

Bioregenerative Dietary Supplementation: Optimizing Brassica Production for Space Travel
Through Light Intensity, Photoperiod, and Harvest Methodology

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DEDICATION

This work is dedicated to my parents, who made it possible for me to pursue an education and encouraged me to do so. Thank you for teaching me to read and to be curious about the world and its inhabitants. It is the best gift I could imagine, and I appreciate it every day. Thank you.

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ABSTRACT

Bioregenerative dietary supplementation, a component of bioregenerative life support, will be necessary for the success of future exploration-length space endeavors. Plants, as autotrophic producers of calories, nutrients, and oxygen, will be indispensable in the development of these systems. Previous work has identified leafy greens from the genus *Brassica* as promising candidates for in-flight production of necessary human nutrients such as vitamins C, B₁, and K₁, in addition to β -carotene, the primary vitamin A precursor in the human diet. These plants also produce lutein and zeaxanthin, two compounds important in protecting the eye from radiation-induced damage, as well as a variety of anthocyanins which act as general antioxidants in the human body, potentially decreasing the damaging effect of free-radicals. Finally, plants accumulate minerals in their tissue, including calcium, potassium, magnesium, and iron; all of which have important implications in the diet of space-faring humans. This thesis seeks to determine 1) which cultivars of *Brassica* (selected from three species within the genus) produce the most promising phytonutrient and morphological profiles for production in space, 2) how light intensity and photoperiod can be manipulated to increase these phytonutrient concentrations, and 3) how a continuous or ‘cut-and-come-again’ harvest strategy can be implemented to maximize phytonutrient yield efficiency. This was tested by growing plants in a controlled environment growth chamber emulating the temperature, humidity, and CO₂ concentration aboard the International Space Station (ISS), and performing a series of phytonutrient extractions and quantifications on the resulting tissue. Of the cultivars evaluated, *B. carinata* ‘Amara’ provides the strongest phytonutrient profile, an aspect of which was the notable production of higher vitamin B₁ concentrations than any cultivar of *B. rapa* var. *nipposinica*. A high light intensity, 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, applied across a 16-hr photoperiod further optimized the profiles of this cultivar and of a complementary cultivar, *B. rapa* var. *nipposinica* ‘Red Hybrid’, for space production. Finally, a continuous harvest protocol increased the phytonutrient production per day per unit of production space when compared to a single harvest approach, and these gains increased over subsequent harvests.

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INTRODUCTION:

VITAMIN DEFICIENCIES ON LONG VOYAGES, AN OLD PROBLEM FOR A NEW AGE

Introduction

The extended and isolated nature of trans-oceanic ship voyages provide two of the most infamous examples of human disease caused by malnutrition, as well as their subsequent treatment by simple dietary inclusions. During the 1700's, or perhaps earlier (Baron, 2009), it was found that sailors who were experiencing scurvy could be treated by the inclusion of citrus in their diet (Carpenter, 1988). In the 1880's, a Japanese naval medical officer by the name of Kanehiro Takaki was able to reduce naval deaths caused by beriberi through the inclusion of meat and vegetables in the diets of sailors (Sugiyama & Seita, 2013). In each case the effective treatment was due to a compound contained in the supplemental foods, respectively vitamins C (ascorbic acid) and B₁ (thiamine).

With the potential of long-distance space travel, these nutritional deficits will again become an issue, compounded by challenges due to the unique environment of space and its effects on human health (Massa et al., 2015). In preparation, NASA has identified a list of nutritional compounds which are expected to be of concern during long distance space flight (Cooper et al., 2017; S. Smith, 2013.; Smith et al., 2005). These bioactive compounds include vitamins C, B₁, and A (retinol) due to their quick degradation in storage (Cooper et al., 2017), and vitamin K (phyloquinone), vitamin D (calciferol), potassium, and calcium, as these compounds and minerals are found in too low of concentrations in the current dry-storage diet (Cooper et al., 2017). Finally, the inclusion of antioxidants, such as carotenoids and anthocyanins, becomes desirable to mitigate the deleterious effects of increased exposure to radiation in space. (Chylack et al., 2009).

