

**CHARACTERIZING LEAFY GREENS GROWTH IN RESPONSE TO CO₂,
RELATIVE HUMIDITY, PHOTON SPECTRUM, AND NITROGEN
CONCENTRATION FOR SPACE PRODUCTION**

by

Emily Kennebeck

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Plant and Soil Sciences

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CONCENTRATION FOR SPACE PRODUCTION**

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ABSTRACT

Space crop production is imperative for space research by adequately supplementing the astronaut diet in future missions. However, the International Space Station (ISS) has unideal environmental conditions for crop growth. To produce the supplementary nutrients and minerals that space-grown crops can provide to the astronaut diet, conditions for optimal growth need to be implemented in future space crop systems. In these ground-based studies, we explored how different light qualities, relative humidity levels, and nitrogen concentrations affected the growth of leafy green species at superelevated CO₂ concentrations, like those seen on the ISS ($\approx 2800 \mu\text{mol}\cdot\text{mol}^{-1}$). The first study assessed the interactions of supplemental far-red (FR) light ($50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and the CO₂ concentration on mustard ‘Amara’. FR light decreased seedling fresh and dry mass at superelevated CO₂ concentrations but did not affect the mature counterparts. Additionally, superelevated CO₂ increased mustard ‘Amara’ fresh and dry mass at both growth stages. The second study assessed the influence of increasing relative humidity (RH) levels from 40% (ambient-ISS levels) to 70% (typical setpoint for growth rooms on Earth) and substituting red and FR light for blue and green light at superelevated CO₂, and the interactions these parameters had on lettuce ‘Outredgeous’ growth. Light, RH, and CO₂ concentration treatments increased seedling and mature fresh and dry mass depending on the level of the other environmental parameters in tandem. The last study assessed the growth and morphology of Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’ baby greens under varying FR photon flux densities (PFDs) and nitrogen (N) concentrations under superelevated CO₂. Both species exhibited typical shade avoidance responses to increasing FR PFDs, although neither showed increases in edible biomass. Increasing

the N concentration generally increased Chinese cabbage ‘Tokyo Bekana’ edible biomass, but not for kale ‘Red Russian’. Additionally, as the FR PFD increased, the positive effect of high N on the shoot fresh mass and total leaf area of Chinese cabbage ‘Tokyo Bekana’ diminished. Collectively, this work demonstrates that the species and cultivar are important considerations when testing growth treatments for use in space crop production. There is no general conclusion as to what environmental treatment is best for all space-grown crops to increase growth.

Chapter 1

MUSTARD ‘AMARA’ BENEFITS FROM SUPERELEVATED CO₂ WHILE ADAPTING TO FAR-RED LIGHT OVER TIME

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Abstract. Compared to the ambient Earth CO₂ concentration ($\approx 415 \mu\text{mol}\cdot\text{mol}^{-1}$), the International Space Station has superelevated CO₂ ($\approx 2800 \mu\text{mol}\cdot\text{mol}^{-1}$), which can be a stressor to certain crops. Far-red light can drive plant photosynthesis and increase extension growth and biomass. However, the effects of far-red light under superelevated CO₂ are unclear. We grew hydroponic mustard ‘Amara’ seedlings in four growth chambers using a randomized complete block design with two CO₂ concentrations (415 and 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$), two lighting treatments, and two blocks at temperature and relative humidity setpoints of 22 °C and 40%, respectively. Each

growth chamber had two lighting treatments at the same total photon flux density of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Under the same blue and green light at $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each, plants received either red light at $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or red + far-red light at $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each. At day 15 after planting, far-red light did not influence shoot fresh or dry mass under $415\text{-}\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 but decreased both parameters by 22%–23% under $2800\text{-}\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 . Increasing the CO_2 concentration increased shoot fresh and dry mass 27%–49% irrespective of the lighting treatment. Far-red light decreased leaf area by 16% under $2800\text{-}\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 but had no effect under $415\text{-}\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 . Increasing the CO_2 concentration increased leaf area by 21%–33% regardless of far-red light. Regardless of the CO_2 concentration, far-red light promoted stem elongation and decreased chlorophyll concentrations by 39–42%. These responses indicate far-red light elicited a crop-specific shade-avoidance response in mustard ‘Amara’, increasing extension growth but decreasing leaf area, thereby reducing light interception and biomass. In addition, CO_2 enrichment up to $2800\text{-}\mu\text{mol}\cdot\text{mol}^{-1}$ increased biomass of mustard ‘Amara’ but decreased biomass of other crops indicating crop-specific tolerance to superelevated CO_2 . In conclusion, mustard ‘Amara’ seedlings benefit from superelevated CO_2 but exhibit growth reduction under far-red light under superelevated CO_2 .

Abbreviations. ETR, electron transport rate; FR, far red; HY5, ELONGATED HYPOCOTYL5; ISS, International Space Station; LAX3, LIKE-AUX1 3; PAR, photosynthetically active radiation; PIF, phytochrome interacting factor; PIN3, PIN-FORMED3; PSI, photosystem I; PSII, photosystem II; R, red; R:FR, ratio of red to far-red light.

Introduction

Space-grown food crops are imperative for advancing space research and exploration by directly supporting astronauts' dietary intake. Currently, the astronaut diet is primarily comprised of pre-packaged and freeze-dried meals that degrade in nutritional quality after 1–3 years in storage (Cooper et al. 2017). Space-grown crops supplement essential minerals and nutrients lost through degradation during storage. Flavorful variety from fresh-grown produce can also alleviate the menu fatigue experienced from underconsumption due to the cyclic menu onboard the International Space Station (ISS) (Tibbetts 2019). However, the unique environment of the ISS imposes a range of environmental stressors, such as microgravity, low relative humidity levels, and highly elevated CO₂ concentrations (i.e., “superelevated”), which can negatively affect crop growth and thus the efficient and consistent fresh food supplementation in the astronaut diet. Therefore, recent space crop production research has focused on understanding crop responses to these stressors and identifying environmental and cultural parameters (e.g., light quality and intensity, growing media, and nutrient delivery) suitable for space-grown crops.

Elevated CO₂ concentrations can, depending on the level, vary in their impacts on crop development. Raising the CO₂ concentration (700–1200 μmol·mol⁻¹) above the ambient-Earth concentration can increase the net photosynthetic rate and crop growth (Bugbee et al. 1994; Gutierrez et al. 2014; Xu 2015). However, CO₂ elevation above this level can decrease plant biomass and delay seed set in spring and winter wheat (*Triticum aestivum*) and decrease RuBisCo concentration and activity in kidney bean (*Phaseolus vulgaris* cv. Linden), potato (*Solanum tuberosum*), lambsquarters

(*Chenopodium album*), cabbage (*Brassica oleracea*), and eggplant (*Solanum melongena*) (Bugbee et al. 1994; Gutierrez et al. 2014; Sage et al. 1989; Xu 2015). The decreased RuBisCo activity directly impacts the Calvin cycle, thus limiting the photosynthetic capacity of plants (Sharkey et al. 2007). When grown at elevated CO₂ concentrations (900–1350 $\mu\text{mol}\cdot\text{mol}^{-1}$), Chinese cabbage (*Brassica rapa* cv. Tokyo Bekana) exhibited a decrease in leaf area and biomass accumulation, and significant chlorosis and necrosis of leaf tissues (Burgner et al. 2019, 2020). Furthermore, the ambient-ISS CO₂ concentration ($\approx 2800 \mu\text{mol}\cdot\text{mol}^{-1}$) is almost seven times that of the ambient CO₂ concentration on Earth's surface (Massa et al. 2017). The superelevated CO₂ concentrations, such as those seen on the ISS, have elicited stress responses in some, but not all, leafy greens. Specifically, superelevated CO₂ concentrations (3000–6000 $\mu\text{mol}\cdot\text{mol}^{-1}$) can further increase fresh mass of lettuce (*Lactuca sativa* cvs. Dragoon and Outredgeous), mustard greens (*Brassica carinata* cv. Amara), pak choi (*Brassica rapa* cv. Extra Dwarf), shungiku (*Glebionis coronaria*), kale (*Brassica napus* cv. Red Russian and *Brassica oleracea* cv. Toscano), and chard (*Beta vulgaris* cv. Barese Swiss) (Wheeler et al. 2021). These findings emphasize the need for a focus on cultivar-specific stress mitigation testing in space crop production research to increase edible biomass production and ensure adequate supplementation of the astronaut diet.

In addition to the CO₂ concentration, light quality plays a significant role in regulating plant growth and development. Photosynthetically active radiation (PAR, 400–700 nm) affects plant growth through plant photosystems (Horton and Ruban 2005; McCree 1981). Far-red (FR) light (700–750 nm) is outside the conventionally defined PAR range but can increase photosynthetic efficiency synergistically with

shorter-wavelength photons, including red (R) light (600–700 nm), by equally exciting photosystems I and II (PSI, PSII) (Zhen and van Iersel 2017). Additionally, the ratio of red to far-red light (R:FR) mediates shade-avoidance responses through phytochromes. Under R light, phytochromes primarily exist in their active form (P_{fr}), translocate into the nucleus, and subsequently suppress phytochrome-interacting factors (PIFs) 4 and 5, which inhibit extension growth (Franklin 2008). In contrast, under FR light, phytochromes partially convert to their inactive form (P_r), which alleviates the suppression of PIFs and promotes extension growth (Sharrock 2008).

The effects of superelevated CO_2 concentration and FR light on leafy greens have been mostly studied separately. Their combined effects and potential interactions are less understood in space crop production. The objective of this ground-based study was to determine how partial substitution of FR light for R light influenced growth, morphology, and pigmentation of mustard green ‘Amara’ under the ambient-Earth CO_2 concentration and the typical ISS superelevated CO_2 concentration. We postulated that FR light would increase plant growth but decrease chlorophyll concentration and pigmentation under the superelevated CO_2 concentration.

Materials and Methods

Plant material and treatments. We grew mustard green ‘Amara’ in four reach-in plant growth chambers (E41L2, Percival Scientific, Perry, IA) in a randomized complete block design with two simultaneous replications from 7 Apr. to 5 May 2022. We rinsed and soaked 240 rockwool plugs (2.5 cm long \times 2.5 cm wide \times 3.5 cm tall; AO 25/40, Grodan, Milton, ON, Canada) with reverse-osmosis water for 10–15 min until saturation before draining the excess water. Subsequently, we sowed two seeds

(Johnny's Selected Seeds, Winslow, ME) per rockwool plug and placed 30 rockwool plugs in each of eight plastic trays (52.0 cm long \times 25.7 cm wide \times 6.1 cm tall). We added reverse-osmosis water up to half of the plug height and covered the trays with transparent humidity domes.

We placed the eight trays on top of lidded bus tubs (50.8 cm long \times 38.1 cm wide \times 12.7 cm tall; Choice Foodservice Equipment Company, Layton, UT) on two shelves in each of the four growth chambers, which delivered four treatments per replication with two CO₂ concentrations (415 and 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$) and, at each CO₂ concentration, two photon spectra (blue + green + R + FR at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each and blue at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ + green at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ + R at 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Fig. 1). The light-emitting diode array with four independent color channels on each shelf provided the same total photon flux density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (400–750 nm) and the same 18-h photoperiod. The adjustment of each lighting treatment was based on 12 spectral measurements at plant height across the treatment area with a spectroradiometer (SS-110, Apogee Instruments, Logan, UT). All chambers maintained the same air temperature of 22.0 ± 0.4 °C and relative humidity of $40.0\% \pm 3.9\%$ throughout the experiment. Chamber environments were recorded by internal chamber sensors and additional third-party temperature and relative humidity sensors (H5072001, Govee, Shenzhen, China) and CO₂ sensors (YEM-40L, GZAIR, Guangzhou, China) at plant height.

On day 4, we removed the humidity domes and subirrigated seedlings by maintaining the nutrient solution level at one-fourth of the plug height. We prepared the nutrient solution by dissolving a premixed base fertilizer (12-4-16 RO FeED, JR Peters, Inc., Allentown, PA) and magnesium sulfate (JR Peters, Inc.) in reverse-

osmosis water (electrical conductivity = $1.30 \pm .05$ dS/m, pH = 5.80 ± 0.01), which initially supplied the following nutrients: nitrogen ($124.82 \text{ mg}\cdot\text{L}^{-1}$), phosphorus ($18.31 \text{ mg}\cdot\text{L}^{-1}$), potassium ($138.13 \text{ mg}\cdot\text{L}^{-1}$), calcium ($72.81 \text{ mg}\cdot\text{L}^{-1}$), magnesium ($48.47 \text{ mg}\cdot\text{L}^{-1}$), sulfur ($39.02 \text{ mg}\cdot\text{L}^{-1}$), boron ($0.12 \text{ mg}\cdot\text{L}^{-1}$), copper ($0.47 \text{ mg}\cdot\text{L}^{-1}$), iron ($1.77 \text{ mg}\cdot\text{L}^{-1}$), manganese ($0.52 \text{ mg}\cdot\text{L}^{-1}$), molybdenum ($0.13 \text{ mg}\cdot\text{L}^{-1}$), and zinc ($0.56 \text{ mg}\cdot\text{L}^{-1}$). We thinned seedlings with tweezers to one plant per plug on day 7 and transplanted six randomly selected seedlings into a mini-hydroponic setup under each treatment on day 15. We grew six plants in two rows of three net pots (6.35 cm diameter \times 5.08 cm tall; Cz Garden Supply) spaced 18 cm apart and embedded in each bus tub containing nutrient solution. Between transplant and the final harvest, we maintained the nutrient solution level at one-half of the plug height by adding nutrient solution daily. We recorded the nutrient solution pH and electrical conductivity in each bus tub daily and maintained the nutrient solution pH at 5.8 ± 0.3 (Table 1).

Data collection and analysis. On the day of transplant (day 15), we quantified growth (shoot fresh and dry mass), morphology (length and width of the longest true leaf, total leaf area, and stem width), and pigmentation (chlorophyll concentration and leaf color indices) of 10 randomly selected seedlings from each treatment. We used a digital caliper (model 41101, Wiha Tools, Monticello, MN) to measure stem width and used a leaf area meter (CI-202, CID Bio-Science, Inc., Camas, WA) to measure the total leaf area. In addition, we used a chlorophyll meter (MC-100, Apogee Instruments, Inc., Logan, UT) to measure the relative chlorophyll concentration of the longest true leaf and used a color reader (CR-10, Konica Minolta Sensing, Ramsey, NJ) to measure leaf color indices in the $L^*a^*b^*$ color space [L^* (0 to 100): 0 = black and 100 = white; a^* (-150 to 150): lower = greener and higher = redder; b^* (-150 to

150): lower = bluer and higher = yellower]. Subsequently, we dried seedlings for 5 d in a drying oven at 65 °C before weighing shoot dry mass. We harvested mature plants from all treatments on day 29 and measured the same parameters as for seedlings, and additionally, root length and root dry mass.

We photographed one representative plant per treatment per replication on day 15 and day 29 for visual comparisons of treatment effects (Fig. 2). We collected environmental parameter data in all growth chambers weekly to ensure they were on target throughout the experiment (Table 1). We conducted pairwise comparisons using the ANOVA and Tukey's honestly significant difference test ($\alpha = 0.05$) in JMP Pro (ver. 16.0.0, SAS Institute, Inc., Cary, NC). We pooled data from two replications when there was no treatment \times replication interaction or when data trends among treatments were consistent across replications.

Results

Shoot and root growth. Under the same lighting treatment at both growth stages, mustard 'Amara' grown at the superelevated CO₂ concentration of 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ were larger than plants grown at the CO₂ concentration of 415 $\mu\text{mol}\cdot\text{mol}^{-1}$. At the seedling stage (days 0–15), increasing the CO₂ concentration from 415 to 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ increased seedling shoot fresh and dry mass by 27–30% with FR light and 49% without FR light (Fig. 3). However, CO₂ concentration did not affect shoot moisture content or shoot height of seedlings. At the mature stage (days 16–29), increasing the CO₂ concentration from 415 to 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ increased shoot fresh and dry mass by 51–71% with FR light and 32–59% without FR light (Fig. 3). At 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂, shoot moisture content decreased by 1% with FR light

and 2% without FR light, when compared to 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. The super-elevated CO₂ concentration with FR light increased shoot height by 20% but did not affect it without FR light.

Plants were taller when grown with FR light than without. At the seedling stage, the addition of FR light decreased shoot fresh and dry mass by 22% and 23%, respectively, under 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ but had no effect at 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. Shoot moisture content increased by 0.4% when FR light was added at 415 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. Additionally, FR light increased seedling shoot height by 40–41% regardless of CO₂ concentration. At the mature stage, FR light did not affect shoot fresh or dry mass at either CO₂ concentration but decreased shoot moisture content by 1% at 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. FR light increased shoot height of mature plants by 539% and 615% at 415- and 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂, respectively. A decrease in the effect of FR light on mustard ‘Amara’ between the seedling and mature stages suggests an environmental adaptation to FR light over time.

Increasing CO₂ concentration from 415 to 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ decreased root length, root dry mass, and the ratio of root to shoot mass by 45%, 35%, and 62%, respectively, without FR light but had no effect with FR light. FR light increased root length by 50% under 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ but had no effect at 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. FR light decreased root dry mass and the ratio of root to shoot mass by 44% and 45%, respectively, under 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ but had no effect at 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ (Table 2).

