditions were set to lower light intensities at 138 µmol m^{-2} s^{-1} (±5%) and 132 µmol m^{-2} s^{-1} (±5%), respectively. This was to better match the amount of PFD-Red and PFD-Blue to better compare the effect of supplemental far-red lighting (Table 1). The PFD-Green varied the most throughout the groups and it is assumed that this had little effect on the results since it was reflected by the plant. The red peak was at 660 nm for all conditions, and the far-red peak was at 730 nm. The complete breakdown of the different spectra is shown below.

	PPFD	PFD-R	PFD-FR	PFD-B	PFD-G	R:FR
SciWhite + Red	150±3	71±2	7±1	24±1	55±1	10.5
SciWhite + Red + Far-red 1:1	138±2	70±1	68±1	24±1	46±4	1.0
SciWhite + Red + Far-red 2:1	132±3	70±2	35±1	24±1	38±1	2.0

Table 1: Breakdown of the light spectrums of each of the three light conditions tested.
Data shown is the mean ± SE. All units are in µmol m^{-2} s^{-1}

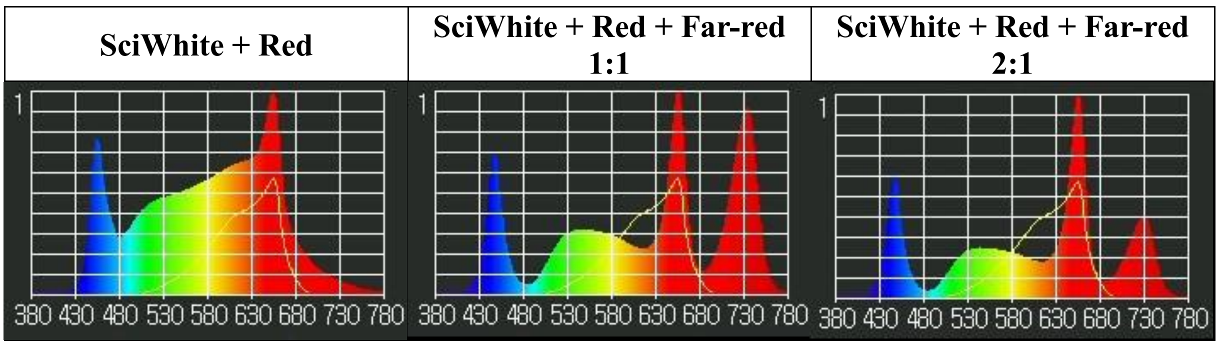

Figure 1: Images of the light spectrum of each of the light conditions, captured on LI-COR LI-80 Spectrometer at 18 inches from the light fixture

Arabidopsis thaliana

At the beginning of the experiment, *Arabidopsis thaliana* 'Col-0 WT' seeds were stratified at 4°C for 4 days to help with germination and to ensure uniformity. After this stratification treatment, (on Day 0) the seeds were sown into 36 cell horticultural trays 5 ½" x 22" (Menards, McKenzie Seed Starter Window Green House Kit – 36 Cell, SKU 2665776). The cell inserts had slits in the bottom to allow for sub-irrigation. The cells were filled with enriched garden soil (Menards, Schultz Flower & Vegetable Garden Soil, SKU 2667805) and sub-irrigated for 10 minutes before being sown with *Arabidopsis thaliana* 'Col-0 WT.' Seeds were spaced with a one cell gap in an alternating diamond pattern to prevent leaves from overlapping (Figure 2A). The trays were then covered with a propagation dome (from the seed starter kit) for one week to keep the seeds moist.

After one week, the propagation domes were removed, and all cells were thinned to contain no more than one plant. Plants were watered weekly through subirrigation by lifting a corner cell insert and filling to 1" with tap water. No fertilizers or pesticides were used in this experiment.