While modern technology offers many solutions for dietary supplementation, the most straightforward way to increase dietary intake of these compounds and minerals remains the consumption of fresh produce on a consistent basis. This approach also allows for the inclusion of interesting flavors and textures in the diet, something that freeze dried foods or supplemental pills do not offer, which is of importance to astronaut health due to menu fatigue (Sirmons et al., 2020). However, simplicity for the consumer does not always equate to simplicity for the producer, and the production of fruits and vegetables on spacecraft introduces a host of challenges that must be faced before fresh produce can be a reliable source of dietary supplementation in space (Romeyn et al., 2019).

Compounds and Minerals of Interest

Anthocyanins

Anthocyanins are a class of flavonoids, containing conjugated double bonds and thus absorbing light in the 500-550 nm range (Landi et al., 2015). Due to this absorption spectrum, they are the source of most red, orange, and purple coloration in plants (Grotewold, 2006). These compounds are highly water soluble and are mostly stored in the vacuoles of the plant cell (Hatier & Gould, 2009) and primarily accumulate in the epidermal tissue (Song et al., 2020). Anthocyanins provide the plant with protection from excessive radiation (Hughes et al., 2007), although it is debated whether this is through an antioxidant effect and the subsequent reduction of reactive oxygen species (ROS) (Zhang et al., 2012), or the absorbing of excess light before it can interact with susceptible oxygen molecules (Landi et al., 2014), or a combination of both mechanisms. However, it is understood that the biosynthesis of anthocyanins is influenced by the presence, intensity, and quality of light (Chalker-Scott, 1999; Dong et al., 1998; Mancinelli, 1990; Hwang et al., 2022).

As a part of the human diet, anthocyanins are suspected to play a role in preventing chronic inflammatory illnesses, diabetes, and obesity (Gonçalves et al., 2021). They are of interest in long term space travel as they act as an antioxidant and may help negate the increased ROS caused by radiation experienced in space, particularly in sensitive areas of the body such as the eye (Shim et al., 2012).

Carotenoids

Plant carotenoids are isoprenoids with a 40-carbon polyene backbone, containing up to 15 conjugated double bonds. They are ubiquitous among photosynthetic plants, as certain carotenoids, including lutein and β -carotene, are part of the antennae structures involved in the light reactions of photosynthesis (Croce et al., 2002; Dall'Osto et al., 2006). As a part of the light harvesting complex, the response of these carotenoids would be expected to increase or decrease with the relative up or down regulation of the photosynthetic machinery.

Lutein and β -carotene, as well as many additional carotenoids, can be found in other plant structures, providing pigment (yellow, red, and orange), photoprotection, or as part of the biosynthesis pathway of other metabolites (Simkin et al., 2004). In these non-photosynthetic roles, they function as secondary metabolites. A key step in the carotenoid biosynthesis pathway

involving phytoene synthase is regulated by factors including light intensity, photoperiod, temperature, and salt concentration (Cazzonelli & Pogson, 2010).

β -carotene is the primary vitamin A (retinol) precursor in the human diet. Vitamin A deficiency is a prominent cause of global child mortality, and supplementation has the potential to reduce its incidence by 25% (Imdad et al., 2011). In adults, vitamin A is required for the maintenance of eye health and is provided primarily by the cleavage of dietary β -carotene. While not important as vitamin A precursors in the human diet, the carotenoids lutein and zeaxanthin are both accumulated in the retina of the eye, providing protection from photodamage (Bernstein et al., 2001). NASA is particularly concerned with β -carotene due to its role in maintaining retinol levels in the body, while interest in lutein and zeaxanthin is due to their activity in mitigating the macular degeneration and cataracts caused by space radiation, in addition to their general antioxidant capability (Cohu et al., 2014). The antioxidant capacity of lutein and zeaxanthin, both in the human eye and in plants, as well as the mechanisms and implications of this activity was recently reviewed quite comprehensively in the journal *Photochem* (Demmig-Adams et al., 2022).