Leaf morphology and coloration. Increasing the CO₂ concentration from 415 to 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ increased seedling leaf length and leaf area by 8% and 21%, respectively, with FR light and 22% and 33%, respectively, without FR light. The CO₂

concentration increase did not affect mature leaf length but increased leaf area by 30% with FR light. At the seedling stage, FR light increased leaf length by 21–37% regardless of CO₂ concentration. Although FR light did not affect leaf area at 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂, it decreased leaf area by 16% at 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂. At the mature stage, FR light increased leaf length by 12% at 415- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ and 13% at 2800- $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ but did not affect leaf area. These results again suggest that mustard ‘Amara’ adapts to the effects of FR light as the cultivar matures. CO₂ concentration did not affect leaf relative chlorophyll concentration. At the seedling and mature stages, FR light decreased relative chlorophyll concentration by 39–42% and 50–51%, respectively. FR light increased foliage brightness (L^*) by 15–20% in seedlings and 25–34% in mature plants. FR light decreased a^* by 42% in seedlings and 27–48% in mature plants. FR light also increased foliage yellowness (b^*) by 81–89% in seedlings and 88–141% in mature plants (Table 2). These results are consistent with the typical shade-avoidance response caused by FR light (Demotes-Mainard et al., 2016).

Discussion

The superelevated CO₂ concentrations on the ISS can act as a stressor towards plant growth as seen when Chinese cabbage ‘Tokyo Bekana’ had stunted growth and severe chlorosis under superelevated CO₂ concentrations (Burgner et al. 2020). Elevated CO₂ concentrations (e.g., 900–1000 $\mu\text{mol}\cdot\text{mol}^{-1}$) decreased RuBisCO concentration and activity in five C₃ species (Sage et al. 1989). A decrease in RuBisCO activity negatively affects nitrogen use efficiency, ultimately decreasing the photosynthetic rate and plant growth (Sage et al. 1989). In this study, increasing the

CO₂ concentration from 415 to 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ increased shoot fresh and dry mass, leaf length, and leaf area of mustard ‘Amara’ seedlings, regardless of FR light. For mature mustard ‘Amara’ plants, this CO₂ concentration increase also increased shoot fresh and dry mass by 32–71% and leaf area by 22–30% of, regardless of FR light. While this result was unexpected given previous findings, it may suggest that mustard ‘Amara’ has a high tolerance to superelevated CO₂ concentrations. In studies on wheat, increasing the CO₂ concentration initially (800–1200 $\mu\text{mol}\cdot\text{mol}^{-1}$) increased growth responses through the CO₂ fertilization effect. Elevating the CO₂ concentration past this point either did not further increase growth or decreased fresh mass and seed set (Bugbee et al. 1994; Gutierrez et al. 2014; Xu 2015). However, the CO₂ fertilization effect's maximum threshold may differ depending on the cultivar studied. For example, eight leafy green cultivars grown under superelevated CO₂ concentrations (1500–6000 $\mu\text{mol}\cdot\text{mol}^{-1}$) exhibited increased fresh and dry mass compared to the ambient CO₂ concentration (400 $\mu\text{mol}\cdot\text{mol}^{-1}$), with no negative effects seen at the highest CO₂ treatments (Wheeler et al. 2021).

Increasing CO₂ concentration from 415 to 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ increased shoot height of mature mustard ‘Amara’ with FR light. In some herbaceous species (e.g., dwarf bean (*Phaseolus vulgaris* cv. ‘Tender Green’), salad burnet (*Sanguisorba minor*), birds-foot trefoil (*Lotus corniculatus*), hoary plantain (*Plantago media*), and kidney vetch (*Anthyllis vulneraria*)), a slightly elevated CO₂ concentration (590 $\mu\text{mol}\cdot\text{mol}^{-1}$) promoted leaf epidermal cell expansion compared to the ambient CO₂ concentration (350 $\mu\text{mol}\cdot\text{mol}^{-1}$) (Ferris and Taylor 1994; Ranasinghe and Taylor 1995). However, for edible gynura (*Gynura bicolor*), wheat, and radish (*Raphanus sativus*), superelevated CO₂ concentrations decreased or did not influence shoot height

(Jiang et al. 2017; Stryjewski and Eraso 2002; Wang et al. 2015). These results support this hypothesis and our findings of increased growth for mustard ‘Amara’ under superelevated, ambient-ISS CO₂ concentrations.

In this study, superelevated CO₂ concentrations decreased root length, root dry mass, and the ratio of root to shoot mass of mustard ‘Amara’. In edible gynura, superelevated CO₂ concentrations (3000 $\mu\text{mol}\cdot\text{mol}^{-1}$) decreased root length but did not influence root dry mass compared to the ambient CO₂ concentration (Wang et al., 2015). In contrast, superelevated CO₂ concentrations increased root mass and lateral root formation in winter wheat and orchid plantlets (*Arachnis hookeriana* \times *Ascocenda* cv. Mokara Yellow and *Cymbidium* cv. Music Hour Maria) compared to the ambient CO₂ concentration (Gouk et al. 1997; Jiang et al. 2017; Norikane et al. 2010). High CO₂ concentrations increased the ratio of root to shoot mass through assimilate partitioning in a variety of herbaceous species (e.g., velvetleaf (*Abutilon theophrasti*), Napa cabbage (*Brassica pekinensis*), Bigelow’s sedge (*Carex bigelowii*), soybean (*Glycine max*), Marsh Labrador Tea (*Ledum palustre*), and cherry tomato (*Lycopersicon esculentum*)) (Farrar and Williams 1991). Taken together, CO₂ concentration has no consistent effect on root and shoot biomass allocation; effects can depend on the plant species, developmental stage, environment, and experimental procedures (Rogers et al. 1996).

We postulated that the effects of FR light would alleviate the growth inhibition from superelevated CO₂ concentrations observed in previous research. In this study, FR light did not influence or decreased the shoot fresh and dry mass and leaf area of mustard ‘Amara’ seedlings and did not affect these growth parameters of mature mustard ‘Amara’, regardless of CO₂ concentration. Similarly, 23 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of

supplemental FR light did not increase shoot fresh mass of mature mustard ‘Amara’ (Spencer et al. 2020). It is unclear why responses of mustard ‘Amara’ to FR light differ from typical shade-avoidance responses induced by FR light (e.g., shoot elongation, leaf extension, and leaf area expansion) (Franklin 2008). When grown under supplemental FR light, leaf lettuce exhibited typical shade avoidance response patterns including increased shoot fresh mass and leaf area (Meng et al. 2019). In comparison, mustard ‘Amara’ is a less volume-efficient crop with a different growing habit; it grows vertically with leaves extending sideways individually instead of the layered-leaf structure in lettuce. These differences in morphology could explain this unusual FR light response. When comparing lettuce cultivars grown with and without supplemental FR light, the head lettuce cultivars ‘Cherokee’ and ‘Little Gem’ responded with the typical growth responses seen under the shade avoidance response. However, the loose-leaf lettuce cultivar ‘Green Salad Bowl’ did not exhibit increases in dry weight under FR light unlike what was seen in the head lettuce cultivars (Liu and van Iersel 2022). This again suggests that morphology plays an integral role in the response of leafy greens to supplemental FR light. FR light could be supplemented for crops that exhibit the shade avoidance response to increase yield such as head lettuce (Spencer et al. 2020), whereas FR light may not be necessary for other less volume-efficient crops to increase yield.

In this study, FR light decreased root dry mass and the ratio of root to shoot mass of mature mustard ‘Amara’. In *Arabidopsis thaliana*, FR light negatively affects lateral root formation and density through the ELONGATED HYPOCOTYL5 (HY5) transcription factor (van Gelderen et al. 2018). Supplemental FR light promoted expression of HY5 in lateral root primordia, which downregulated

PIN-FORMED3 (PIN3) and LIKE-AUX1 3 (LAX3) that control the auxin flow from the shoot toward the root (van Gelderen et al. 2018). This could explain the increases in root length seen under some treatments with FR light to ensure adequate nutrient uptake when lateral root formation may be suppressed through the above mechanism.

In mustard ‘Amara’, FR light had more pronounced influence on growth of seedlings than mature plants. This suggests that mustard ‘Amara’ can acclimate to FR light over time. In arabidopsis, exposure to FR light for long periods of time resulted in overstimulation and subsequent partial degradation of PSI and an increase in PSII antenna size to equalize the electron transport rates (ETRs) of both photosystems (Hu et al. 2021). After being placed under actinic lighting, strong PSII photoinhibition was observed from the PSII ETR bottleneck caused by the PSI reduction (Hu et al. 2021). Increasing the antenna size makes PSII even more susceptible to damage from high-light intensity stress than its high baseline sensitivity, furthering photoinhibition and the ETR bottleneck (Shi et al. 2022). PSII photoinhibition decreases the photosynthetic rate, which could explain the negative or lack of influence from FR light on fresh and dry mass and leaf area in mustard ‘Amara’ seedlings. However, this does not explain the difference in the effect of FR light on the same measured parameters at the mature stage. The PSII photorepair system for high light-intensity damaged PSII structures may explain this difference (Shi et al. 2022). Repairing a damaged (or in this case, altered) PSII structure mainly involves the disassembly of the PSII complex, replacement of the D-1 reaction center subunit, and the reassembly of the whole structure. Studies *in vivo* have found that the D-1 subunit is the main component to become damaged during photoinhibition of PSII. FtsH and Deg protease families are mainly responsible for the degradation of the damaged subunit. The entire

PSII structure is then disassembled, and a replacement subunit is selected for reassembly into the newly repaired PSII (Nixon et al. 2010). The replacement of the altered PSII structure through this photorepair system would alleviate the ETR bottleneck with an increase in functional photosystems. This step might not have occurred at the seedling phase for mustard ‘Amara’, but as the plants matured further into their growth cycle, this repair mechanism could have been implemented.

In summary, we observed unique responses of mustard ‘Amara’ to FR light and CO₂ concentration in space-relevant conditions (e.g., temperature and relative humidity level). The typical shade avoidance response elicited by FR light influences plant growth patterns toward elongated shoots, expanded leaf area, and overall biomass accumulation (Franklin 2008). However, mustard ‘Amara’ grown under FR light does not follow these growth trends. Specific cultivar morphology appears to play a role in the growth response to FR light. Leafy green varieties with a stacked canopy, such as in head lettuce, do follow the typical shade avoidance response patterns. Loose-leaf lettuces and other less volume-efficient cultivars, such as mustard ‘Amara’, do not exhibit this same response possibly due to decreased light penetration into the plant canopy (Liu and van Iersel 2022). As such, we suggest conducting ground testing with cultivars under FR light before approval for use in-flight to determine the necessity of including the additional light waveband in space crop production systems. In earlier studies, superelevated CO₂ concentrations found in ambient-ISS conditions negatively affected leafy green growth by directly decreasing RuBisCO activity and concentration (Sage et al. 1989). We have shown that the most common CO₂ fertilization effect curve (with maximum positive responses observed \approx 800–1200 $\mu\text{mol}\cdot\text{mol}^{-1}$) is not representative of mustard ‘Amara’ (Bugbee et al. 1994;

Gutierrez et al. 2014; Xu 2015). Superelevated CO₂ concentrations increased mustard ‘Amara’ growth regardless of the lighting treatment. In other ground testing studies, mustard ‘Amara’ also exhibited increased growth at higher CO₂ concentrations than the level used in this study (Wheeler et al. 2021). With this present study, we advise the implementation of ground testing with FR light and ISS-simulated environmental parameters to observe cultivar-specific responses and the nutritional quality of the crops. This continued research will ensure adequate mineral and nutrient supplementation of the astronaut diet through space crop production.

Table 1. Environmental parameters of four treatments across two replications. The first number under Treatment designates the targeted CO₂ concentration for the environmental treatment. The following “-FR” or “+FR” designates the absence or presence of far-red light in the treatment, respectively. T: top shelf, B: bottom shelf.

Chamber Shelf	Replication One				Replication Two			
	2 T	B	1 B	T	3 B	T	4 T	B
Target CO ₂	415	415	2800	2800	415	415	2800	2800
Light Treatment	-FR	+FR	-FR	+FR	-FR	+FR	-FR	+FR
Environmental Data								
Temperature (°C)	21.37 ± 0.22	21.88 ± 0.17	21.60 ± 0.13	21.14 ± 0.18	21.92 ± 0.12	21.33 ± 0.35	20.65 ± 0.28	21.62 ± 0.10
Relative Humidity (%)	42.12 ± 1.17	42.96 ± 0.94	43.58 ± 1.38	42.40 ± 1.53	43.67 ± 2.43	42.99 ± 3.21	42.94 ± 1.48	43.02 ± 0.88
CO ₂ Concentration (μmol·mol ⁻¹)	429.08 ± 15.60		2722.27 ± 39.55		424.13 ± 4.44		2739.74 ± 17.82	
Nutrient Solution Data								
pH	6.09 ± 0.11	6.08 ± 0.08	6.09 ± 0.11	6.09 ± 0.11	6.10 ± 0.08	6.08 ± 0.10	6.08 ± 0.06	6.08 ± 0.07
Electrical Conductivity (dS/m)	1.39 ± 0.02	1.40 ± 0.03	1.34 ± 0.02	1.35 ± 0.01	1.37 ± 0.02	1.38 ± 0.02	1.36 ± 0.02	1.39 ± 0.01

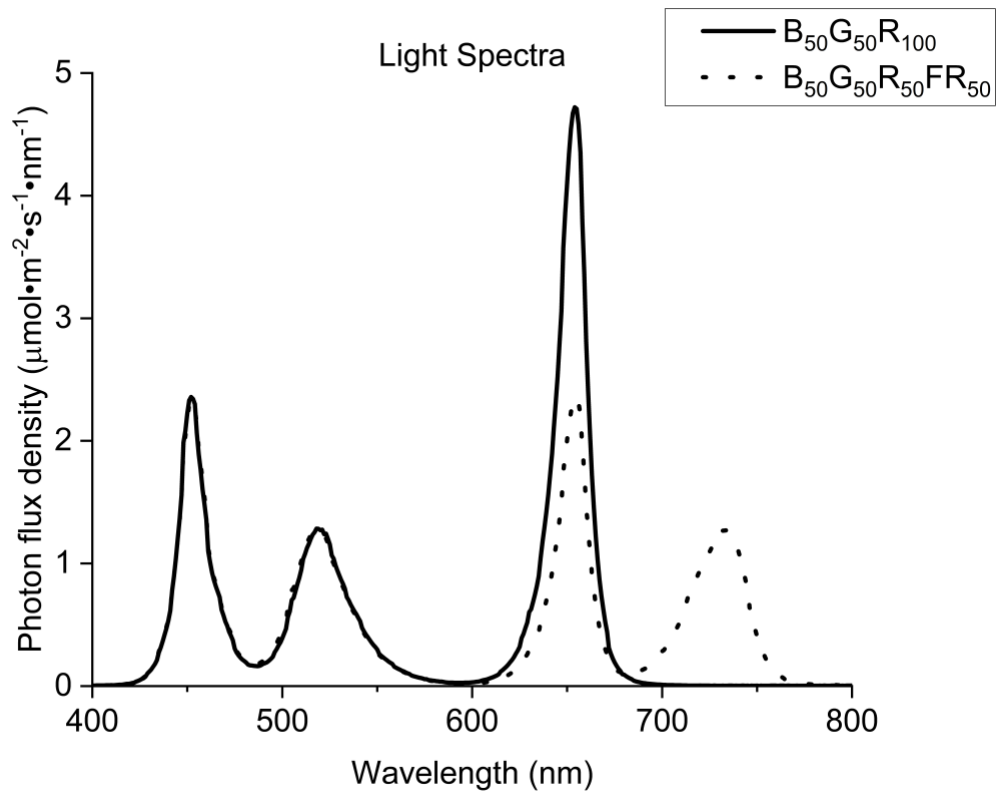


Figure 1. Photon spectra of two lighting treatments consisting of blue (B), green (G), and red (R) light, with and without far-red (FR) light. The photon flux density (in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of each waveband is signified by its subscript.