Starting on Day 9, the number of leaves on each plant was recorded 5 days a week, this included both basal and stem leaves. Beginning on Day 14, the length and width of the longest leaves on each plant were measured twice a week. Once stems emerged, the height of the stem was measured twice a week. All measurements were taken using a miniature tape measure, measurements started from the 10mm line to prevent any errors from the metal lip of the tape measure. All graphs are formatted so that the dot is the average, and the error bars are equivalent to the standard error, this was chosen since the sample sizes between the experiments varied.

Flowering dates were recorded on a plant-by-plant basis, and the median flowering date was used for all trials.

Figure 2: Images of planting style for *Arabidopsis thaliana* (A) and *Lactuca sativa* ‘Rex’ (B) showing the alternating diamond pattern with one cell of space between each to prevent leaf overlap

Lactuca sativa ‘Rex’

At the beginning of the experiment, on Day ‘0’, *Lactuca sativa ‘Rex’* were sown into peat seed-starting pellets (Jiffy, Biodegradable Seed Starting Peat Pellets, #J3R36G). The peat pellets were placed into 36 cell horticultural trays 5 ½” x 22” (Menards, McKenzie Seed Starter Window Green House Kit – 36 Cell, SKU 2665776). The cell inserts had slits in the bottom to allow for sub-irrigation. Seeds were spaced with a one cell gap in an alternating diamond pattern to prevent leaves from overlapping (Figure 2B). Each flat was watered with a nutrient solution containing 1 gallon of water and 10 mL of Base B (Humboldts Secret, Base B, B01IM88KKI), this solution had a pH of 5.9±0.1 and an EC of 800±100 µS/cm. The trays were then covered with a propagation dome (from the seed starter kit) for four days.

After four days, the propagation domes were removed. Beginning on Day 7, the number of leaves was recorded five days per week. Additionally, the length and width of all leaves greater than 1 cm in length were recorded twice per week beginning on Day 7. Flats were filled as needed with the nutrient solution. No pesticides were used in this experiment.

After 21 days, eight of the plants (SWR, n = 3: SWRFR1:1, n = 2: SWRFR2:1, n = 3) were randomly transferred to a left side of a vertical hydroponics tower under standard lighting conditions (SciWhite, 450±50 µmol m^{-2} s^{-1} PFD). One plant from each condition was transferred to the right side of the tower. The nutrient solution in this tower contained Base A, Base B, and Golden Tree (Humboldts Secret, B01IM88KKI). Nutrients were added as needed to water in order to create a nutrient solution of 5.6-6.0 pH and an EC of 1800-2400 µS/cm, as advised in HortAmericas “The Guide for Growing Leafy Greens” (HortAmericas). At 40 days after planting, all plants were harvested and their fresh weight was recorded and analyzed.

Results + Discussion

As shown in Figure 3, the length and width of *Arabidopsis thaliana* grown under supplemental far-red lighting were larger than those grown without supplemental far-red lighting. While this experiment has a small sample size (SWR, n = 7: SWRFR1:1, n = 13) it gives a good estimation of what the difference in leaf length under the two conditions would be. At 5 weeks, the longest leaf in *Arabidopsis thaliana* under SWRFR1:1 was 3 mm longer and 1 mm wider than under SWR conditions. After 3 weeks, both the length and width began to level off, as this when stems were formed and more of the plant's energy went to producing stems than growing leaves. This research follows other research on far-red lighting, as it promotes leaf elongation and expansion (Legendre). The benefit of longer and wider leaves varies by crop, and the ideal ratio of Red:Far-red light depends on both plant species and cultivar.