Vitamin B₁ (Thiamine)

Thiamine (vitamin B₁) is a water-soluble compound which serves as a general cofactor in plants (Krampitz, 1969) and is concentrated in green tissue and seeds (Kong et al., 2008). The high concentration in seeds is likely due to its function as a cofactor in the biosynthesis of several amino acids (valine, leucine, isoleucine) (Goyer, 2010). The expression of these thiamine biosynthesis genes can also be upregulated by environmental factors including light intensity, osmotic stress, infection, and hypoxia, with light being the most impactful naturally occurring environmental trigger (Tunc-Ozdemir et al., 2009).

Thiamine serves as a multipurpose enzymatic cofactor in mammals. It is involved in cellular metabolism and energy production, including the transketolase reaction during the production of NADPH, and several reactions involving the oxidative decarboxylation of α -keto acids (Beltramo et al., 2008). Thiamine deficiencies can lead to breakdown of neurotransmitter anabolism (Butterworth, 1989), potentially explaining the neuropathic symptoms of beriberi. Only small amounts of thiamine are stored in the human liver, and it has a short half-life once absorbed, requiring daily intake to maintain healthy levels (Sriram et al.,

2012). Thiamine is, however, sensitive to heat, light, and oxidation, meaning that any processing (including cooking) is likely to lower thiamine levels in food (Rucker, 2001). This combination of instability in storage and the lack of biological reserves in the human body make supplementation for long-duration space missions necessary.

Vitamin C (Ascorbic Acid)

Ascorbic acid (vitamin C) is a water-soluble compound ubiquitous in plants and is detectable in all plant tissue, even the cell wall. This molecule freely converts between its cationic and anionic forms, ascorbic acid and ascorbate, dependent on solution pH. While concentrations are dependent on the genotype of the plant and can vary throughout developmental stages (Felicetti & Mattheis, 2010), they are also influenced by environmental factors including light (Li et al., 2009, Logan et al., 1996). Ascorbate/ascorbic acid concentrations are correlated with anthocyanin concentrations, as low ascorbate reduces the capacity for anthocyanin accumulation as shown in *Arabidopsis* mutants deficient in ascorbate synthesis (Page et al., 2012).

Unlike other mammals, primates (including humans) lack the ability to express the l-gluconolactone oxidase enzyme, a key step in endogenous ascorbic acid production, and require dietary ascorbic acid to avoid scurvy (Drouin et al., 2011). Scurvy is defined as vitamin C deficiency, with symptoms widespread and serious, as ascorbic acid performs many roles as an enzymatic cofactor (Rucker, 2001). In addition to being a necessary dietary inclusion, vitamin C is highly unstable and degrades rapidly under storage and processing (Ajibola et al., 2009). Thus, it is important to have a consistent source of fresh fruits or vegetables to avoid the onset of scurvy.

Vitamin K₁ (Phylloquinone)

Vitamin K refers to several structurally related fat-soluble compounds. Phylloquinone (vitamin K₁) is the form of vitamin K produced in plastids throughout the plant kingdom (Oostende et al., 2008). It is highly conserved due to its function as a cofactor in the electron transport chain of photosystem I (Snyder et al., 1991). The involvement in photosystem I suggests that phylloquinone levels would increase as light increases and as electron flow from Photosystem II increases, as these stimuli can upregulate production of photosystem I (Tarento et al., 2018).

In the animal kingdom vitamin K serves as a blood coagulant factor and facilitates the binding of calcium to bone tissue, as well as an antioxidant role in protecting neurons (Li et al., 2003). It is stored in fat cells of the liver and is then available for utilization over a longer period compared to vitamins C and B₁. Dietary vitamin K needs are quite low, however once a deficiency is reached, the results are serious (Rucker, 2001).

Calcium

Calcium, as a mineral, is not produced in the plant but is required for proper growth and is accumulated in the cells during normal development. In ionic form (Ca²⁺), it is used during synthesis of the cell wall middle lamella. It is also used during mitotic spindle formation and as a secondary messenger during plant signaling responses (Taiz et al., 2015). Calcium uptake is passive (Higinbotham et al., 1967), thus, the major influences on calcium content in plant tissue are Ca²⁺ availability, transpiration rate, and plant genetic factors. Calcium has an antagonistic effect on the uptake of both potassium and magnesium cations (Jakobsen, 1993).