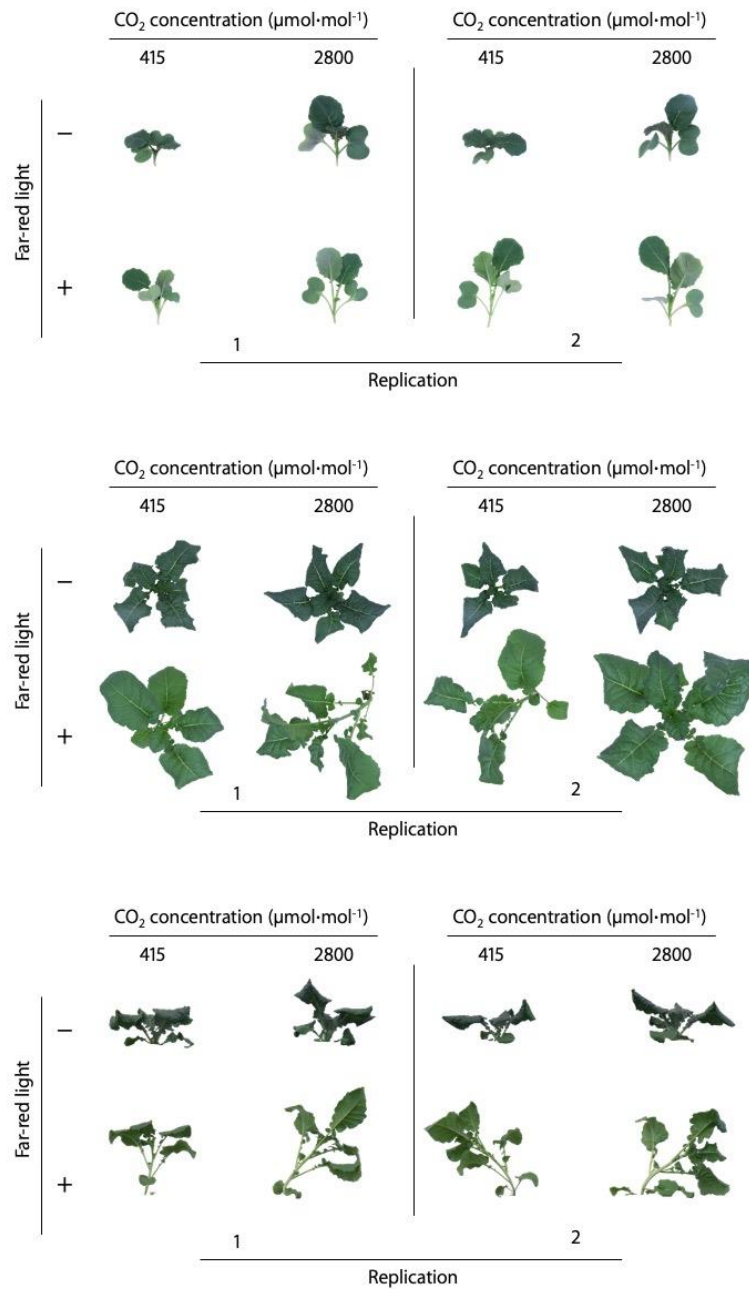


Figure 2. Images of representative plants from two experimental replications. Mustard ‘Amara’ seedlings and mature plants were grown under lighting treatments with (+) and without (-) far-red light under two CO₂ concentrations (415 and 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$).

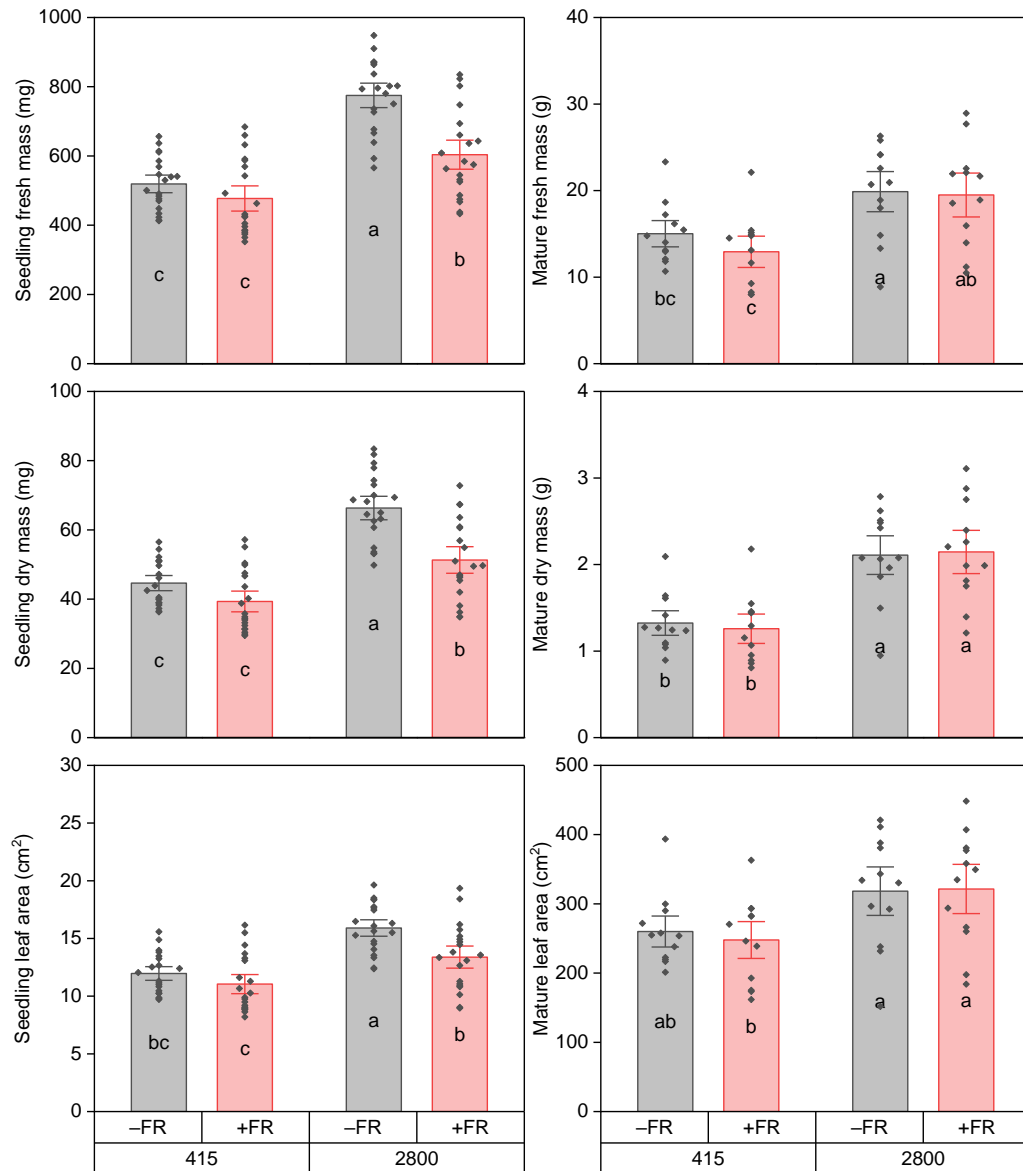


Figure 3. Shoot fresh and dry mass and leaf area of mustard ‘Amara’ at the seedling (day 15) and mature (day 29) growth stages. Plants were grown without (–) and with (+) far-red (FR) light at two CO₂ concentrations (415 and 2800 μmol·mol⁻¹). Means followed by different letters within each column are statistically different by Tukey’s honest significant difference test at P ≤ 0.05.

Table 2. Destructive measurements of the biomass, morphology, and chlorophyll and coloration of mustard ‘Amara’ at seedling (day 15) and mature (day 29) growth stages. Root morphology of mature plants is also described. Acronyms signify fresh mass (FM), dry mass (DM), moisture content (MC), shoot height (SH), leaf length (LL), leaf area (LA), and root length (RL). Mustard ‘Amara’ was grown at two CO₂ concentrations (415 and 2800 μmol·mol⁻¹), and under two lighting treatments (Light) without (–FR) or with (+FR) far-red light. Different letters within each column are significantly different by Tukey’s honest significant difference test at P ≤ 0.05.

Treatment		Seedling			Mature		
		Biomass					
CO ₂	Light	Shoot FM (mg)	Shoot DM (mg)	Shoot MC (%)	Shoot FM (mg)	Shoot DM (mg)	Shoot MC (%)
415	–FR	519.15 ± 16.96 c	44.62 ± 1.46 c	91.39 ± 0.10 b	15.02 ± 1.01 bc	1.32 ± 0.09 b	91.20 ± 0.12 a
415	+FR	477.17 ± 24.25 c	39.33 ± 1.99 c	91.75 ± 0.07 a	12.92 ± 1.20 c	1.26 ± 0.11 b	90.18 ± 0.20 b
2800	–FR	774.98 ± 23.56 a	66.33 ± 2.26 a	91.44 ± 0.13 ab	19.88 ± 1.55 a	2.11 ± 0.15 a	89.26 ± 0.34 bc
2800	+FR	603.67 ± 28.05 b	51.31 ± 2.56 b	91.51 ± 0.13 ab	19.49 ± 1.69 ab	2.15 ± 0.17 a	88.84 ± 0.26 c
Treatment		**	**	*	**	**	**
Replication (Rep.)		NS	NS	NS	NS	NS	NS
Trt. × Rep.		**	*	*	NS	NS	NS
Treatment		Morphology					
CO ₂	Light	SH (cm)	LL (cm)	LA (cm ²)	SH (cm)	LL (cm)	LA (cm ²)
415	–FR	1.03 ± 0.03 b	3.67 ± 0.09 d	11.96 ± 0.40 bc	2.11 ± 0.12 c	15.33 ± 0.36 c	260.02 ± 14.92 ab
415	+FR	1.44 ± 0.05 a	5.02 ± 0.15 b	11.05 ± 0.55 c	13.48 ± 0.73 b	17.12 ± 0.53 ab	247.77 ± 17.76 b
2800	–FR	1.10 ± 0.02 b	4.48 ± 0.08 c	15.91 ± 0.48 a	2.26 ± 0.13 c	16.11 ± 0.49 bc	318.30 ± 23.32 a

2800	+FR	1.56 ± 0.06 a	5.42 ± 0.14 a	13.39 ± 0.64 b	16.16 ± 0.52 a	18.22 ± 0.58 a	321.46 ± 23.70 a		
Treatment		**	**	**	**	**	**		
Replication (Rep.)		*	NS	NS	NS	NS	NS		
Trt. × Rep.		NS	**	**	*	NS	NS		
Treatment		Chlorophyll (Chl.) and coloration (L^* , a^* , b^*)							
CO ₂	Light	Chl.	L^*	a^*	b^*	Chl.	L^*	a^*	b^*
415	-FR	35.79 ± 0.59 a	21.59 ± 0.43 b	-4.86 ± 0.08 a	7.31 ± 0.12 b	62.56 ± 2.74 a	13.12 ± 0.76 c	-3.72 ± 0.16 a	4.09 ± 0.30 b
415	+FR	20.68 ± 0.26 b	24.91 ± 0.37 a	-6.93 ± 0.08 b	13.82 ± 0.17 a	31.13 ± 1.51 b	17.64 ± 0.64 ab	-4.72 ± 0.06 b	7.71 ± 0.18 a
2800	-FR	36.98 ± 0.69 a	21.32 ± 0.50 b	-4.82 ± 0.10 a	7.36 ± 0.25 b	66.33 ± 2.43 a	14.80 ± 0.52 bc	-3.21 ± 0.09 a	3.30 ± 0.17 b
2800	+FR	22.41 ± 0.41 b	25.57 ± 0.39 a	-6.87 ± 0.09 b	13.35 ± 0.27 a	32.29 ± 2.28 b	18.48 ± 0.67 a	-4.75 ± 0.13 B	7.95 ± 0.33 a
Treatment		**	**	**	**	**	**	**	**
Replication (Rep.)		NS	*	*	NS	NS	NS	NS	NS
Trt. × Rep.		NS	NS	NS	NS	NS	NS	*	NS
Treatment		Mature root morphology							
CO ₂	Light	Root DM (mg)		RL (cm)		Root:Shoot			
415	-FR	60.33 ± 6.20 a		25.28 ± 2.16 a		0.047 ± 0.005 a			
415	+FR	34.07 ± 6.80		20.08 ± 1.77		0.026 ± 0.005			

2800 -FR	b 39.06 ± 5.43	ab 13.97 ± 0.84	b 0.018 ± 0.002
2800 +FR	b 36.62 ± 5.76	a 20.98 ± 2.94	b 0.016 ± 0.002
Treatment	**	**	**
Replication (Rep.)	NS	*	NS
Trt. × Rep.	NS	NS	NS

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Chapter 2

LETTUCE GROWTH IS AFFECTED BY FAR-RED LIGHT, RELATIVE HUMIDITY, AND CO₂ DEPENDING ON THE LEVEL OF ENVIRONMENTAL PARAMETERS IN TANDEM

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Abstract. The unique environment onboard the International Space Station can be stressful for crop growth, inhibiting adequate yield to supplement the minerals and nutrients of the pre-packaged astronaut diet lost during long-term storage. However, changes in the photon spectrum and relative humidity levels can influence plant growth and secondary metabolism. In this ground-based experiment, we grew lettuce ‘Outredgeous’ in four reach-in growth chambers with two photon spectra (blue (B),

green (G), red (R), and far-red (FR) light at $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each; and B, and G light at $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each plus R and FR light at $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each), two relative humidity (RH) levels (40% and 70%), and two CO_2 concentrations (415 and $2800 \mu\text{mol}\cdot\text{mol}^{-1}$). Light, RH, and CO_2 concentration treatments increased seedling and mature growth parameters depending on the level of the other environmental parameters in tandem. For example, decreasing the (B+G):(R+FR) light ratio in treatments with 40% RH increased fresh and dry mass by 53–65% and 42–44% for seedlings and mature plants, respectively, at $415 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 , while those at $2800 \mu\text{mol}\cdot\text{mol}^{-1}$ were not affected. Treatments at 70% RH only affected seedling fresh mass with decreases of 23% at $2800 \mu\text{mol}\cdot\text{mol}^{-1}$ with no effect at $415 \mu\text{mol}\cdot\text{mol}^{-1}$. Increasing RH and CO_2 concentrations and decreasing the (B+G):(R+FR) light ratio generally increased most measured growth parameters, aside from fresh and dry mass which were variably affected by the (B+G):(R+FR) light ratio depending on the combination of the other parameters per environmental treatment. Secondary metabolism was measured through phenolic compound and anthocyanin concentrations in mature lettuce leaves. These secondary metabolite concentrations were similar across the environmental treatments indicating that FR light, at the higher level of $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, increased mature lettuce biomass without detracting from the measured nutritional composition. These parameter interactions on lettuce growth indicate the need for continued ground-testing of environmental treatments per crop species and cultivars for growth in a space environment.

Abbreviations. B, blue; (B+G):(R+FR), ratio of blue and green to red and far-red light; B:R, ratio of blue to red light; EC, electrical conductivity; FR, far-red; G, green; ISS, International Space Station; LED, light-emitting diode; PIF, phytochrome interacting factor; PSII, photosystem II; R, red; R:FR, ratio of red to far-red light; RH, relative humidity; VPD, vapor pressure deficit

Introduction

Space crop production research is essential to ensure food safety and security for astronauts on the International Space Station (ISS) and in future missions. The current astronaut diet is primarily made up of pre-packaged, freeze-dried meals that run on a cyclical schedule and lead to menu fatigue (Tibbetts 2019). These meals can also start to degrade in nutritional quality after just 1–3 years in storage (Cooper et al. 2017). Fresh, space-grown crops would solve menu fatigue by providing more flavorful variety to the astronaut diet and supplement essential vitamins and minerals lost due to nutritional degradation. However, the the ISS and future stations have unique environmental stressors for crop growth, including superelevated CO₂ concentrations and low relative humidity levels (Burgner et al. 2019, 2020; Massa et al. 2017).

The effects of FR light on plant morphology and photosynthesis are relevant to space crop production under unideal growing conditions. Far-red (FR; 700–750 nm) light exists beyond the photosynthetically active radiation (PAR; 400–700 nm) but has biological significance in plant growth and development. The red (R) to FR light ratio

(R:FR) influences the activity of photoreceptors, especially phytochromes. When phytochromes absorb R light, they partially convert from the inactive form (P_r) to the active form (P_{fr}), which represses transcription factors that promote stem, leaf petiole, and leaf blade extension. When phytochromes absorb FR light, they revert to P_r , which alleviates the suppression of those transcription factors and thus induces the shade-avoidance response (Sharrock 2008). FR light is primarily absorbed by photosystem II (PSII), leading to an unequal excitation between the photosystems. However, FR light interacts synergistically with light of shorter wavelengths to achieve equal excitation and thus enhance photosynthetic efficiency (Zhen and van Iersel 2017).

Besides R and FR light, blue (B) and green (G) light also influence plant growth and development. The photon flux density of B light influences plant photomorphogenesis and secondary metabolism. High B light (e.g., 60–100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) decreases stem elongation and leaf expansion but increases the accumulation of secondary metabolites such as anthocyanins (Meng et al. 2020; Mickens et al. 2018). Anthocyanin accumulation in response to high light provides photoprotection, influences organoleptic attributes, and increases nutritional value (Page et al. 2012). However, increased secondary metabolites can produce undesirable, bitter flavors in leaf tissue, deterring consumers regardless of added nutritional value (Kopsell et al. 2015). Comparatively, low B light (e.g., 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) promotes extension growth and biomass (Meng et al. 2020). Under low B light, phytochrome-interacting factors (PIF) 4 and 5 interact with cryptochromes 1 and 2 to promote extension growth and elicit the shade-avoidance response (Pedmale

et al. 2016). Compared to B and R light, G light has higher reflectance and transmission and lower absorptance (Smith 2017; Vogelmann 1989). However, it penetrates deeper into the leaf to reach spongy mesophyll cells, where light scattering creates extended paths to improve absorption and thus photosynthetic efficiency (Smith 2017; Vogelmann 1989).