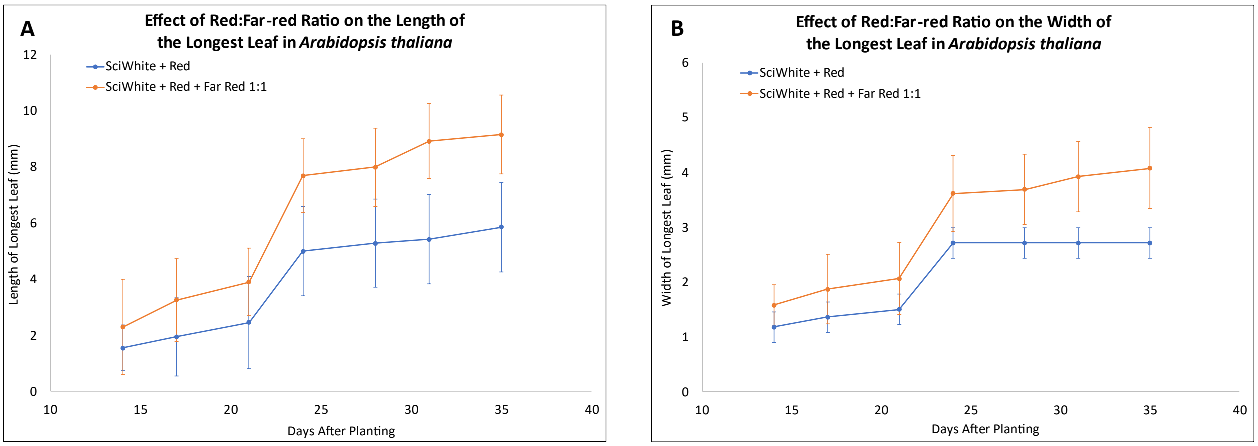

Figure 3: The effect of Red:Far-red ratio on the length (A) and width (B) of the longest leaf in *Arabidopsis thaliana* The data indicates mean ± SE

The ratio of leaf length/width was roughly 10% higher for plants grown under SWRFR1:1 than under SWR (Figure 4B). Under the additional far-red light, plants grew proportionately narrower leaves than under SWR conditions. This additional far-red lighting could be useful for plants that would benefit from longer leaves. At ratios of Red:Far-red at 1:1 the leaf length is increased more than leaf width, which could cause issues for plants that rely on their leaves for stability and could cause harvestable crops to change in appearance compared to the same crop under less intense far-red light. The median flowering time for *Arabidopsis thaliana* grown under SWRFR1:1 was 12 days earlier than under SWR conditions, at 28 and 40 days respectively (Figure 4C). Another study conducted on *Arabidopsis thaliana* also showed reduced time to flower under far-red lighting, although that report tested the effect of only far-red lighting, with a 730nm peak (Deppe). Previous studies have also shown that far-red lighting deficiencies can inhibit flowering in plants (Runkle). Supplemental far-red lighting could be incredibly beneficial for horticultural plants with long flowering times as reducing this time could lead to shorter growing cycles and more harvests per year.