In humans (*Homo sapiens*) calcium is required for proper bone and dental health. It is also required for nerve function and muscle contraction, including rhythmic heart contractions (Kuo & Ehrlich, 2015). Much of the calcium in the body is stored in the skeletal system and, when needed, is drawn into the bloodstream for muscle function and other non-structural needs (Nordin, 1997). Bone loss under microgravity is a known issue (Smith et al., 2005), and dietary calcium can prevent the leeching of skeletal calcium by maintaining sufficient blood concentrations for non-structural needs. However, this is further complicated by the risk of kidney stones, as dietary and demineralized calcium from bone-loss can quickly lead to supersaturation of calcium in the urine, an indicator of the conditions required for kidney stone formation (Smith et al., 2014).

Potassium

Potassium is the most abundant cation present in plant tissue, appearing as K⁺, and playing a widespread set of roles in osmotic regulation (providing turgor pressure and stomatal control), signaling, and as an enzymatic cofactor (Pandey & Mahiwal, 2020). Because K⁺ concentration influences osmotic status, the concentration in the cytoplasm is maintained at a relatively consistent level, however, levels in the vacuole vary based on environmental availability (Zörb et al., 2014).

Potassium is the predominant cation in human intracellular fluid and is key to many processes including maintaining cellular pH homeostasis (Lee Hamm et al., 2013), establishing proper electrochemical gradients for nerve and muscle signaling (Palmer & Clegg, 2016), and maintaining healthy blood pressure (He & MacGregor, 1999).

Magnesium

Magnesium is another cation found in plant tissues, playing a role in enzymatic activation during respiration and DNA synthesis, and being the central component of the chlorophyll molecule (Taiz et al., 2015). Magnesium uptake is primarily passive, being influenced most by environmental levels and plant transpiration (Mayland & Wilkinson, 1989). Plants are also able to store considerable amounts of magnesium in the vacuole without inducing toxicity (Hawkesford et al., 2012), meaning higher transpiration rates may increase total plant concentrations if it is environmentally available.

In the human body magnesium helps regulate blood pressure, blood sugar, and nerve function, and helps create bone in conjunction with calcium (NIH Fact Sheet, 2021). While magnesium deficiency has been linked to bone loss (Rude et al., 2009), it has not been flagged as a mineral currently lacking in the astronaut diet, but a steady source will be beneficial during long-term space transit (Massa et al., 2015).

Iron

Iron is considered a plant micronutrient, only needed in small amounts, but without which the plant would be unable to produce chlorophyll or carry out several other enzymatic functions. Chlorosis, or the loss of green pigmentation in the leaves due to low chlorophyll concentrations, is the most common symptom of iron deficiency in plants (Taiz et al., 2015).

Iron, while beneficial and necessary as a part of the diet, does not require further supplementation in the current space diet. Typically, it is difficult to induce iron-toxicity in humans, however, higher levels of iron are correlated with bone loss in microgravity, and as such it is desirable to avoid excessive intake beyond current consumption (Zwart et al., 2013).

Fresh Source Vs. Dry Source

As mentioned in several places above, many of these nutritional compounds degrade during processing or storage (Cooper et al., 2017). It is thus crucial to maintain a source of fresh

produce to supplement the existing space diet to avoid symptoms of deficiencies and maintain astronaut health (Board and NRC, 2011). Additionally, sourcing vitamins and minerals from fresh produce allows for positive synergistic effects and avoids accidental toxicities that can occur if too many pill-based supplements are taken on a consistent basis (Tran & Demmig-Adams, 2007). This area of research carries special importance outside of space travel, as many questions remain on the variable bioavailability of different nutrients in the human diet and how their matrix affects uptake (Tan & Clements, 2021; Kamiloglu et al., 2021; Shkempi & Huppertz, 2022).

Genetic, Environmental, and Cultural Factors

As established in the previous paragraphs, any long-term space venture will require a consistent source of fresh produce to provide a complete nutritional profile and mitigate the negative effects of space on the human body. It is desirable to produce these vegetables in the most efficient way possible, providing the highest concentrations of vitamins and minerals in the smallest amount of plant tissue. Genetic and environmental factors, as well as production practices, all have the potential to alter growth rate, plant morphology, and phytonutrient concentrations. These parameters are potential levers that can be manipulated in the quest to produce a consistent supply of vitamins and minerals *in situ* for the supplementation of the dry space diet.