CO₂ affects photosynthesis in light-independent reactions, which use ATP and NADPH produced from the light-dependent reactions to fix CO₂ as carbohydrates via RuBisCo (Raines 2003). Although CO₂ enrichment increases the net photosynthetic rate when CO₂ is the limiting factor for carbon fixation (Sharkey et al. 2007), for many plants, there is a species- and cultivar-specific optimal CO₂ concentration that saturates growth promotion (Bugbee et al., 1994; Gutiérrez et al., 2014; Sage et al., 1989; Xu, 2015). Elevated CO₂ concentrations beyond the optimal concentration can decrease RuBisCo concentration and activity, thereby decreasing the net photosynthetic rate and growth of some crops such as Chinese cabbage ‘Tokyo Bekana’ (Burgner et al. 2019, 2020; Sage et al. 1989). However, other leafy greens (e.g., lettuce (*Lactuca sativa* cvs. Dragoon and Outredgeous), mustard greens (*Brassica carinata* cv. Amara), pak choi (*Brassica rapa* cv. Extra Dwarf), shungiku (*Glebionis coronaria*), kale (*Brassica napus* cv. Red Russian and *Brassica oleracea* cv. Toscano), and chard (*Beta vulgaris* cv. Barese Swiss)) respond positively to elevated CO₂ concentrations, which emphasizes the need to screen and identify sensitive and tolerant crops for space production (Wheeler et al. 2021).

Relative humidity (RH) influences plant transpiration and thus water and ion uptake, water balance, and transpirational cooling (Gruda 2005). Suitable RH levels for leafy green growth are between 50% and 70% (Brechtner et al. 2013). Low RH (e.g., in a high vapor pressure deficit (VPD) of 2–6 kPa) promotes plant transpiration and thus increases water loss, which triggers stomatal closure to conserve water but hinders CO₂ influx and thus photosynthesis and growth (Amitrano et al. 2019; Sulman et al. 2016). VPD is the difference between relative humidity and air vapor pressure at any given temperature, whose changes are sensed through plant leaves, and drives transpiration (Amitrano et al. 2019; Sulman et al. 2016). The low RH (\approx 40%) onboard the ISS can hinder plant productivity and limit crop growth (Massa et al. 2017). However, low RH can increase nutritionally relevant secondary metabolites in lettuce (*var. capitata*), such as anthocyanins (Amitrano et al. 2021).

To our knowledge, the combined effects of the light quality, CO₂ concentration, and RH on leafy greens growth are not well understood but merit research because they influence photosynthesis and biomass accumulation through different mechanisms (Bauer et al. 2013; Franklin 2008; Galvão et al. 2015; Sage et al. 1989). Here, we conducted a ground-based experiment to investigate: 1) whether increasing RH and substituting R and FR light for G and B light would increase the growth and morphology of hydroponic lettuce at superelevated CO₂; and 2) whether the light spectrum, CO₂ concentration, and RH would have interactions.

Materials and methods

We grew lettuce ‘Outredgeous’ in a complete randomized block design under eight environmental treatments in two replications across time. For each replication, we sowed two seeds in each of 400 rockwool plugs (2.5 cm long \times 2.5 cm wide \times 3.5 cm tall; AO 25/40, Grodan, Milton, ON, Canada), which were rinsed and soaked in reverse-osmosis water to remove lingering surface surfactants from rockwool production. We placed 50 seeded plugs in each of eight plastic trays (52 cm long \times 25.7 cm wide \times 6.1 cm tall), added reverse-osmosis water to one half of the plug height, and then covered each tray with a clear humidity dome. We placed the trays on top of lidded bus tubs (50.8 cm long \times 38.1 cm wide \times 12.7 cm tall; Choice Foodservice Equipment Company, Layton, UT) under eight environmental treatments in four reach-in growth chambers, each with two identical vertical shelves equipped with color-tunable light-emitting diode arrays (E41L2; Percival Scientific, Perry, IA). The environmental treatments were the combinations of two light spectra (B, G, R, and FR light at $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each; and B, and G light at $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each plus R and FR light at $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ each), two RH levels (40% and 70%), and two CO₂ concentrations (415 and $2800 \mu\text{mol}\cdot\text{mol}^{-1}$) (Fig. 4 and Table 3). All treatments delivered the same total photon flux density (400–750 nm) of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under an 18-h photoperiod. We measured and set up each light spectrum with 12 spectral scans across the growing area using a spectroradiometer (SS-110; Apogee Instruments, Logan, UT). All chambers maintained a constant air temperature setpoint of 22 °C. Both third-party sensors and the internal chamber sensors were used to ensure the accuracy of the environmental parameters. Commercial temperature and

RH sensors (MX1101; Onset, Bourne, MA) were placed on each chamber shelf on top of a wooden block under a radiation shield to log data every 15 min. A CO₂ concentration sensor (YEM-40L; GZAIR, Guangzhou, China) was placed on the top shelf of every chamber to log data every 10 min.

We removed the humidity domes on day 4 and subirrigated the seedlings to one fourth of the plug height with a nutrient solution starting day 5. The nutrient solution consisted of a base fertilizer mix (12-4-16 RO FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (JR Peters, Inc.) dissolved in reverse-osmosis water (electrical conductivity (EC) = 1.30 ± 0.05 dS/m; pH = 5.80 ± 0.01), which supplied the following nutrients: nitrogen ($124.82 \text{ mg}\cdot\text{L}^{-1}$), phosphorus ($18.31 \text{ mg}\cdot\text{L}^{-1}$), potassium ($138.13 \text{ mg}\cdot\text{L}^{-1}$), calcium ($72.81 \text{ mg}\cdot\text{L}^{-1}$), magnesium ($48.47 \text{ mg}\cdot\text{L}^{-1}$), sulfur ($39.02 \text{ mg}\cdot\text{L}^{-1}$), boron ($0.12 \text{ mg}\cdot\text{L}^{-1}$), copper ($0.47 \text{ mg}\cdot\text{L}^{-1}$), iron ($1.77 \text{ mg}\cdot\text{L}^{-1}$), manganese ($0.52 \text{ mg}\cdot\text{L}^{-1}$), molybdenum ($0.13 \text{ mg}\cdot\text{L}^{-1}$), and zinc ($0.56 \text{ mg}\cdot\text{L}^{-1}$). On day 7, we manually thinned seedlings to one plant per plug with tweezers. On day 14, we transplanted 12 randomly selected seedlings in their rockwool plugs to two bus tubs per treatment filled with the above nutrient solution. We placed each seedling plug in one of six net pots (6.35 cm diameter \times 5.08 cm tall; Cz Garden Supply) within each bus tub lid. From day 14 to 29, we maintained the nutrient solution level at one-half of the plug height. We recorded the pH and EC of the solution within each bus tub daily and maintained the solution pH at 5.8 ± 0.3 .

On the day of transplant (day 14), we conducted destructive measurements of 10 randomly selected seedlings per environmental treatment. We collected data on

growth (shoot fresh and dry mass), morphology (stem length, length of the longest leaf, and total leaf area) and relative chlorophyll concentration and leaf pigmentation using the $L^*a^*b^*$ color space. We measured total leaf area with a portable leaf area meter (CI-202; CID Bio-Science, Inc., Camas, WA). We also used a chlorophyll meter (MC-100, Apogee Instruments, Inc., Logan, UT) to measure relative chlorophyll concentration and a color reader (CR-10; Konica Minolta Sensing, Ramsey, NJ) to take color measurements in the $L^*a^*b^*$ color space. L^* is a brightness scale from 0 to 100, with 0 representing black and 100 representing white. Both a^* and b^* range from -150 to 150, with a^* representing a G–R color scale and b^* representing a B–yellow color scale. Shoot dry mass was recorded after drying plant material in an oven at 65 °C for 7 d. On day 29, besides the same parameters as for seedlings, we collected mature plant data on plant diameter, root length, and root dry mass. In addition, we completed phytochemical concentration extractions and measurements from representative mature plant leaves. We freeze-dried plant tissue (model no. 5.2.16; Harvest Right, North Salt Lake, UT) and ground the tissue of three plants from each treatment with a mortar and a pestle. We followed the pH differential method to determine anthocyanin concentration and the spectrophotometric method to determine phenolic concentration (Ainsworth and Gillespie 2007; Lee et al. 2005).

At each harvest, we took images of one representative plant per environmental treatment for visual treatment comparisons (Fig. 5). We downloaded the environmental data from the internal chamber sensors at the end of each replication. We also downloaded the third-party sensor data weekly to ensure that the

environmental parameters were maintained in each treatment throughout the experiment. We analyzed plant data with the Tukey's honestly significant difference test ($\alpha = 0.05$) in JMP Pro (ver. 16.0.0; SAS Institute Inc., Cary, NC). We pooled data from two replications when there was no treatment \times replication interaction or when data trends among treatments were consistent across replications.

Results

Light. Increasing FR and R light while decreasing B and G light generally increased seedling shoot height, leaf length, total leaf area, L^* , and b^* . Decreasing (B+G):(R+FR) light decreased relative chlorophyll concentrations and a^* . Increasing the FR light intensity in seedlings grown at 40% RH, increased shoot fresh and dry mass from 53–65% at $415 \mu\text{mol}\cdot\text{mol}^{-1}$ while those at $2800 \mu\text{mol}\cdot\text{mol}^{-1}$ were not affected (Fig. 6). However, at 70% RH, increasing the incidence of FR light decreased fresh mass by 23% at $2800 \mu\text{mol}\cdot\text{mol}^{-1}$ and had no effect at $415 \mu\text{mol}\cdot\text{mol}^{-1}$. Decreasing (B+G):(R+FR) only affected seedling shoot height by increasing it 26% in plants grown at $415 \mu\text{mol}\cdot\text{mol}^{-1}$ and 40% RH (Table 4). $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light increased leaf length by 20–36% at low RH at both CO_2 concentrations compared to $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light. Total leaf area increased by 69% as FR light increased at low RH and low CO_2 concentrations. Increasing FR light decreased relative chlorophyll concentration by 8% in treatments with $415 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 and 40% RH, and by 10% in treatments with $2800 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 and 70% RH. L^* readings were affected by FR light in most treatments with increases of $50\text{--}80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of

FR light leading to a 21–23% increase in L^* readings, or foliage brightness. A^* readings were also predominantly affected by decreasing (B+G):(R+FR) with decreases from 161–1561% signifying greener leaves. B^* readings generally increased by 28–43% as (B+G):(R+FR) decreased signifying yellower leaves. In mature plants, increasing R and FR light from 50–80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ while decreasing G and B light from 50–20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased shoot fresh mass and dry mass, canopy diameter, and total leaf area in most treatments. In some treatments, FR light increases decreased mature root length. Increasing the FR light intensity in treatments with 40% RH increased mature shoot fresh and dry mass by 42–44% but those in treatments with 70% RH were not affected at 415 $\mu\text{mol}\cdot\text{mol}^{-1}$. Treatments at 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ showed no significant differences as (B+G):(R+FR) decreased. Plant canopy diameter increased by 11–12% as FR light increased under 415 $\mu\text{mol}\cdot\text{mol}^{-1}$, with no effect under 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$. Total leaf area increased as FR light intensity increased by 40% in treatments with 40% RH at a low CO_2 concentration. Mature root length decreased by 25% as (B+G):(R+FR) decreased in treatments with high CO_2 concentrations and 40% RH.

RH. For seedlings, increasing RH from 40% to 70% generally increased shoot fresh mass, dry mass, shoot height, leaf length, total leaf area, and b^* . Increasing RH decreased relative chlorophyll concentration and a^* in some cases. Increasing RH increased seedling shoot fresh mass by 59–83% at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light, but not at 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light, regardless of CO_2 concentration. Dry mass increased by 46–47% at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light regardless of CO_2 concentration

as relative humidity increased, treatments at $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light were not affected (Fig. 6). Shoot height increased by 22–23% as the RH increased under low FR light levels regardless of the CO_2 concentration. Increasing the RH simultaneously increased leaf length by 30–43% at low FR levels regardless of CO_2 concentration (Table 4). Additionally, increasing RH increased seedling leaf area by 65–77% in treatments with low levels of FR light. Increasing the RH decreased relative chlorophyll concentrations in most treatments by 12–19%, aside from treatments grown at $415 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 and $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light. RH decreased a^* foliage readings by 185–1239% in treatments with low FR intensities and increased b^* values at high CO_2 concentrations by increasing b^* by 22–36%. In mature plants, RH increased canopy diameter and leaf length in most environmental treatments. In some treatments, RH increased shoot fresh mass and dry mass. Fresh and dry mass increased by 26–28% in treatments with $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light and $2800 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 . Increasing the RH increased the canopy diameter overall by 11–16%. Increasing the RH increased leaf length in most treatments by 10–13% except for treatments with a high CO_2 concentration and a high FR light intensity.

CO_2 . CO_2 had a positive individual effect on most measured parameters for seedlings and mature plants. In seedlings, increasing the CO_2 concentration increased shoot fresh and dry mass by 46–48% at 70% RH and $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light, and increased shoot dry mass by 46% at 40% RH and $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light (Fig. 6). In mature plants, increasing the CO_2 concentration increased shoot fresh and dry mass by 23–61% across all environmental treatments. Increasing the CO_2 concentration

increased the canopy diameter by 10% at 70% RH and low FR light, but not under the other treatments (Table 4). Treatments with low FR and RH had a 41% increase in total leaf area as the CO₂ concentration increased.

Discussion

Light. In our work, we postulated that using FR light would enhance production under low relative humidity levels and superelevated CO₂ concentrations through the shade avoidance response. This response elicited by FR light leads to stem elongation, leaf area expansion, and overall biomass accumulation (Demotes-Mainard et al. 2016; Franklin 2008; Zou et al. 2019). These morphological expressions could counteract the negative effects of low relative humidity and superelevated CO₂ concentrations as these parameters affect photosynthesis and subsequent biomass accumulation through separate genetic pathways (Bauer et al. 2013; Franklin 2008; Galvão et al. 2015, Sage et al. 1989). FR light affected both seedling and mature plants variably, and not continuously for some growth parameters. For example, in seedlings, FR light had no significant effects on shoot biomass, but FR light did significantly affect all parameters for mature shoot biomass, with no effect on mature root biomass. This increase in response over time could be attributed to the amount of time needed for FR light responses to become detectable in lettuce plants, though unlikely. However, the changes in the B:R light ratio in combination with FR light could also explain these results. Lettuce ‘Rex’ and ‘Cherokee’, when grown for 12 days, did not exhibit differences in fresh mass when 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light was added to a

high and low B:R light ratio (Meng and Runkle 2019). Additionally, the effects of FR light on lettuce ‘Rex’ and ‘Rouxai’ were attenuated with a low B:R ratio and a high photosynthetic photon flux density (Meng and Runkle 2019). Low levels of B light induce similar morphologies to the shade avoidance response, as seen in arabidopsis (*Arabidopsis thaliana*) (Pedmale et al. 2016). Therefore, it seems that the effects of FR light on lettuce growth depend on the amount of B light present in the environment. Additionally, the bus tub lids containing lettuce ‘Outredgeous’ treated with $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light, were much more crowded with enlarged lettuce than those grown under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light. The increase in neighbor shading (beginning day 24) would strengthen the FR shade avoidance response, and not just due to the spectral treatment with an increased incidence of FR light. As the lettuce grows, the FR light response would become more evident in all treatments than when the lettuce is freshly transplanted.

RH. At both growth stages, RH influenced almost all measured growth parameters. Lettuce ‘Outredgeous’ had increased growth and development when grown under 70% RH compared to the ambient-ISS RH of 40%. Low RH levels, such as those seen on the ISS, contribute to increased transpiration and plant water loss, ultimately leading to stomatal closure (Amitrano et al. 2019; Mencuccini 2003). Stomatal closure prevents subsequent gas exchange and thus the light-independent reactions of photosynthesis are delayed due to lack of CO₂ fixation (Amitrano et al. 2019). Decreases in seedling relative chlorophyll concentration with increasing RH is surprising and, to our knowledge, has not been reported. In seedlings, increasing RH

increased shoot height, leaf length, and total leaf area. Similarly, when R:FR is decreased, plants follow the shade avoidance response, with rapid changes in morphology (Franklin 2008). This rapid change toward elongated growth creates a ‘dilution effect’ where the expansion in growth decreases the amount of chlorophyll per area (Zhen and Bugbee 2020). Although RH and FR light affect photosynthesis through different pathways, in this case, these pathways may have the same effect on seedling chlorophyll concentrations (Bauer et al. 2013; Demotes-Mainard et al. 2016; Franklin 2008). The lack of RH influence on mature relative chlorophyll concentration may relate to the attenuated growth responses of mature lettuce to RH when compared to seedlings. All environmental treatments for seedling growth and morphology increased with increasing RH level. However, RH produced variable responses for mature growth and morphology across the environmental treatments. This decrease could be an environmental adaptation to the respective RH level. The fresh mass of kale (*Brassica oleracea* var. *alboglabra* Bailey) responded to five RH levels at week 2 but did not at week 4, indicating that leafy greens can acclimate to RH (Chowdhury et al. 2021).

*CO*₂. Superelevated CO₂ increased lettuce fresh and dry mass and, in some cases, canopy diameter and leaf area at both growth stages. In a similar study, when compared to the 400 μmol·mol⁻¹ treatment, lettuce ‘Outredgeous’ and other leafy greens had greater fresh and dry mass at superelevated concentrations (1500, 3000, and 6000 μmol·mol⁻¹) with no negative growth effects (Wheeler et al. 2021). Leaf expansion is commonly seen under elevated CO₂ concentrations for some plants (Prior

et al. 2004; Pritchard et al. 2001). It is not known whether elevated CO₂ concentrations increased lettuce leaf expansion by increasing leaf cell turgor pressure, cell expansion, and/or cell division, as this mechanism varies between species (Madsen 1968; Pritchard et al. 2001; Ranasinghe and Taylor 1995; Sasek and Strain 1989; Taylor et al. 1994).