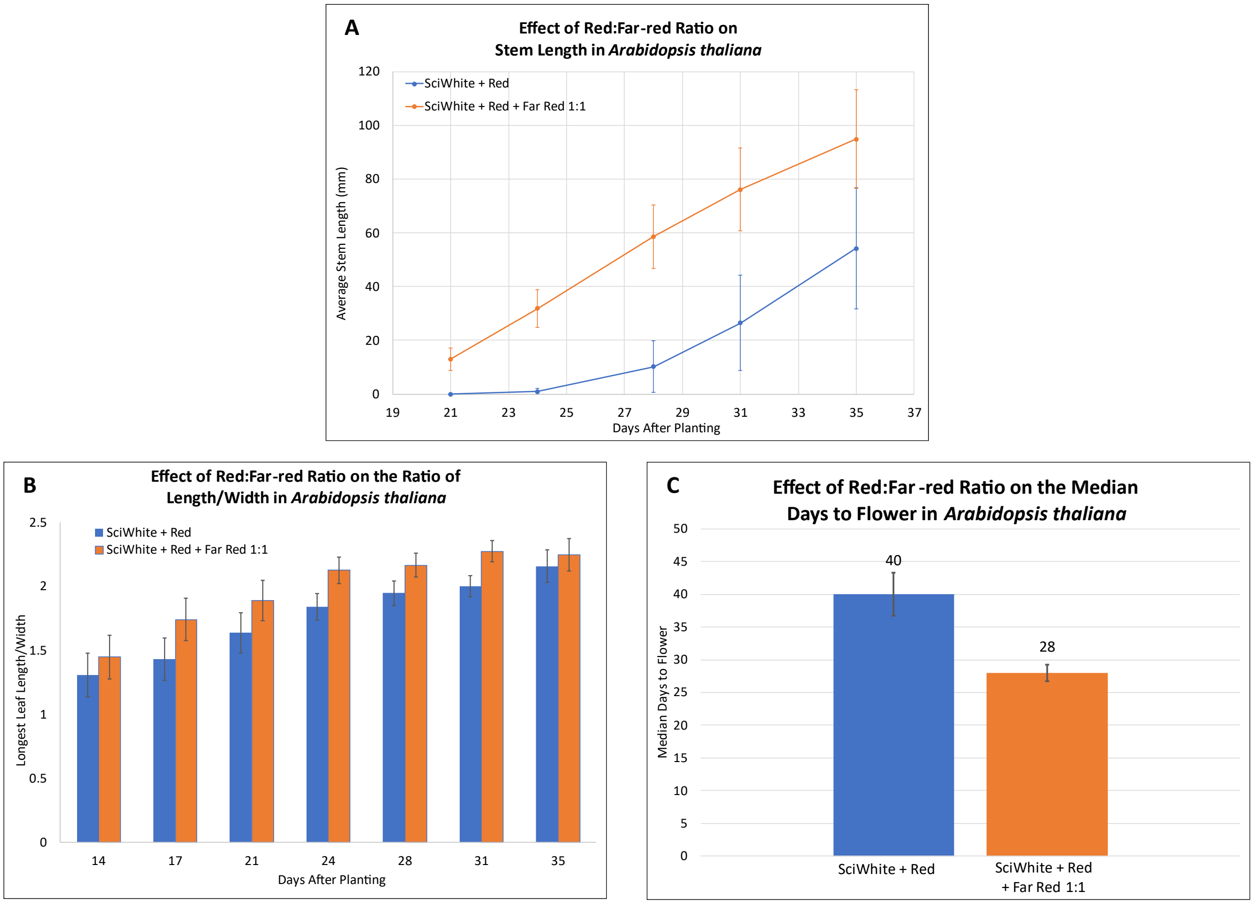

Figure 4: Effect of Red:Far-Red ratio on Stem Length (A) Leaf Length/Width (B) and median days to flower (C) in *Arabidopsis thaliana*
The data indicates the mean ± SE (A & B), and the median ± SE (C)

From Days 21 to 35, *Arabidopsis thaliana* plants grown under the SWRFR1:1 condition had 30 to 40 mm longer stems than plants grown under the SWR condition. This is consistent with previous experiments in multiple plants species that found far-red deficiencies can suppress stem elongation (Runkle). Over the first 28 days, *Arabidopsis thaliana* grown under SWRFR1:1 had more leaves than under SWR conditions (Figure 5A). After this point, the two had roughly the same number of leaves. 28 days was the median flowering date for plants grown under the SWRFR1:1 condition which is likely why no new leaves were formed after this point. On the contrary, *Lactuca sativa* 'Rex' plants grown under SWR, SWRFR1:1, and SWRFR2:1 did not vary significantly (Figure 5B). This is likely due to the small sample size of n = 4, 3, and 4, respectively, in this experiment and so there was not enough data to accurately differentiate the number of leaves on. Previous studies have shown differences in number of leaves between varying amounts of Red:Far-red light (Lee).