Cultivars of Mustard Greens, Including Mizuna

Mustard greens have previously been targeted as promising candidates for fresh vitamin supplementation during space travel (Massa et al., 2015). Mustards and other leafy greens are desirable due to their rapid growth, manageable size, and broad nutrient profile. The mustard *Brassica rapa* var. *chinesis* (Chinese Cabbage) was grown on the international space station but showed signs of stress including chlorosis and necrosis (Burgner et al., 2019). Other *Brassica* species were subsequently grown on the station with better results including *Brassica rapa* var. *nipposinica* (mizuna) in 2017-2019, *Brassica napus* ‘Red Russian’ (kale) in 2018 and 2021, *Brassica juncea* ‘Wasabi’ (wasabi mustard) in 2019 and 2021, and *Brassica carinata* ‘Amara’ (Ethiopian kale) in 2021 (Johnson et al., 2021).

Brassica rapa var. *nipposinica* is a group of mustards, commonly referred to as mizuna, that was identified by NASA as having the most promising nutritional and organoleptic profile

after Chinese cabbage (Massa et al., 2015). Mizuna has been grown since the early Kyoto period in Japan, with a characteristic jagged leaf shape and slightly spicy flavor. Mibuna, a closely related group of cultivars (considered by many to still be *Brassica rapa* var. *niposinica*), diverged from the jagged-leafed mizuna 200 years ago and has a milder flavor and smooth leaf margin (Kawakatsu et al., 2021).

Another mustard of particular interest, *Brassica carinata* (Ethiopian kale), has been utilized and studied primarily as an oilseed crop. However, it has a high antioxidant and phenolic level compared to some mustards (Frazie et al., 2017) and has been a crop of interest for space-based growing due to its performance in preliminary taste and biomass metrics (Bhuiyan et al., 2018).

High CO₂ Environment

The international space station (ISS) operates at an elevated CO₂ concentration of 2,800 ppm or more, which is about six times higher than atmospheric concentration (James et al., 2011; Massa et al., 2016). These CO₂ levels have been the subject of human health research but have not been a topic of concern regarding plant production in space until more recently, arising due to the irregular growth of Chinese cabbage on the space station (Burgner et al., 2019). Plants require CO₂ to undergo photosynthetic reactions, and indoor growers have been supplementing their CO₂ above atmospheric concentration since the 1980's. Increased CO₂ allows for increased performance of the enzyme RuBisCO, which can catalyze either a carboxylation or an oxygenation reaction, pushing the reaction kinetics in the direction of the metabolically preferable carboxylation reaction. Additionally, CO₂ supplementation can result in elevated levels of oligo- and mono- saccharides, amino acids, and total flavonoid content (Levine et al., 2008), all metrics that can improve crop quality. However, this supplementation does not typically surpass levels much higher than 1,000 ppm (Mortensen, 1987). As higher levels of CO₂ concentration are reached, the benefits begin to taper off and can become detrimental (Madsen, 1974), with findings in wheat showing the steepest decline between 1,000 and 2,600 ppm (Reuveni, 1997).

There are a variety of hypotheses on why some plants begin to show stress under extremely elevated CO₂, including reduced dark respiration rate (Reuveni, 1997) and failure to close stomata during the nocturnal period (Wheeler et al., 1999). The failure of stomata to close

during the night period is less of a concern in hydroponic production, as water is readily available, although the recycling of evaporated water aboard space craft does require additional energy. The cause of the non-linear effect of CO₂ supplementation is still an open area of research, and it is likely that elevated CO₂ response varies from species to species and may interact with different abiotic and biotic conditions including water availability, light intensity, and temperature as these factors all play into metabolic and photosynthetic rates (Xu et al., 2016).