Parameter interactions. Two and three-way interactions were more impactful for lettuce at the seedling stage than for mature plants. The two-way interaction of FR × RH was most frequently identified as significant in lettuce seedlings. FR light and high incidences of RH increased stem elongation in tomato cultivars (*Solanum lycopersicum*) (Retana-Cordero et al. 2022). FR light and RH have individually increased stem elongation through the shade avoidance response and perhaps through increased cell turgor, respectively (Azuma et al. 1997; Hirai et al. 2000; Hwang et al. 2020; Retana-Cordero et al. 2022). In the present experiment, even if FR light did not affect a measured parameter for ‘Outredgeous’ seedlings, the interaction of FR × RH was still significant. As FR and RH each increase there is an increase in plant growth and development. The signaling pathways that respond to these parameters and mediate growth expansion may be different, however, they may interact depending on the intensity of FR light and the level of RH. RH × CO₂ was another two-way interaction of significance in measured seedling parameters. *Chrysanthemum morifolium* cv. ‘Fiesta’ when grown at high and low parameters of RH and CO₂ concentration, exhibited a significant positive RH × CO₂ interaction on plant growth within the first two weeks that disappeared at the four- and six-week marks of the

experiment, following the trend seen in the present data (Gislerød and Nelson 1988). Increases in both RH and CO₂ concentration increased the total stomatal aperture allowing increased gas exchange and thus an increase in growth and development (Amitrano et al. 2019; Gislerød and Nelson 1988). The current study shows that for ‘Outredgeous’ seedlings, two-way interactions had more instances of significance, including RH × CO₂, whereas the mature plants had only one instance of RH × CO₂ significance. This tapering off of two-way interaction influences could indicate a decrease in the growth rate, or acclimation to the environment itself. FR × CO₂ was no exception to this tapering off but was the most prevalent two-way interaction on mature lettuce growth. To our knowledge, there was no previous work combining FR light intensity and CO₂ concentration interactions on plant growth. However, the individual effects of these two parameters were additive for plant growth at both growth stages. This two-way interaction present at the mature stage could be evident of the increased neighbor shading occurring in the high FR light treatments. As FR light increases photosynthetic efficiency by acting synergistically with lower wavelengths of PAR, CO₂ would also be utilized at an increased rate to match the increase in light-dependent photosynthetic reactions (Sharkey et al. 2007; Zhen and van Iersel 2017). The one instance of the three-way interaction of FR × RH × CO₂ having a significant influence indicates that although there is an amplification of individual effects in each two-way interaction, the specific two-way interaction does not vary in its effect when combined with the third variable for interaction and is therefore not significant.

Tipburn of inner young leaves occurred in many mature lettuce plants (Fig. 4) and was unaffected by environmental treatments. As tipburn is a response to rapid growth and calcium-deficiency in leaves, and increases in response to light intensity, this could be related to the nutrient solution provided within each treatment (Sago 2016). The nutrient solution was only tested for pH and EC, and not for the individual mineral content during the experiment. We postulate that due to the increased, elongated growth under FR light, and the lack of knowledge surrounding the mineral content within each treatment's nutrient solution, that there was not enough calcium present to prevent tipburn and subsequent necrosis in our lettuce (Demotes-Mainard et al. 2016). Future work should consider analysis of the mineral constitution of the nutrient solution used to ensure adequate nutrition of the crop, and to prevent negative impact.

Overall, increasing any of the individual parameters in this study increased most lettuce growth and morphology measurements at the seedling and mature stages. FR light at the higher level of $80 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ increased mature biomass without detracting from the measured nutritional composition as typically seen under increased instances of FR light (Spencer et al 2020). This shows that FR light may be included in future space crop systems to enhance the yield in some crop species without reducing the nutrients and minerals used to supplement the astronaut diet. When in combination with FR or CO_2 , increasing RH amplified their positive effects. A system with RH control should be considered in the future of space crop production. Although there is concern of the effects of superelevated CO_2 concentrations on crop growth

during spaceflight based on previous literature (Bugbee et al. 1994; Burgner et al. 2019, 2020; Gutiérrez et al. 2014; Sage et al. 1989; Xu 2015), there were no negative effects of ambient-ISS CO₂ concentrations on lettuce as, based on this and past studies, this lettuce cultivar appears to tolerate superelevated CO₂ concentrations (Wheeler et al. 2021). This emphasizes the need to ground-test individual species for their readiness under ambient-ISS conditions.

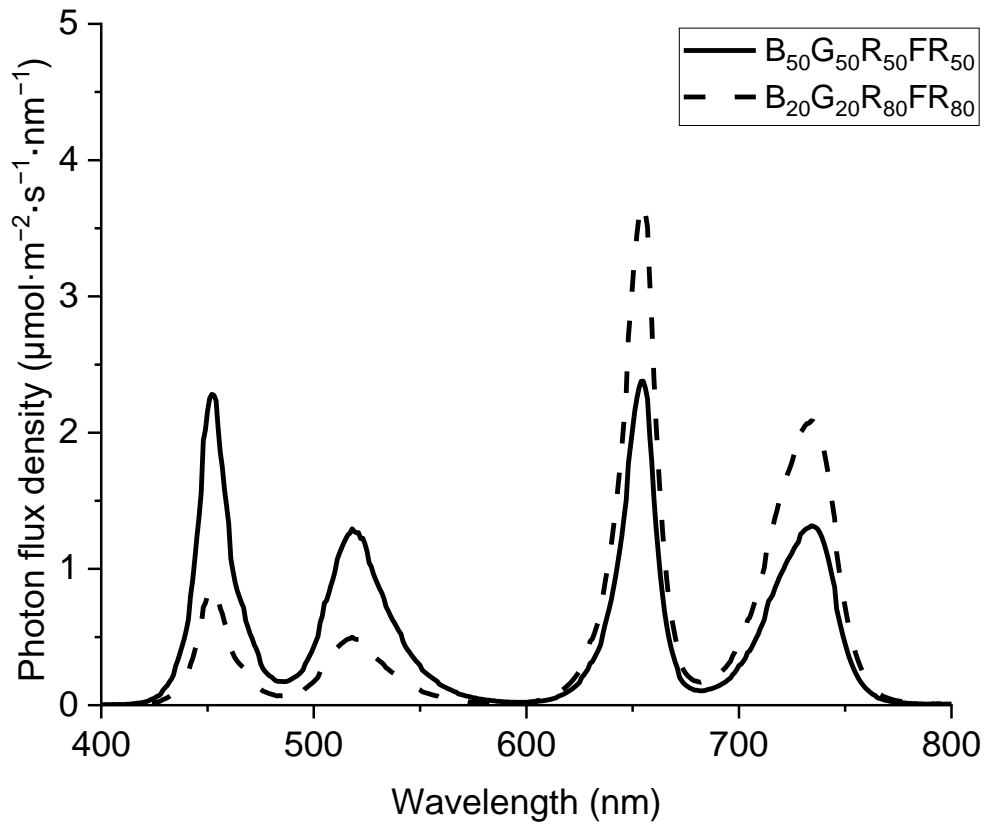


Figure 4. Distributions of two light spectra at the same total photon flux density. Each letter signifies an abbreviated waveband [e.g., blue (B), green (G), red (R), and far-red (FR)]. The photon flux density (in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) follows each waveband abbreviation in subscript.

Table 3. The environmental parameters of two experimental replications. Each treatment name is designated by the CO₂ concentration followed by the relative humidity level and far-red (FR) light intensity in subscript (e.g., 415-40 FR50: a CO₂ concentration of 415 $\mu\text{mol}\cdot\text{mol}^{-1}$, relative humidity level of 40%, and a FR light intensity of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Environmental parameters are represented by the reported average and standard deviation. T: top shelf, B: bottom shelf.

		Replication One							
Chamber Shelf	1		2		3		4		
	T	B	T	B	T	B	T	B	
	415-40 FR ₅₀	415-40 FR ₈₀	2800-70 FR ₈₀	2800-70 FR ₅₀	415-70 FR ₅₀	415-70 FR ₈₀	2800-40 FR ₈₀	2800-40 FR ₅₀	
		Environmental Data							
Temperature (°C)	21.74 ± 0.57	21.82 ± 0.65	21.86 ± 0.41	22.09 ± 0.35	21.81 ± 0.40	22.19 ± 0.35	21.72 ± 0.56	22.05 ± 0.50	
Relative Humidity (%)	43.91 ± 6.08	43.29 ± 5.72	70.66 ± 1.82	69.84 ± 1.40	70.75 ± 1.40	69.12 ± 1.28	43.51 ± 6.37	42.37 ± 5.97	
CO ₂ Concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$)	395.95 ± 17.40		2754.11 ± 16.89		445.70 ± 20.27		2792.13 ± 14.24		
		Nutrient Solution Data							
pH	5.86 ± 0.26	5.88 ± 0.22	5.91 ± 0.22	5.97 ± 0.19	5.91 ± 0.21	6.01 ± 0.23	5.87 ± 0.25	5.87 ± 0.25	
Electrical Conductivity (dS/m)	1.50 ± 0.09	1.47 ± 0.07	1.39 ± 0.04	1.38 ± 0.02	1.41 ± 0.06	1.37 ± 0.06	1.43 ± 0.03	1.44 ± 0.05	
		Replication Two							
Chamber Shelf	1		2		3		4		
	T	B	T	B	T	B	T	B	
	2800-70 FR ₈₀	2800-70 FR ₅₀	415-40 FR ₅₀	415-40 FR ₈₀	2800-40 FR ₈₀	2800-40 FR ₅₀	415-70 FR ₅₀	415-70 FR ₈₀	

	Environmental Data							
Temperature (°C)	21.84 ± 0.30	21.95 ± 0.32	21.92 ± 0.46	21.48 ± 0.43	21.61 ± 0.28	22.20 ± 0.40	21.92 ± 0.25	21.90 ± 0.29
Relative Humidity (%)	70.90 ± 2.79	69.95 ± 2.70	46.60 ± 7.63	47.60 ± 7.27	45.28 ± 6.76	43.99 ± 6.48	70.84 ± 2.79	70.50 ± 2.65
CO ₂ Concentration (μmol·mol ⁻¹)	2785.95 ± 10.25		406.12 ± 62.57		2824.51 ± 13.94		402.22 ± 64.00	
	Nutrient Solution Data							
pH	5.88 ± 0.20	5.91 ± 0.21	5.88 ± 0.19	5.80 ± 0.24	5.77 ± 0.28	5.74 ± 0.21	5.78 ± 0.27	5.85 ± 0.25
Electrical Conductivity (dS/m)	1.35 ± 0.02	1.34 ± 0.02	1.50 ± 0.08	1.47 ± 0.06	1.43 ± 0.03	1.46 ± 0.04	1.45 ± 0.05	1.40 ± 0.03

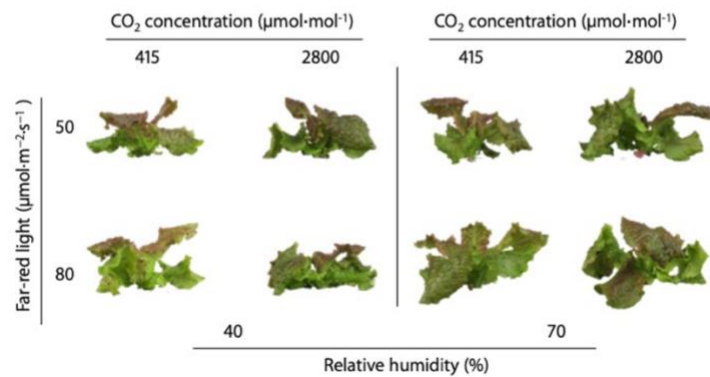
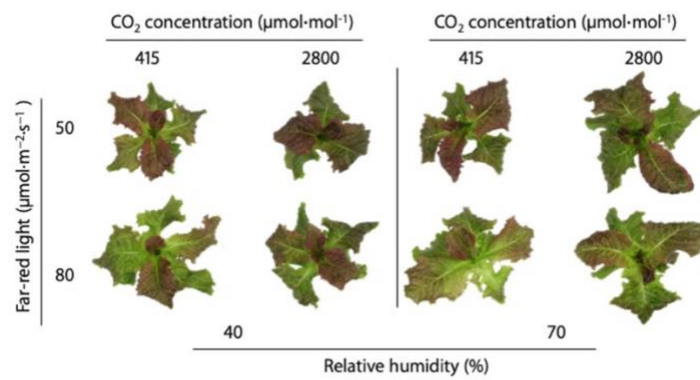
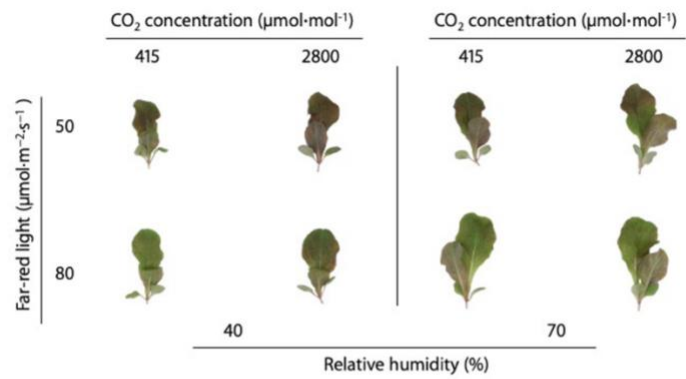


Figure 5. Representative images of lettuce ‘Outredgeous’ at the seedling (day 14) and mature (day 29) stages grown under eight environmental treatments, including two light spectra with 50 and 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light, two relative humidity levels of 40% and 70%, and two CO₂ concentrations of 415 and 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$.

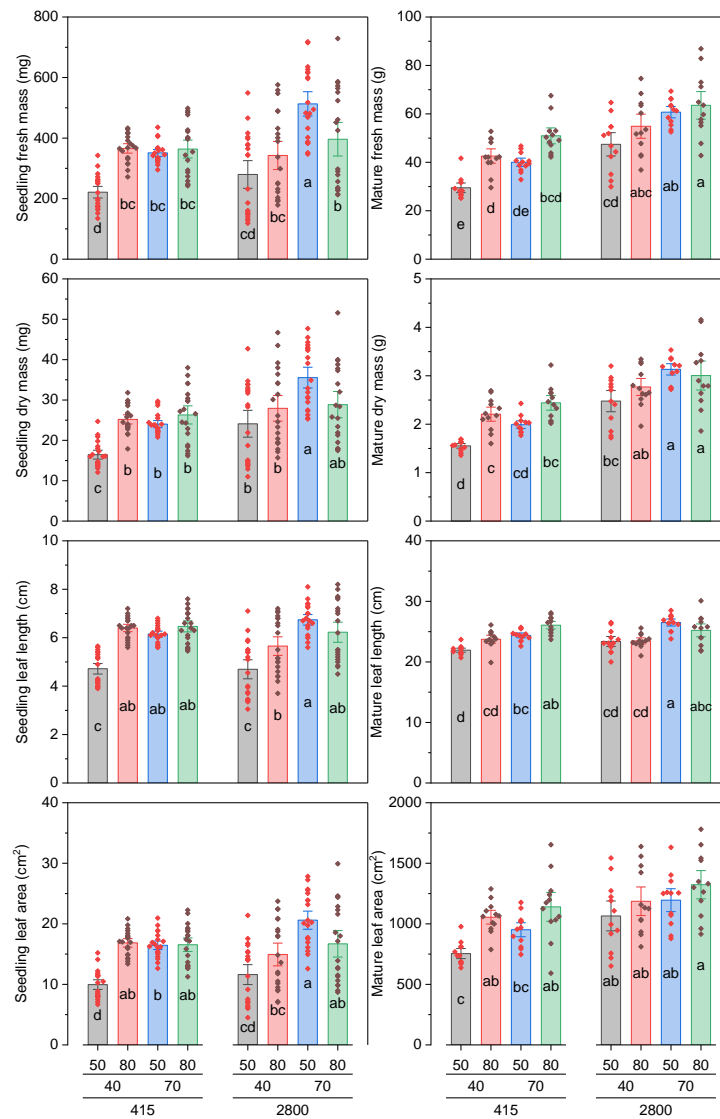


Figure 6. Shoot fresh and dry mass, longest leaf length, and total leaf area of lettuce ‘Outredgeous’ at seedling (day 14) and mature (day 29) growth stages. X-axis tiers designate that lettuce was grown under two light spectra with 50 and 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light (first tier), two relative humidity levels of 40% and 70% (second tier), and two CO_2 concentrations of 415 and 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$ (third tier). Different letters within each panel are significantly different by Tukey’s honest significant difference test at $P \leq 0.05$.

Table 4. Measurements on the biomass, morphology, and chlorophyll and pigmentation of lettuce 'Outredgeous' at seedling (day 14) and mature (day 29) growth stages. The root morphology and measured nutritional composition of mature plants is also described. Measurement abbreviations for fresh mass (FM), dry mass (DM), moisture content (MC), shoot height (SH), longest leaf length (LL), total leaf area (LA), canopy diameter (CD), relative chlorophyll concentration (Chl.), and root length (RL) have been used. 'Outredgeous' was grown at two light spectra with 50 (FR₅₀), and 80 (FR₈₀) $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light, two relative humidity levels at 40% and 70%, and two CO₂ concentrations at 415 and 2800 $\mu\text{mol}\cdot\text{mol}^{-1}$. Treatment names are designated by the CO₂ concentration followed by the relative humidity level and far-red light intensity in subscript (e.g., 415-40 FR₅₀: a CO₂ concentration of 415 $\mu\text{mol}\cdot\text{mol}^{-1}$, relative humidity level of 40%, and a FR light intensity of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Different letters within each column are significantly different by Tukey's honest significant difference test at $P \leq 0.05$ of pooled data of two experimental replications. For treatment and replication significance, * and ** represent significance at $P \leq 0.05$ and $P \leq 0.001$, respectively.