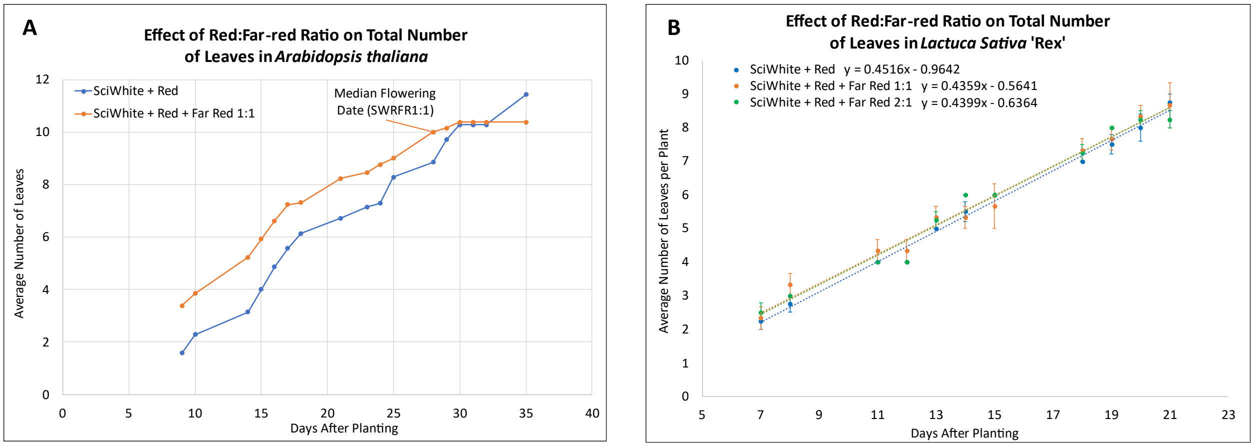

Figure 5: Total number of leaves in *Arabidopsis thaliana* (A) & *Lactuca sativa* 'Rex' (B) grown under varying amounts of supplemental far-red lighting.
The data indicates the mean (A), and the mean ± SE (B)

As shown in Figure 8, even with the small sample size of this experiment, there were significant differences between plants grown under SWR and plants grown under SWRFR1:1 or SWRFR2:1. The longest leaf length under SWRFR1:1 and SWRFR2:1 was over 30 mm longer at three weeks compared to plants grown under SWR (Figure 6A). This is consistent with Figure 3, the comparison of SWR and SWRFR1:1 in *Arabidopsis thaliana,* as well as the results of previous studies (Legendre). The average leaf length followed the longest leaf length in that the SWR condition was significantly shorter than SWRFR1:1 or SWRFR2:1. The average leaf length in the SWRFR1:1 and SWRFR2:1 conditions were similar to the longest leaf length in SWR which shows how large of a difference was observed in this study (Figure 6A). Plants grown under SWRFR1:1 and SWRFR2:1 had wider leaves than plants grown under SWR conditions (Figure 6B). This was true for both the average width and the width of the longest leaf. This is consistent with previous studies that found leaf width to increase with the amount of far-red light (Legendre) and the same was found in *Arabidopsis thaliana* (Figure 3B). No significant differences were noticed between the SWRFR1:1 and SWRFR2:1 conditions. More studies would need to be conducted to determine the optimal ratio of Red:Far-red lighting.