Light Intensity

Light is the primary driver of photosynthesis, and as such, it is the primary energy source for any metabolic function that occurs in photosynthetic plants (Arnon, 1960). Increased light intensity has been shown to alter biomass, growth rate, and leaf structure (Jiang et al., 2011; Zelitch, 1982), as well as increase carbohydrate concentrations (Adedipe & Ormrod, 1975). Additionally, the synthesis of secondary carotenoids (Haldimann et al., 1995), anthocyanins (Irshad et al., 2018), and other antioxidant compounds (Szymanska & Kruk, 2010) are upregulated under high light stress.

Light intensity affects levels of nutritionally important compounds in a variety of cultivated *Brassica*. Phenolic concentrations are positively correlated with light intensity (Craver et al., 2017), while mineral nutrient concentrations decrease with increasing light intensity (Gerovac et al., 2016). Total carotenoid concentrations do not change in *Brassica rapa* var. *parachinensis* (choy sum) when light intensity is increased from 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, potentially due to an increase in secondary, radical scavenging, carotenoids mirrored by a decrease in primary, light harvesting, carotenoids (Huang et al., 2021).

Photoperiod

Photoperiod as an aspect of the plant environment can be considered as 1) a component of the daily light integral (DLI), which describes the number of photons a plant receives over a 24-hr period, or 2) a separate environmental signal which can be received by non-photosynthetic receptors. These receptors play a role in developmental initiation and cell signaling, and the alteration of the photoperiod can induce non-desirable effects during vegetable production, such as early flowering (Taiz et al., 2015). Increasing the photoperiod may unexpectedly decrease fresh biomass, as seen in lettuce (-18%) and chicory (-47%) when the photoperiod is increased

from 16 to 24 hrs (Pennisi et al., 2020), or cause tissue damage as seen in the development of chlorotic leaf tissue in tomatoes grown under a 24-hr photoperiod (Demers et al., 1998). This indicates that while photoperiod can be used as a tool to increase the total photon exposure, it can have unintended side effects through the disruption of circadian rhythms or other biological processes.

In *Brassica* microgreens a continuous (24 hr) photoperiod increases fresh mass, dry mass, and the concentrations of flavonoids and anthocyanins (Shibaeva et al., 2022). This increase in antioxidant compounds is a response to the increase in ROS created by constant light exposure. However, another study reported that while temperature and photoperiod positively influenced *Brassica oleracea* var. *italica* (broccoli) developmental rate, neither factor affected vitamin C (ascorbic acid) concentration (Mølmann et al., 2015). The difference in response between ascorbic acid and other antioxidants, such as flavonoids or anthocyanins, could be due to its involvement in primary metabolic processes that are necessary under both conditions or simply be due to genetic differences.

Daily Light Integral (DLI)

Light intensity and photoperiod both play individual roles in stimulating plant growth, development, and phytochemistry, however, the interplay of the two factors can be further elucidated as the DLI, or the total number of photons received by a plant in a 24-hr period. For example, mizuna produces 18.7% more dry mass when grown under a longer 22-hr photoperiod at low light intensity than when grown at an equivalent DLI over a 10-hr period (Palmer & van Iersel, 2020). This is in part due to the increased quantum yield efficiency at lower light intensities (Elikins & van Iersel, 2020). Quantum yield efficiency is of particular interest to crop production in space, as lower light intensities across a continuous photoperiod make energy budgeting easier to integrate into the already complex spacecraft energy utilization.

Multi-Harvest Logistics and Cost Benefit Balance

A primary concern when producing vegetables in a limited space is that of yield over time. Maximum yield in a single harvest may not be the optimal approach if multiple lower yields can be achieved from a single plant before a second plant reaches maturity. This ‘cut and come again’ approach also provides a steady harvest cadence by skipping the slow germination phase on subsequent harvests. NASA explored options for a ‘cut and come again’ system,

however, the response was cultivar dependent and further optimization and testing is needed (Bunchek et al., 2021). Multiple variables, including the effects on biomass yield, phytonutrient concentration, and plant health still need exploration before implementation of a continuous harvest system on the ISS.