Treatment (Trt)	Seedling			Mature		
	Biomass			Biomass		
	Shoot FM (mg)	Shoot DM (mg)	Shoot MC (%)	Shoot FM (g)	Shoot DM (g)	Shoot MC (%)
415-40 FR ₅₀	221.27 ± 12.84 d	16.46 ± 0.73 c	92.41 ± 0.19 b	29.47 ± 1.32 e	1.55 ± 0.03 d	94.67 ± 0.14 b
415-40 FR ₈₀	365.84 ± 10.40 bc	25.20 ± 0.76 b	93.10 ± 0.13 ab	42.58 ± 1.99 d	2.21 ± 0.10 c	94.80 ± 0.08 ab
415-70 FR ₅₀	351.10 ± 7.71 bc	24.02 ± 0.58 b	93.15 ± 0.09 a	40.01 ± 1.16 de	1.99 ± 0.05 cd	95.01 ± 0.08 ab
415-70 FR ₈₀	363.64 ± 19.75 bc	26.31 ± 1.50 b	92.78 ± 0.11 ab	50.93 ± 2.19 bcd	2.44 ± 0.10 bc	95.18 ± 0.15 ab
2800-40 FR ₅₀	279.88 ± 30.54 cd	24.11 ± 2.22 b	91.02 ± 0.22 c	47.43 ± 3.20 cd	2.48 ± 0.15 bc	94.74 ± 0.08 ab
2800-40 FR ₈₀	342.51 ± 30.92 bc	27.93 ± 2.12 b	91.60 ± 0.19 c	54.89 ± 3.32 abc	2.77 ± 0.12 ab	94.88 ± 0.15 ab
2800-70 FR ₅₀	512.97 ± 26.81 a	35.55 ± 1.71 a	93.02 ± 0.12 ab	60.70 ± 1.56 ab	3.13 ± 0.08 a	94.83 ± 0.09 ab

2800-70 FR ₈₀	396.25 ± 36.97 b	28.86 ± 2.16 ab	92.41 ± 0.23 b	63.52 ± 3.81 a	3.01 ± 0.20 a	95.25 ± 0.19 a
CO ₂ Trt	**	**	**	**	**	NS
RH Trt	**	**	**	**	**	*
FR Trt	NS	NS	NS	**	**	*
FR × RH	**	**	**	NS	NS	
RH × CO ₂	*	NS	**	NS	NS	
FR × CO ₂	*	*	NS	NS	*	
FR × RH × CO ₂	NS	NS	NS	NS	NS	
Replication	**	**	**	**	**	*
Trt. × Rep.	**	**	**	*	*	NS

Morphology

	SH (cm)	LL (cm)	LA (cm ²)	CD (cm)	LL (cm)	LA (cm ²)
415-40 FR ₅₀	0.55 ± 0.02 bc	4.72 ± 0.15 c	9.95 ± 0.53 d	31.39 ± 0.59 d	21.93 ± 0.22 d	754.11 ± 27.73 c
415-40 FR ₈₀	0.69 ± 0.02 a	6.40 ± 0.10 ab	16.85 ± 0.47 ab	35.29 ± 0.76 bc	23.73 ± 0.44 cd	1054.44 ± 38.03 ab
415-70 FR ₅₀	0.67 ± 0.02 a	6.15 ± 0.08 ab	16.45 ± 0.47 b	35.91 ± 0.51 bc	24.38 ± 0.27 bc	950.84 ± 38.57 bc
415-70 FR ₈₀	0.69 ± 0.03 a	6.47 ± 0.15 ab	16.55 ± 0.74 ab	39.95 ± 0.75 a	26.07 ± 0.41 ab	1140.10 ± 79.42 ab
2800-40 FR ₅₀	0.52 ± 0.02 c	4.70 ± 0.26 c	11.62 ± 1.10 cd	34.19 ± 0.81 cd	23.38 ± 0.55 cd	1064.99 ± 82.30 ab
2800-40 FR ₈₀	0.59 ± 0.03 abc	5.65 ± 0.26 b	14.93 ± 1.25 bc	34.78 ± 1.02 cd	23.47 ± 0.33 cd	1186.16 ± 78.84 ab

2800-70 FR ₅₀	0.64 ± 0.02 ab	6.74 ± 0.15 a	20.60 ± 1.00 a	39.68 ± 0.97 a	26.51 ± 0.39 a	1195.22 ± 63.06 ab		
2800-70 FR ₈₀	0.64 ± 0.03 ab	6.23 ± 0.28 ab	16.70 ± 1.46 ab	38.65 ± 1.13 ab	25.22 ± 0.71 abc	1323.09 ± 77.56 a		
CO ₂ Trt	*	NS	NS	*	NS	**		
RH Trt	**	**	**	**	**	*		
FR Trt	*	**	*	*	NS	**		
FR × RH	*	**	**	NS	NS	NS		
RH × CO ₂	NS	*	NS	NS	NS	NS		
FR × CO ₂	NS	*	*	**	**	NS		
FR × RH × CO ₂	NS	NS	NS	NS	NS	NS		
Replication (Rep.)	NS	**	**	NS	*	*		
Trt. × Rep.	*	**	**	*	*	*		
Chlorophyll and coloration								
	Chl.	<i>L</i> *	<i>a</i> *	<i>b</i> *	Chl.	<i>L</i> *	<i>a</i> *	<i>b</i> *
415-40 FR ₅₀	6.87 ± 0.15 a	20.40 ± 0.74 cd	0.18 ± 0.29 ab	14.49 ± 0.55 cd	8.18 ± 0.44	15.05 ± 2.64	6.83 ± 0.42 a	4.88 ± 0.75
415-40 FR ₈₀	6.29 ± 0.13 bc	24.84 ± 1.31 a	-2.63 ± 0.45 cde	19.68 ± 0.89 ab	7.07 ± 0.23	16.56 ± 2.99	5.44 ± 0.58 ab	5.45 ± 1.30
415-70 FR ₅₀	6.06 ± 0.11 cd	21.26 ± 0.67 bcd	-2.05 ± 0.25 cde	17.21 ± 0.44 bc	8.20 ± 0.41	15.73 ± 2.79	5.83 ± 0.64 ab	4.47 ± 0.69
415-70 FR ₈₀	5.96 ± 0.11 cd	21.90 ± 0.70 abc	-3.72 ± 0.20 e	20.04 ± 0.49 ab	7.35 ± 0.39	16.84 ± 3.02	5.08 ± 0.77 ab	8.18 ± 1.27
2800-40 FR ₅₀	7.08 ± 0.17 a	18.29 ± 0.59 d	1.35 ± 0.41 a	12.06 ± 0.62 d	8.08 ± 0.53	14.79 ± 2.66	5.87 ± 0.48 ab	4.23 ± 0.86

2800-40 FR ₈₀	6.83 ± 0.13 ab	22.18 ± 0.68 abc	-1.72 ± 0.52 cd	17.24 ± 0.77 bc	7.92 ± 0.56	16.52 ± 3.02	5.75 ± 0.64 ab	5.99 ± 1.49
2800-70 FR ₅₀	6.13 ± 0.13 c	20.26 ± 0.78 cd	-1.15 ± 0.36 bc	16.45 ± 0.57 c	8.70 ± 0.56	14.70 ± 2.48	5.94 ± 0.63 ab	3.77 ± 0.92
2800-70 FR ₈₀	5.52 ± 0.10 d	24.82 ± 0.88 ab	-3.00 ± 0.48 de	21.10 ± 0.79 a	7.90 ± 0.53	15.48 ± 3.18	3.69 ± 0.59 b	7.13 ± 1.77
CO ₂ Trt	NS	NS	**	*	NS	NS	NS	NS
RH Trt	**	NS	**	**	NS	NS	NS	NS
FR Trt	**	**	**	**	*	NS	*	*
FR × RH	NS	NS	*	NS	NS	NS	NS	NS
RH × CO ₂	*	*	NS	*	NS	NS	NS	NS
FR × CO ₂	NS	NS	NS	NS	NS	NS	NS	NS
FR × RH × CO ₂	*	NS	NS	NS	NS	NS	NS	NS
Replication (Rep.)	NS	**	**	**	NS	**	*	**
Trt. × Rep.	*	**	**	**	NS	NS	NS	*

Mature root morphology

	Root DM (mg)	RL (cm)	Root:Shoot
415-40 FR ₅₀	106.22 ± 12.13	29.20 ± 1.87 ab	0.068 ± 0.007 a
415-40 FR ₈₀	109.35 ± 12.65	23.85 ± 1.17 ab	0.049 ± 0.005 ab
415-70 FR ₅₀	105.57 ± 5.79	27.94 ± 1.03 ab	0.053 ± 0.003 ab
415-70 FR ₈₀	124.90 ± 15.44	28.56 ± 1.86 ab	0.051 ± 0.006 ab
2800-40 FR ₅₀	138.88 ± 13.13	30.23 ± 1.94 a	0.055 ± 0.003 ab

2800-40 FR ₈₀	144.88 ± 20.98	22.66 ± 1.71 b	0.051 ± 0.006 ab
2800-70 FR ₅₀	142.87 ± 13.11	25.53 ± 1.39 ab	0.045 ± 0.004 b
2800-70 FR ₈₀	156.40 ± 18.80	27.53 ± 1.36 ab	0.051 ± 0.005 ab
CO ₂ Trt	*	NS	NS
RH Trt	NS	NS	NS
FR Trt	NS	*	NS
FR × RH	NS	**	NS
RH × CO ₂	NS	NS	NS
FR × CO ₂	NS	NS	NS
FR × RH × CO ₂	NS	NS	NS
Replication (Rep.)	**	NS	**
Trt. × Rep.	**	*	*
Phytochemical composition			
	Phenolic concentration (µg/mg)	Anthocyanin concentration (mg/g)	
415-40 FR ₅₀	47.80 ± 2.21 a	2.79 ± 0.21	
415-40 FR ₈₀	39.65 ± 1.63 abc	3.43 ± 0.26	
415-70 FR ₅₀	43.86 ± 1.94 ab	4.56 ± 1.27	
415-70 FR ₈₀	38.50 ± 2.04 bc	4.84 ± 0.92	
2800-40 FR ₅₀	42.23 ± 2.15 abc	4.12 ± 0.75	

2800-40 FR ₈₀	38.97 ± 1.82 bc	3.95 ± 0.72
2800-70 FR ₅₀	37.28 ± 2.27 bc	3.09 ± 0.23
2800-70 FR ₈₀	34.14 ± 1.31 c	3.34 ± 0.14
CO ₂ Trt	*	NS
RH Trt	*	NS
FR Trt	**	NS
FR × RH	NS	NS
RH × CO ₂	NS	*
FR × CO ₂	NS	NS
FR × RH × CO ₂	NS	NS
Replication (Rep.)	*	NS
Trt. × Rep.	NS	NS



Figure 7. Tipburn of inner leaves of lettuce 'Outredgeous'. It was present in all environmental treatments.

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Chapter 3

FAR-RED LIGHT AND NITROGEN CONCENTRATIONS ELICIT CROP-SPECIFIC RESPONSES IN BABY GREEN SPECIES AT SUPERELEVATED CO₂

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Abstract. Baby greens are rising in popularity in the consumer market due to their intense flavor and leaf size. The short life cycles and early response times to environmental stimuli make baby greens ideal for growth treatment analysis for use in controlled environment agriculture (CEA) and space crop production. Additionally, far-red (FR) light has been used in microgreen and baby green research to enhance stem elongation and leaf area expansion, but not in tandem with nitrogen (N)

concentration effects on plant growth. N is essential for plant growth and in this ground-based study, we aimed to characterize the growth and morphology of Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’ baby greens under four FR photon flux densities (PFDs) [blue₂₅ + green₂₅ + 1) red₁₅₀; 2) red₁₂₅ + far red₂₅; 3) red₁₀₀ + far red₅₀; or 4) red₇₅ + far red₇₅ (Subscript indicates its PFD in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)] and three N concentrations (75, 125, and 175 $\text{mg}\cdot\text{L}^{-1}$) under superelevated CO₂, equivalent to levels seen in spaceflight ($\approx 2800 \mu\text{mol}\cdot\text{mol}^{-1}$). As FR PFDs increased typical shade avoidance morphologies increased with Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’ exhibiting leaf length increases of 20–26% and 31–61%, respectively. Edible biomass did not increase with increasing FR PFDs for either species. Increasing N concentrations increased Chinese cabbage ‘Tokyo Bekana’ fresh and dry mass increased by 32–59% and 37–74%, respectively, except under 25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light, where shoot fresh mass increased by 55% with increasing N concentration from 75 to 125 $\text{mg}\cdot\text{L}^{-1}$, and shoot dry mass was not affected. Increasing N concentrations did not affect kale ‘Red Russian’ growth. Furthermore, the positive influence of high N concentrations on Chinese cabbage ‘Tokyo Bekana’ diminished as the FR PFD increased. The two crops in this study exhibited varying responses to both FR light and N concentrations, indicating the need for crop-specific environmental testing for use in either CEA or space crop production.

Abbreviations. B, blue; B:R, ratio of blue to red light; EC, electrical conductivity; ePAR, extended photosynthetically active radiation; FR, far-red; ISS, International

Space Station; N, nitrogen; PAR, photosynthetically active radiation; PFD, photon flux density; R, red; R:FR, ratio of red to far-red light

Introduction

Baby greens, while not being a legally defined term, are rising in popularity amongst consumers and, consequently, have increased production in the produce market (Saini et al. 2016; Treadwell et al. 2020). These greens are young and full of flavor and contain increased levels of health-promoting compounds compared to their mature counterparts (Martínez-Sánchez et al. 2012; Saini et al. 2016; Waterland et al. 2017). Baby greens are generally defined as young plants between microgreens and mature stages with at least two pairs of true leaves (Saini et al. 2016; Waterland et al. 2017). Having increased flavor and beneficial compounds, baby greens make perfect candidates for use in spaceflight where the astronaut diet is primarily comprised of pre-packaged, freeze-dried food that begins to degrade in nutritional value after 1–3 years in storage (Tibbetts 2019). Foods with increased flavor would also be beneficial for use in space travel as astronauts can lose some of their ability to taste and smell as a side effect of fluid redistribution as the human body enters microgravity (Linda Herridge, National Aeronautics and Space Administration, 2021; Taylor et al. 2020). However, the environment onboard the International Space Station (ISS) provides some unique parameters that can hinder desired crop growth. For example, the low relative humidity levels and superelevated CO₂ concentrations at ambient-ISS conditions can decrease crop yield and produce chlorosis and necrosis in some leafy

greens, such as tomato (*Solanum lycopersicum* cv. ‘DiFen’), lettuce (*Lactuca sativa* var. *capitata*), and Chinese cabbage (*Brassica rapa* var. *chinensis* cv. ‘Tokyo Bekana’) (Amitrano et al. 2019, 2021; Burgner et al. 2019, 2020; Massa et al. 2017). Baby greens also exhibit responses to environmental stimuli at their early developmental stage, reducing experimental durations and expediting data collection. However, these shorter harvest periods would require additional seed and growth media to adequately supplement the astronaut diet, ultimately increasing the expense due to the heavier payload.

The lighting environment also influences plant growth and development. Far-red (FR) light (700–800 nm) exists adjacent to the traditional photosynthetically active radiation (PAR) spectra (400–700 nm). However, recent research has shown that far-red light is effective at driving photosynthesis (Zhen et al. 2021). This light waveband, in tandem with red (R) light, forms the R:FR ratio, which elicits various physiological responses from plants through plant phytochrome photoreceptors (Demotes-Mainard et al. 2016; Franklin 2008). Once a R photon is detected, a phytochrome undergoes a conformational change toward its active form (P_{fr}) (Sharrock 2008). This form actively suppresses genetic expressions for stem elongation, leaf area expansion, and overall biomass accumulation; all typical morphology changes seen under the shade avoidance response (Demotes-Mainard et al. 2016; Franklin 2008; Zou et al. 2019). When a FR photon is detected, the phytochrome undergoes a subsequent conformational change to the inactive form (P_r), thereby alleviating the suppression of the above genetic expressions (Sharrock 2008). By increasing edible biomass, FR light

could potentially increase crop yield when grown under unideal conditions, like those on the ISS.