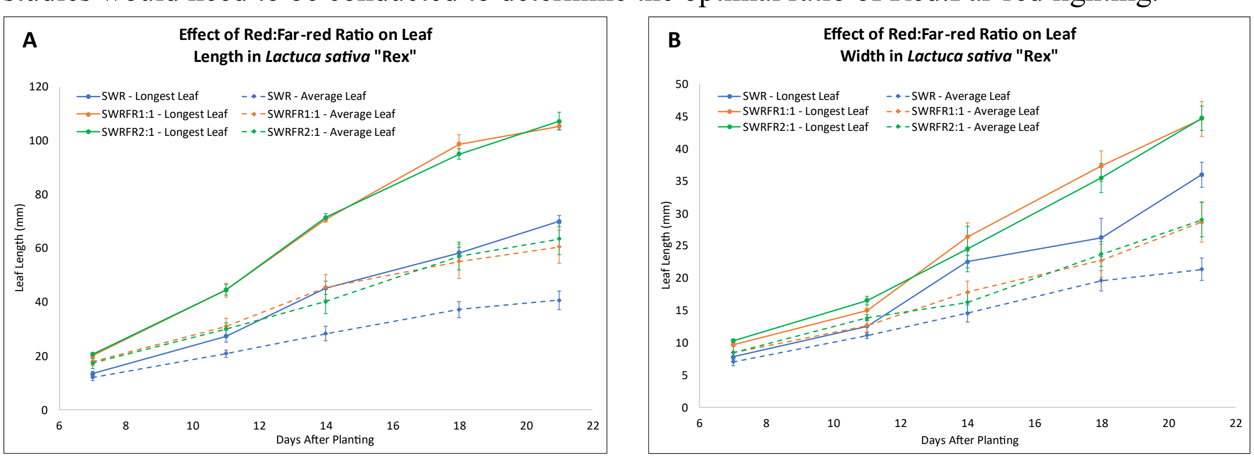

Figure 6: Effect of ratio of Red:Far-red light on Leaf Length (A) and Leaf Width (B) in *Lactuca sativa* 'Rex'
The data indicates mean ± SE

Lettuce plants grown under SWRFR1:1 and SWRFR2:1 grew at a higher ratio of leaf length/width than those under SWR conditions (Figure 7A). This is consistent with other studies on lettuce and other crops that showed increased leaf length/width when subjected to supplemental far-red light (Zhang) as well as the results from *Arabidopsis thaliana* (Figure 4B). The effect of leaf length/width varies by species as some plants rely more on their leaves for stability and for biological processes than others. For lettuce and other crops produced for human consumption the bag appeal, harvest weight, and taste must also be considered. To try and test one of these variables, after 3 weeks in each of their respective lighting conditions, all plants were transferred to a vertical hydroponics tower under SciWhite lighting where they were grown for 19 days. Ideally, these plants would have been allowed to grow for 2-3 more weeks under this condition, however this was the last day of our internship and plants had to be harvested on this day. Eight of the plants were randomly dispersed on the left-hand side and one plant from each condition was transferred to the right-hand side of the tower at different vertical positions. Previous tests had shown that the vertical position on the tower did not affect the fresh harvest weight (Bradford). 40 days after planting, all plants were harvested from the tower and the fresh harvest weight was recorded. Only plants grown on the left-hand side of the tower were used for this analysis, as all plants grown on the left-hand side of the tower were larger than plants grown on the right-hand side, which unfortunately shrunk our already small sample size. Lettuce plants grown for 3 weeks under the SWRFR1:1 and SWRFR2:1 conditions had greater fresh harvest weights than those that were initially grown under SWR conditions, with SWRFR2:1 having a greater fresh harvest weight than SWRFR1:1 (Figure 7B). Based on the results of this experiment, it is assumed that lettuce plants could be grown under spectrum specific LED lighting for the first 2-3 weeks of their life before being transferred to a greenhouse for the remainder of their life cycle. This would allow lettuce farmers to use LED lighting in a more cost-effective manner than growing under LED throughout their entire life cycle. Additionally, lettuce plants are much smaller during the first few weeks of their life cycle than they are before harvest allowing farmers to purchase fewer lights to satisfy their needs. Experiments with much larger sample sizes over the full length of the lettuce life cycle would prove useful to determine how big of an improvement growing under LEDs supplemented with Far-red light for the beginning of the life cycle has on the fresh harvest weight.