The species, number of leaves harvested, frequency of harvests, and environmental stressors are all factors in plant response to repeated harvests. Shunigiku greens (*Glebionis coronaria*), from which all but the youngest 4-6 leaves were harvested every 14 days for a total of three harvests, provided 50% more biomass than two single harvest growth cycles in the same period. However, pak choi responded negatively and produced 50% less biomass while ‘Amara’ greens and ‘Red Russian’ kale showed similar biomass production independent of harvest methodology (Bunchek et al., 2021). Also, basil (Corrado et al., 2020) and lettuce (Carillo et al., 2020) showed changes in phytonutrient concentrations after successive harvests.

Developing leaves transition from being a sink to a source (Foyer & Paul, 2001), thus, it may be beneficial to avoid harvesting all the mature leaf tissue during implementation of continuous harvest to avoid exhausting starch repositories. Harvesting may also induce the upregulation of certain primary and secondary metabolites which respond to tissue damage as part of an herbivory response (Ponzio et al., 2017). Finally, the management of hydroponic nutrient delivery becomes more complex in an extended production system and plants may be more likely to deplete macronutrients before all micronutrients are absorbed, potentially inducing nutritional toxicities or antagonisms upon redosing.

Conclusions

The need for a steady source of unstable or deficient vitamins and minerals in the diet of those embarking on long term space voyages is undeniable. Plants provide a compact and consistent solution to this problem, however, many aspects of how to grow these plants in space remain to be determined. The interplay of different metabolites under specific environmental conditions makes it difficult to identify a single treatment that provides an ideal approach, however, the interplay between high CO₂, light intensity, and photoperiod can provide insights into how to increase concentrations of phytonutrients of interest. More difficult is the question of how a continuous harvest system impacts phytonutrient concentrations in plants grown under

ISS-like conditions. It is likely that specific testing will need to occur for each cultivar that will be grown in space, as results are genotype specific.

CHAPTER 1:
BIOREGENERATIVE DIETARY SUPPLEMENTATION IN SPACE: BRASSICA RAPA
VAR. NIPPOSINICA AND OTHER MUSTARDS

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Abstract

Despite the precise environmental manipulation enabled by controlled environment agriculture (CEA), plant genotype remains a key factor in producing desirable traits. *Brassica rapa* var. *nipposinica* (mizuna) is a leading candidate for supplementing deficiencies in the space diet, however, which cultivar of mizuna will respond best to the environment of the International Space Station (ISS) is unknown. It is also unclear if there are more inter-varietal (mizuna - mustards) or intra-varietal (mizuna - mizuna) differences in response to the ISS environment. Twenty-two cultivars of mustard greens, including 13 cultivars of mizuna, were grown under ISS-like conditions to determine which would provide the greatest yield and highest concentrations of carotenoids, anthocyanins, calcium, potassium, iron, magnesium, ascorbic acid, thiamine, and phyloquinone. The experiment was conducted thrice, and data were analyzed to determine which cultivar is most suited for further optimization of space-based cultivation. It was found that phyloquinone and β -carotene concentrations did not vary between cultivars, while all other metrics of interest showed some variation. ‘Amara’ mustard (*B. carinata*) provided the best overall nutritional profile, despite its low biomass yield of 36.8 g, producing concentrations of 27.85, 0.40, and 0.65 mg·g⁻¹ of ascorbic acid, thiamine, and lutein, respectively. Of the mizuna cultivars evaluated, open pollinated mibuna provided the best profile, while ‘Red Hybrid’ mizuna provided a complimentary profile to that of ‘Amara’, minimally increasing dietary iron while providing beneficial anthocyanins lacking in ‘Amara’.

Introduction

Proper nutritional intake is required to maintain astronaut health during exploration-length space missions (Massa et al., 2015). This becomes increasingly problematic on longer voyages, as the current freeze-dried diet does not provide sufficient levels of certain dietary nutrients (potassium, calcium, magnesium, and vitamin K₁), while others degrade during storage and require consistent fresh supplementation (vitamin C, vitamin B₁, and β -carotene) (Cooper et al., 2017). Potassium and calcium are both important for proper muscle and nerve function (Parikh & Webb, 2012), while calcium and magnesium are integral for bone structure (Flynn, 2003; Long & Romani, 2014). Scurvy is defined as vitamin C (ascorbic acid) deficiency, while beriberi is defined as vitamin B₁ (thiamine) deficiency, both being diseases which can be fatal if not treated by supplementation (Jukes, 1989). Vitamin K₁, referred to as phyloquinone in plants, is important in human nutrition as a cofactor in blood coagulation (Basset et al., 2017). Finally, β -carotene is the primary dietary precursor to vitamin A, and as such is important for proper eye and immune health (Olson & Hayaishi, 2009).