Nutrient availability can also influence crop growth and morphology. Nitrogen (N) is an essential macronutrient for crop growth and is available for uptake by plants in its inorganic forms of nitrate and ammonia (Lam et al. 1996). N plays a substantial role in amino acid and subsequent structural and enzymatic protein synthesis (Kirkby 1981; Lam et al. 1996; Leghari et al. 2016). Metabolically active cells, like those involved in meristematic activity and photosynthesis, contain a larger amount of N per dry weight compared to cells involved in storage and transport (Lawlor et al. 1988, 2001). N influences the light-dependent and independent reactions of photosynthesis as a main component of the involved proteins, thus making crop growth N-dependent (Evans and Seemann 1989; Greenwood et al. 1991; Lawlor et al. 2001).. The electrical conductivity (EC) of the nutrient solution used in a hydroponics system can also affect crop growth. Increasing EC from 0.5–0.9 $\text{dS}\cdot\text{m}^{-1}$ and 0.5-1.2 $\text{dS}\cdot\text{m}^{-1}$, led to an increase in fresh mass for lettuce ‘Batavia-Caipira’ and basil (*Ocimum basilicum* cv. ‘Emily’), respectively, whereas further increases to 2 $\text{dS}\cdot\text{m}^{-1}$, decreased fresh mass (Hosseini et al. 2021). Furthermore, high EC levels (up to 10 $\text{dS}\cdot\text{m}^{-1}$) led to increased leaf nitrate content in most tested lettuce cultivars (‘Kiber’, ‘Attirai’, ‘Rouxai’, ‘Cencibel’, and ‘Sintia’) while decreasing the fresh mass of all tested cultivars compared to lettuce grown at low EC levels (0.5–1.3 $\text{dS}\cdot\text{m}^{-1}$) (Kappel et al. 2021; Samarakoon et al. 2006).

N has a crucial impact on plant growth patterns, perhaps having the ability to regulate plant response to light-induced morphological changes, such as those from FR light. This finding would begin to address the relationship between fertilizer composition and the degree of plant growth response to environmental stimuli. In this ground-based experiment, we aimed to characterize the growth and morphology of baby greens under varying FR photon flux densities (PFDs) and N concentrations under superelevated CO₂. We postulated that increasing the FR PFD and/or the N concentration would synergistically increase biomass and extension growth of baby greens.

Materials and methods

We grew baby greens of Chinese cabbage ‘Tokyo Bekana’ and kale (*Brassica napus* cv. ‘Red Russian’) in a randomized complete block design with two simultaneous replications (Johnny’s Selected Seeds, Winslow, ME). We conducted this experiment at the University of Delaware’s Delaware Indoor Ag Lab (Newark, DE) from 9 to 31 Jan. 2023. We sowed two seeds in each of 336 rockwool plugs (2.5 cm long × 2.5 cm wide × 3.5 cm tall; Grodan, The Netherlands) per cultivar. Before seeding, we rinsed and soaked all rockwool plugs in reverse-osmosis water to remove any lingering surfactants from the manufacturing process. After seeding, we placed 14 rockwool plugs into each plastic container and covered them with transparent lids (22 cm long × 15 cm wide × 4.5 cm tall). Each experimental replication was conducted in two reach-in plant growth chambers (E41L2, Percival Scientific, Perry, IA). Plants were grown under four light spectra [blue₂₅ + green₂₅ + 1) red₁₅₀; 2) red₁₂₅ + far red₂₅;

3) red₁₀₀ + far red₅₀; or 4) red₇₅ + far red₇₅] and three N concentrations (75, 125, and 175 mg·L⁻¹) (Fig. 8). The subscript following each waveband is its PFD in μmol·m⁻²·s⁻¹. The temperature, relative humidity, and CO₂ concentration were held constant at ambient-ISS conditions at 22 °C, 50%, and 2800 μmol·mol⁻¹, respectively, under a 24-h photoperiod (Table 5). We set up lighting treatments by taking 12 PFD measurements per treatment with an extended PAR (ePAR) quantum sensor (PQ-612, Apogee Instruments, Logan, UT) and spectral distributions with a spectroradiometer (SS-110, Apogee Instruments, Logan, UT). Environmental parameters were monitored by third-party sensors as well as internal chamber sensors. We placed a temperature and relative humidity sensor (MX1101, Onset, Bourne, MA) on a wooden block under a radiation shield in the middle of each treatment shelf to log data every 15 min. Additionally, we placed a CO₂ sensor (YEM-40L, GZAIR, Guangzhou, China) on the top shelf of each chamber to log data every 10 min. We downloaded environmental data once a week to ensure the targeted environmental parameters.

On day 4, we removed the transparent lids to expose the seedlings to the air. On day 5, we began to manually thin the seedlings to one plant per plug as they germinated. From day 5 to the termination of the experiment, we fertigated the seedlings with their respective nutrient solutions varying in N concentration. Each nutrient solution was made with a base fertilizer (5-12-26 Part A, JR Peters, Allentown, PA) and calcium nitrate (15-0-0 Part B, JR Peters) dissolved in reverse-osmosis water with an adjusted pH of 5.80 ± 0.02 . All treatments received the same amount of base fertilizer (1000 mg·L⁻¹) and varying amounts of calcium nitrate (167,

500, and 833 mg·L⁻¹) to deliver the three N concentrations of 75, 125, and 175 mg·L⁻¹, respectively). The level of nutrient solution was maintained at one-fourth of the rockwool plug height.

We conducted a destructive harvest on six randomly selected baby greens per treatment per cultivar per replication at canopy closure (on day 18 for Chinese cabbage ‘Tokyo Bekana’ and day 21 for kale ‘Red Russian’). We collected measurements on biomass (shoot fresh and dry mass), morphology (shoot height, longest leaf length, and total leaf area), and relative chlorophyll concentration. Plant material was dried at 65 °C for 7 d before dry mass measurements. We photographed a representative plant per treatment per cultivar per replication for visual treatment comparisons (Fig. 9). Additionally, we downloaded environmental data from the internal chamber sensors and third-party sensors throughout the experiment. We analyzed plant data with mean separations in pair-wise comparisons using an ANOVA and Tukey’s honestly significant difference test ($\alpha = 0.05$) in JMP Pro (ver. 16.0.0, SAS Institute Inc., Cary, NC). We pooled data from two replications when there was no treatment \times replication interaction or when data trends among treatments were consistent across replications.

Results

FR light. The FR PFD affected all measured parameters for both Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’. For Chinese cabbage ‘Tokyo Bekana’, increasing the FR PFD generally increased leaf length and decreased relative chlorophyll concentration. As the FR PFD increased from 0 to 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, leaf

length increased by 20–26%, and the relative chlorophyll concentrations decreased by 44–47% (Table 6). For kale ‘Red Russian’, FR generally increased shoot height and leaf length and decreased relative chlorophyll concentration. FR decreased shoot fresh mass and total leaf area in a few treatments. Shoot fresh mass decreased by 30% as FR increased at the N concentration of 125 mg·L⁻¹ (Fig. 10). As the FR PFD increased, shoot height increased by 27–32%. Additionally, leaf length increased by 31–61% as FR increased, except at the N concentration of 125 mg·L⁻¹. Total leaf area decreased by 35% as FR increased from 0 to 25 μmol·m⁻²·s⁻¹ in treatments at the N concentration of 125 mg·L⁻¹ N. Relative chlorophyll concentration decreased by 52–58% with increased FR.

N concentration. N concentration affected most of the measured growth parameters of Chinese cabbage ‘Tokyo Bekana’ but did not affect those of kale ‘Red Russian’. For Chinese cabbage ‘Tokyo Bekana’, the N concentration generally increased shoot fresh and dry mass and increased longest leaf length and total leaf area in a few treatments. As N concentration increased from 75 to 175 mg·L⁻¹, Chinese cabbage ‘Tokyo Bekana’ shoot fresh mass increased by 32–59%, except under 25 μmol·m⁻²·s⁻¹ of FR light, where shoot fresh mass increased by 55% with increasing N concentration from 75 to 125 mg·L⁻¹ but did not change with increasing N concentration from 125 to 150 mg·L⁻¹ (Fig. 10). Shoot dry mass increased by 37–74% as N concentration increased, except under 25 μmol·m⁻²·s⁻¹ of FR light. Leaf length increased by 15% with increasing N concentration from 75 to 175 mg·L⁻¹ under 75 μmol·m⁻²·s⁻¹ of FR light. Leaf length also increased by 16% as N concentration

increased from 75 to 125 mg·L⁻¹ under 25 μmol·m⁻²·s⁻¹ of FR light (Table 6). Total leaf area increased with increasing N concentration by 40% and 35% under 0 and 75 μmol·m⁻²·s⁻¹ FR light, respectively. Under 25 μmol·m⁻²·s⁻¹ of FR light, total leaf area increased by 37% when N concentration increased from 75 to 125 mg·L⁻¹.

Discussion

FR light. Both Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’ exhibited typical morphologies akin to the shade avoidance response under increasing FR PFDs. Increased leaf length and stem elongation and decreased relative chlorophyll concentration are typical responses to increasing FR light in a variety of plants (Demotes-Mainard et al. 2016; Franklin 2008; Legendre and van Iersel 2021). However, in this study, FR light did not affect edible biomass (shoot fresh and dry mass) or total leaf area of either crop, except that increasing the FR PFD decreased kale ‘Red Russian’ in a few instances as fresh mass decreased by 30% as FR increased at the N concentration of 125 mg·L⁻¹ and total leaf area decreased by 35% as FR increased from 0 to 25 μmol·m⁻²·s⁻¹ in treatments at the N concentration of 125 mg·L⁻¹ N. Similarly, supplemental FR light did not affect the biomass of basil (*Ocimum basilicum*), cabbage (*Brassica oleraceae* var. *capitata*), or kale microgreens (Hooks et al. 2022). However, supplemental FR light increased the shoot fresh and dry mass of lettuce (*Lactuca sativa* cv. ‘Red Cross’) baby greens (Li and Kubota 2008). The fact that microgreens, of the same species in the current study, did not exhibit appreciable changes in edible biomass in the presence of FR light may be a sign that it is still too early in the growth cycle to observe the typical responses to FR light on

biomass accumulation during the micro- and baby green stages (Hooks et al. 2022; Zou et al. 2019). The results with lettuce ‘Red Cross’ demonstrate that this delay or change in response might also vary between cultivars and species, and some baby greens will show morphological changes to FR light (Li and Kubota 2008). However, this “delay” in response could also be due to the changing FR PFD in combination with a changing blue (B):R ratio. The effects of FR light on plant growth depend on the amount of B light present, as exhibited by the lack of change in fresh mass of lettuce ‘Rex’ and ‘Cherokee’ when grown under a high and low B:R ratio with $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light (Meng and Runkle 2019). In arabidopsis (*Arabidopsis thaliana*), low levels of B light signal morphological changes similar to the shade avoidance response elicited by FR light (Pedmale et al. 2016). The interaction of these signaling pathways could attenuate the effect of the other as seen in lettuce ‘Rex’ and ‘Rouxai’ where a low B:R ratio and a high photosynthetic photon flux density attenuated the morphological effects of FR light (Meng and Runkle 2019).

N concentration. N concentration had varied effects on biomass accumulation between Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’. In Chinese cabbage ‘Tokyo Bekana’, as the N concentration increased, plant edible biomass and most morphological parameters increased. Similarly, increased growth with increasing N concentration was observed in winter wheat (*Triticum aestivum*), French beans (*Phaseolus vulgaris*), tall fescue (*Festuca arundinacea* cv. ‘Clarine’), and potatoes (*Solanum tuberosum*) (Greenwood et al. 1991). Because N plays a significant role in protein and enzyme synthesis and photosynthesis, increasing available N can increase

photosynthetic activity and thus growth (Lawlor et al. 2001). However, N concentration did not affect the biomass and morphology of kale ‘Red Russian’. N availability positively correlates with the growth of crops (e.g., winter wheat) until it becomes N-saturated (Bock and Hergert 1991; Lawlor et al. 2001). Therefore, the lack of N responses in kale ‘Red Russian’ could be explained by lower N-saturation thresholds than for Chinese cabbage ‘Tokyo Bekana’.

FR × N concentration interaction. As the FR PFD increased, the positive effect of N concentration on the shoot fresh mass and total leaf area of Chinese cabbage ‘Tokyo Bekana’ biomass diminished (Table 6). For those of kale ‘Red Russian’, however, there were no FR × N concentration interactions. FR light can decrease N assimilation, which in turn affects the synthesis of enzymes and proteins necessary for continual plant growth and development (Demotes-Mainard et al. 2016). Additionally, nitrate reductase activity in squash (*Cucurbita maxima*) exhibited phytochrome-mediated decreases when exposed to FR light pulses or R followed by FR light pulses, compared to solely R light (Rajasekhar et al. 1988). Catalyzation from nitrate reductase is the first step in assimilating inorganic N for plant use and is therefore essential for growth (Campbell 1999). A decrease in nitrate reductase activity due to FR light may explain the decreasing influence of N concentration in Chinese cabbage ‘Tokyo Bekana’ under an increasing FR PFD. In contrast, kale ‘Red Russian’ growth was not affected by N concentration and showed typical morphological responses to increasing FR light.

Chlorosis was present in both crops under all treatments, especially at the lowest N concentration of $75 \text{ mg}\cdot\text{L}^{-1}$. This can be attributed to a deficiency in N, which is critical in chlorophyll development (Shimshi 1967). In addition, Chinese cabbage ‘Tokyo Bekana’ showed necrosis under all treatments. Chinese cabbage ‘Tokyo Bekana’ also had decreased, chlorotic, and necrotic growth when grown at super-elevated CO_2 concentrations in arcillite growing media (Burgner et al. 2019; 2020).

The two crops in this study exhibited varying responses to both FR light and N concentration. Chinese cabbage ‘Tokyo Bekana’ was more susceptible to growth influences from either variable than kale ‘Red Russian’. Chinese cabbage ‘Tokyo Bekana’ appears to require optimal growing conditions for desired yield and quality, while kale ‘Red Russian’ is more tolerant of adverse or less than optimal conditions for other leafy greens as seen in this study. Additionally, Chinese cabbage ‘Tokyo Bekana’ was most affected by the decrease in N assimilation produced by the interaction of increasing FR PFDs and N concentrations, while kale ‘Red Russian’ was not. When considering any crop treatment for use in spaceflight, it is imperative that intensive testing be done in replicated environments that are as close to ambient-ISS conditions as possible, as crop responses to environmental parameters are varied and can be cultivar-specific.

Table 5. Temperature, relative humidity, and CO₂ concentration of each of four spectral treatments varying in far-red (FR) light intensity. The subscript following FR is its photon flux density in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Each parameter is reported through its average and standard deviation. T: top shelf, B: bottom shelf.

Replication Chamber Shelf Light Treatment	Environmental Data							
	1				2			
	1		2		3		4	
	T	B	T	B	T	B	T	B
	FR ₅₀	FR ₀	FR ₂₅	FR ₇₅	FR ₀	FR ₇₅	FR ₂₅	FR ₅₀
Temperature (°C)	21.78 ± 0.12	22.25 ± 0.10	21.95 ± 0.16	22.11 ± 0.23	21.87 ± 0.15	22.21 ± 0.14	21.73 ± 0.10	22.14 ± 0.13
Relative Humidity (%)	50.74 ± 1.06	49.07 ± 1.01	50.46 ± 1.30	49.53 ± 1.19	50.65 ± 1.22	49.15 ± 0.95	50.10 ± 1.01	49.19 ± 0.94
CO ₂ Concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$)	2763.07 ± 185.16		2727.89 ± 248.99		2830.29 ± 108.71		2775.99 ± 227.59	

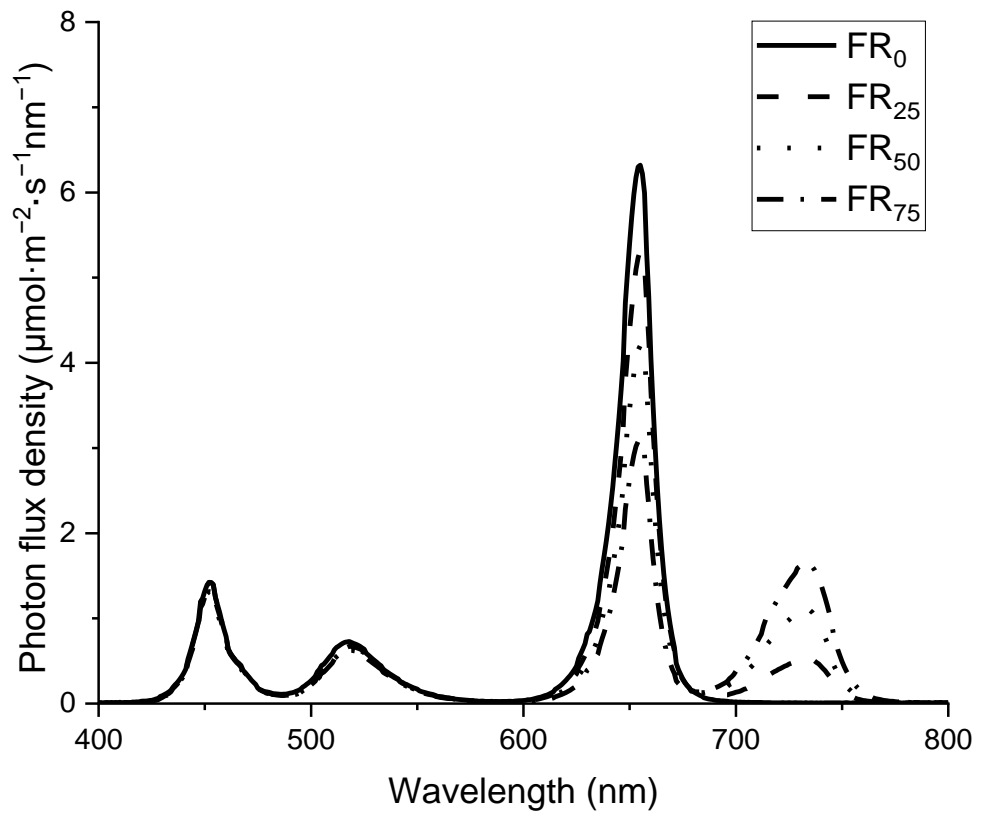


Figure 8. Spectral graphs (400–800 nm) for four lighting treatments with varying intensities of far-red (FR) light (700–800 nm). Each subscript in the legend represents the FR photon flux density of the lighting treatment in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Table 6. Destructive measurements of biomass, morphology, and relative chlorophyll concentrations for Chinese cabbage ‘Tokyo Bekana’ and kale ‘Red Russian’ baby greens. Acronyms for shoot fresh mass (FM), shoot dry mass (DM), shoot moisture content (MC), shoot height (SH), longest leaf length (LL), and total leaf area (LA) are used. Treatment names are described using the far-red (FR) photon flux density (subscript, in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) followed by the nitrogen concentration in ($\text{mg}\cdot\text{L}^{-1}$), (e.g., FR₀-75 represents an environmental treatment with 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR light and 75 $\text{mg}\cdot\text{L}^{-1}$ of nitrogen). Measurement results are reported by the average and standard error. Letters represent significant differences using Tukey’s honest significant difference test at $P \leq 0.05$.