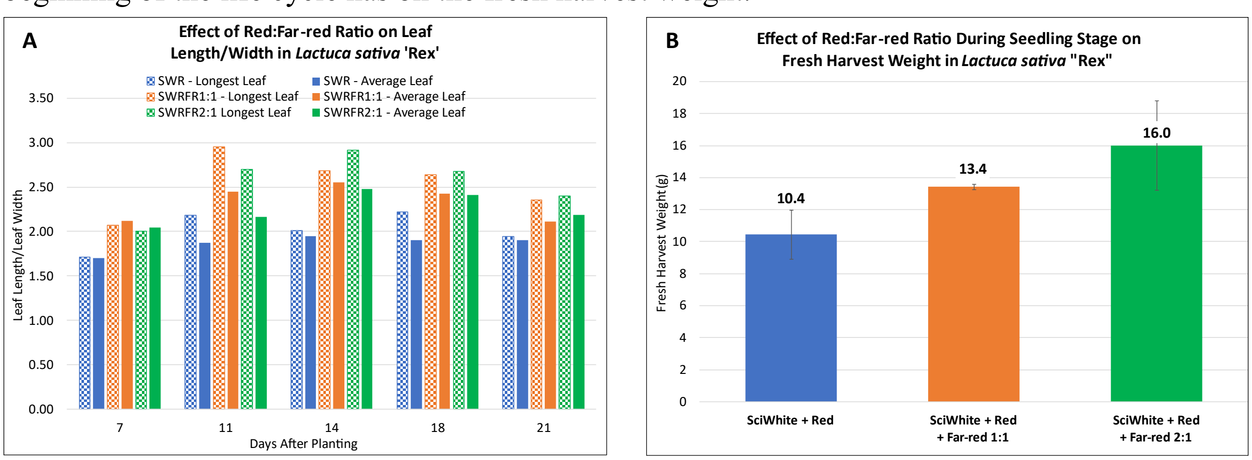

Figure 7: Effect of Red:Far-red Ratio During Seedling Stage on Leaf Length/Width (A, Data indicates the mean) and on Fresh Harvest Weight (B, Data indicates the mean ± S.E.) in *Lactuca sativa* 'Rex'

Plants grown under SWRFR1:1 and SWRFR2:1 appeared visually larger than plants grown under SWR at 18 and 40 days after planting (Figure 8). They also had noticeably longer leaf structure at both time points, especially in the oldest leaves. At 18 days, plants grown under SWRFR1:1 and SWRFR2:1 were a slightly lighter shade of green compared to the SWR treatment. This difference seemed to disappear at 40 days, likely because the plants were grown under the same lighting for the final 19 days of the experiment.

Figure 8: Images of *Lactuca sativa* ‘Rex’ at 18 (Top) and 40 (bottom) Days After Planting
3 out of 4 plants were randomly selected for SWR and SWRFR2:1, all 3 plants were photographed for SWRFR1:1

Conclusion & Recommendations

When comparing leaf length, leaf width, number of leaves, stem height, flowering date, and fresh harvest mass there were significant differences between plants grown under supplemental far-red lighting and those that did not receive additional far-red light.

Plants grown under 1:1 and 2:1 ratios of Red:Far-red light grew larger, flowered quicker, and had higher fresh harvest weights after being transferred to the same conditions partway through their lifecycle. There did not appear to be significant differences between the 1:1 and 2:1 conditions, although further experimental replications would be useful in determining if there is a discernible difference between these two conditions. Plants grown under this intense of Red:Far-red ratio did have significant morphological differences, most notably when comparing leaf length/leaf width, as compared to plants grown under SciWhite + Red and SciWhite. The Red:Far-red ratios used in this experiment are too high to recommend for a general light fixture that is designed to be used across a wide variety of plants, as the morphological characteristics are too extreme compared to plants grown under white LEDs or sunlight. Further testing could be conducted to design the optimum ratio of Red:Far-red light that could be used for a wide variety of plants. Research conducted by the scientific community found that Red:Far-red ratios around 6:1 were ideal for boosting plant growth while not significantly affecting the morphology of plants (Dorokhov, Kim, Legendre).

Ideally, a light fixture containing supplemental far-red lighting would be developed as an additional lighting option rather than a replacement of SciWhite lighting. Far-red lighting has been shown to benefit crops that produce fruits and flowers such as tomatoes, cucumbers, strawberries, peppers, and cannabis and could be marketed to researchers interested studying these crops (Danziger, Kim, Runkle, Zahedi, Zhang). Supplemental far-red lighting causes negative morphological effects in leafy greens such as lettuce, spinach, and basil and should not be marketed to these researchers (Lee, Kong).

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