Treatment (Trt)	‘Tokyo Bekana’			‘Red Russian’		
	Biomass					
	Shoot FM (mg)	Shoot DM (mg)	Shoot MC (%)	Shoot FM (mg)	Shoot DM (g)	Shoot MC (%)
FR ₀ -75	1794.56 ± 66.43 bcde	137.88 ± 8.66 bcd	92.31 ± 0.38 ab	2055.56 ± 167.91 b	245.13 ± 25.16 ab	88.30 ± 0.40 ab
FR ₀ -125	2223.49 ± 176.31 abc	166.33 ± 15.88 abc	92.60 ± 0.39 ab	2936.48 ± 167.40 a	325.67 ± 23.27 a	88.94 ± 0.29 ab
FR ₀ -175	2756.00 ± 170.97 a	205.45 ± 12.75 a	92.43 ± 0.37 ab	2466.23 ± 263.81 ab	290.58 ± 29.42 ab	88.14 ± 0.49 ab
FR ₂₅ -75	1465.39 ± 82.22 de	116.12 ± 8.55 de	92.13 ± 0.26 ab	1927.93 ± 64.38 b	225.67 ± 10.93 ab	88.34 ± 0.28 ab
FR ₂₅ -125	2264.88 ± 124.50 abc	150.32 ± 8.32 bcd	93.32 ± 0.25 a	2045.80 ± 133.87 b	224.23 ± 19.55 ab	89.14 ± 0.39 ab
FR ₂₅ -175	1863.68 ± 141.68 bcde	150.194 ± 9.57 bcd	91.80 ± 0.30 b	2062.36 ± 140.08 b	239.26 ± 15.61 ab	88.29 ± 0.43 ab

FR ₅₀₋₇₅	1745.03 ± 73.04 cde	123.34 ± 7.27 cde	92.92 ± 0.31 ab	1971.55 ± 97.53 b	227.91 ± 15.86 ab	88.54 ± 0.37 ab
FR ₅₀₋₁₂₅	1954.93 ± 84.18 bcd	148.48 ± 8.39 bcd	92.42 ± 0.21 ab	2044.08 ± 313.72 b	223.61 ± 31.70 ab	88.76 ± 0.33 ab
FR ₅₀₋₁₇₅	2304.78 ± 157.74 ab	168.43 ± 8.24 ab	92.56 ± 0.26 ab	1991.24 ± 172.84 b	227.54 ± 20.10 ab	88.49 ± 0.35 ab
FR ₇₅₋₇₅	1374.69 ± 50.84 e	93.66 ± 5.69 e	93.21 ± 0.25 a	2119.10 ± 117.02 ab	216.41 ± 12.68 b	89.77 ± 0.27 a
FR ₇₅₋₁₂₅	1841.58 ± 73.97 bcde	125.14 ± 6.05 bcde	93.20 ± 0.17 a	2043.11 ± 145.01 b	248.93 ± 19.26 ab	87.85 ± 0.42 b
FR ₇₅₋₁₇₅	2184.35 ± 79.91 bc	163.18 ± 5.99 abc	92.50 ± 0.21 ab	2148.73 ± 200.87 ab	262.83 ± 26.77 ab	87.83 ± 0.29 b
FR Trt	**	**	NS	*	*	NS
N ppm Trt	**	**	*	NS	NS	NS
FR × N ppm	*	NS	*	NS	NS	*
Replication (Rep.)	NS	NS	NS	NS	NS	*
Trt. × Rep.	**	**	*	**	**	*
Morphology						
	SH (cm)	LL (cm)	LA (cm ²)	SH (cm)	LL (cm)	LA (cm ²)
FR ₀₋₇₅	0.69 ± 0.04 abc	7.88 ± 0.14 ef	42.30 ± 1.37 bcd	1.42 ± 0.06 c	8.58 ± 0.25 f	41.11 ± 2.76 b
FR ₀₋₁₂₅	0.70 ± 0.04 abc	8.59 ± 0.21 def	48.91 ± 3.05 abc	1.42 ± 0.08 c	10.95 ± 0.24 cde	62.33 ± 4.09 a

FR ₀ -175	0.71 ± 0.04 abc	8.57 ± 0.29 def	59.01 ± 4.32 a	1.42 ± 0.08 c	9.90 ± 0.29 def	52.86 ± 5.79 ab
FR ₂₅ -75	0.70 ± 0.05 abc	7.53 ± 0.18 f	36.30 ± 1.77 d	1.37 ± 0.05 c	9.81 ± 0.17 ef	41.23 ± 2.61 b
FR ₂₅ -125	0.66 ± 0.04 bc	8.74 ± 0.29 cde	49.76 ± 2.87 abc	1.30 ± 0.07 c	10.30 ± 0.27 def	40.58 ± 3.33 b
FR ₂₅ -175	0.61 ± 0.05 c	7.75 ± 0.28 ef	39.60 ± 2.67 cd	1.23 ± 0.06 c	10.16 ± 0.29 def	41.38 ± 3.32 b
FR ₅₀ -75	0.64 ± 0.03 bc	9.15 ± 0.23 cd	44.17 ± 2.02 bcd	1.48 ± 0.07 bc	11.18 ± 0.34 cde	42.69 ± 3.22 b
FR ₅₀ -125	0.67 ± 0.04 bc	9.14 ± 0.15 cd	44.27 ± 2.05 bcd	1.48 ± 0.10 bc	11.54 ± 0.80 bcd	43.93 ± 7.01 ab
FR ₅₀ -175	0.64 ± 0.03 bc	9.82 ± 0.41 abc	53.20 ± 4.24 ab	1.48 ± 0.07 bc	11.55 ± 0.34 bcd	41.91 ± 3.47 b
FR ₇₅ -75	0.73 ± 0.03 abc	9.43 ± 0.13 bcd	38.96 ± 1.42 cd	1.80 ± 0.08 ab	13.81 ± 0.28 a	47.52 ± 4.18 ab
FR ₇₅ -125	0.82 ± 0.06 ab	10.41 ± 0.24 ab	46.94 ± 2.15 abcd	1.88 ± 0.12 a	12.06 ± 0.36 bc	45.11 ± 2.51 ab
FR ₇₅ -175	0.87 ± 0.05 a	10.83 ± 0.27 a	52.51 ± 1.86 ab	1.83 ± 0.09 ab	13.00 ± 0.39 ab	47.24 ± 4.71 ab
FR Trt	**	**	*	**	**	*
N ppm Trt	NS	**	**	NS	NS	NS
FR × N ppm	NS	*	*	NS	**	NS
Replication	NS	NS	NS	NS	*	**
Trt. × Rep.	NS	**	**	*	**	**
Relative chlorophyll concentration						
FR ₀ -75	9.55 ± 0.67 bcd			43.03 ± 4.26 a		
FR ₀ -125	10.32 ± 0.67 abc			43.18 ± 2.12 a		

FR ₀ -175	11.84 ± 0.85 ab	40.54 ± 3.51 a
FR ₂₅ -75	9.13 ± 0.71 cde	29.79 ± 1.22 bc
FR ₂₅ -125	9.86 ± 0.48 abcd	26.89 ± 1.40 bcd
FR ₂₅ -175	12.53 ± 0.77 a	30.65 ± 1.15 b
FR ₅₀ -75	7.23 ± 0.56 defg	21.93 ± 1.59 bcd
FR ₅₀ -125	7.83 ± 0.49 cdef	23.05 ± 1.07 bcd
FR ₅₀ -175	8.36 ± 0.37 cdef	19.83 ± 0.82 d
FR ₇₅ -75	5.06 ± 0.24 g	18.26 ± 0.61 d
FR ₇₅ -125	5.68 ± 0.29 fg	20.55 ± 1.34 cd
FR ₇₅ -175	6.66 ± 0.42 efg	19.14 ± 0.92 d
FR Trt	**	**
N ppm Trt	**	NS
FR × N ppm	NS	NS
Replication	NS	NS
Trt. × Rep.	NS	*

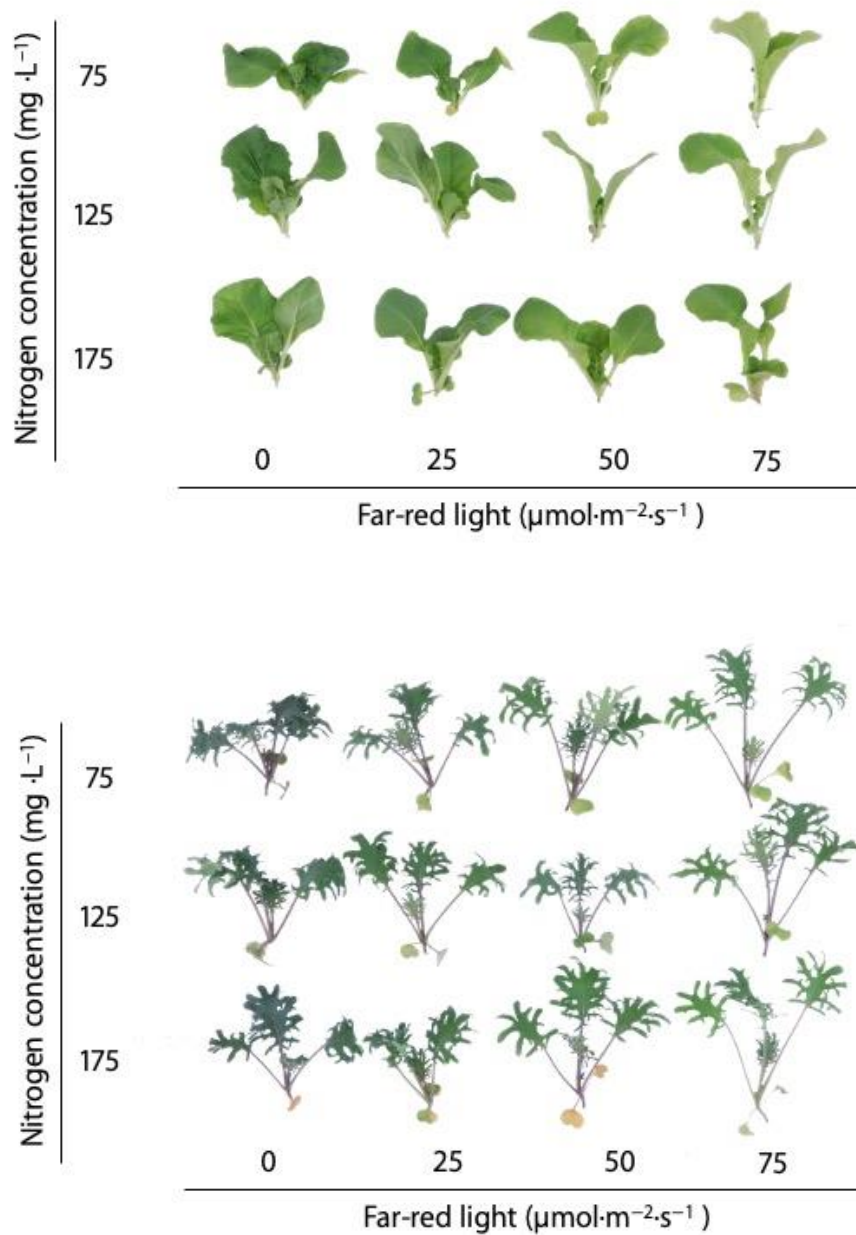


Figure 9. Representative images of Chinese cabbage ‘Tokyo Bekana’ (top) and kale ‘Red Russian’ (bottom) baby greens grown at four far-red light photon flux densities (0, 25, 50, and 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and three nitrogen concentrations (75, 125, and 175 $\text{mg}\cdot\text{L}^{-1}$).

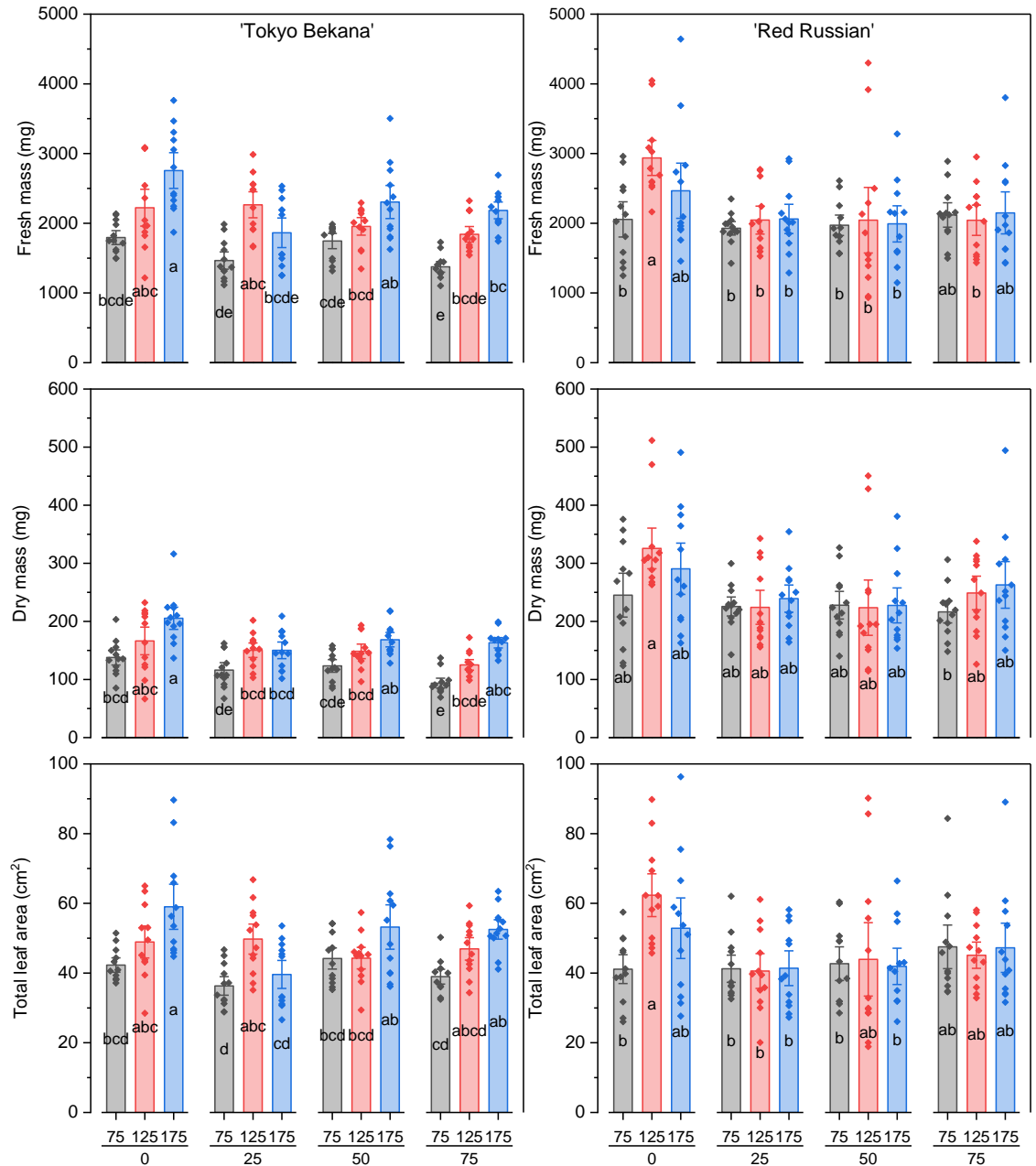


Figure 10. Edible biomass (shoot fresh and dry mass, and total leaf area) measurements of Chinese cabbage 'Tokyo Bekana' and kale 'Red Russian' baby greens. Bars depict the average of the data measured (scatter) with standard error bars.

Letters represent significant differences using Tukey's honest significant difference test at $P \leq 0.05$. Treatments are differentiated using the the nitrogen concentration (75, 125, and 175 $\text{mg}\cdot\text{L}^{-1}$; first tier) followed by the far-red (FR) photon flux density (0, 25, 50, and 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; second tier)

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