Growing and eating fresh produce remains the most straightforward way to obtain these compounds and minerals, in addition to providing the benefits of non-essential phytonutrients such as lutein, zeaxanthin, and anthocyanins, as well as avoiding toxicities that can occur during the overutilization of pill-based supplements (Chylack et al., 2009; Tran & Demmig-Adams, 2007). Mizuna (*Brassica rapa* var. *nipposinica*) is a promising candidate for growth aboard spacecraft for supplementing the astronaut diet, due to its nutritional and organoleptic profile, quick production cycle, compact growth habit, and resilience to elevated CO₂ concentrations (Massa et al., 2015). This variety of *Brassica* has a long history of use in Japan and according to local culture is divided into mizuna (jagged leafed cultivars) and mibuna (smooth leafed cultivars), with mizuna traditionally being used in soups, and mibuna being pickled, although in more recent times both have been used as a component in salads in their raw form (Kawakatsu et al., 2021).

Early screening compared several leafy greens (*Brassica rapa* var. *nipposinica*, *Brassica rapa* spp. *chinensis*, *Beta vulgaris*, *Lactuca sativa*, *Spinacia oleracea*) in which mizuna performed second best, after Chinese cabbage (*Brassica rapa* spp. *chinensis*) (Massa et al., 2015). However, upon growth in space Chinese cabbage performed poorly, developing chlorosis and necrotic tissue (Burgner et al., 2019). Follow-up testing included production of additional

leafy greens (*Brassica carinata*, *Brassica napus*, *Brassica juncea*, and *Raphanus sativus*) (Johnson et al., 2021), and utilization of environmental parameters more similar to those aboard the International Space Station (ISS), such as supplementing the carbon dioxide levels to 2,800 $\mu\text{mol}\cdot\text{mol}^{-1}$ (Burgner et al., 2020). Mizuna continued to perform well, however, the group contains many cultivars which will perform variably under different environmental conditions. A knowledge of these differences, including differential phytonutrient production and mineral uptake, under an ISS-like environment will be beneficial for future dietary supplementation.

The purpose of this study is to determine how different cultivars of mizuna and related mustards, including cultivars of *Brassica juncea* and *Brassica carinata*, react to an environment like that of the ISS and to determine which cultivars provide optimal biomass, phytonutrient, and mineral yields in a compact space. It is possible that as increased data become available on which phytonutrients are more heavily concentrated in each cultivar, leafy green blends (salads) could be tailored to individual needs. This information will also allow for the identification of cultivars of mizuna and other *Brassica* that are most apt for further environmental and cultural optimization.

Materials and Methods

Experimental Design

The experiment was organized in a randomized complete block design with 10 plants of each cultivar randomized across 8 hydroponic systems. The experiment was conducted three times over time. At harvest, morphological data were collected on 10 plants per cultivar per replication. Biochemical data were collected on 5 randomly selected plants per cultivar per replication, while the remaining 5 plants were utilized for mineral analysis.

Plant Production

Seeds of twenty-two cultivars from three species of *Brassica* (Table 1.1) were sown in 162-cell bound coco-peat cubes (Preforma plugs; Jiffy Growing Solutions, Lorain, OH) and placed in a walk-in growth chamber (TC2, Environmental Grow Chambers; Chagrin Falls, OH). The temperature was $21.2 \pm 1.3^{\circ}\text{C}$ and the relative humidity was $58 \pm 8\%$, both measured and logged every 10 minutes by a shielded temperature and relative humidity sensor (Hobo MX2300; OnSet, Bourne, MA). The temperature and relative humidity during this experiment were similar to those on

Table 1.1. List of *Brassica* cultivars grown, their cultivar names or a description in non-cultivar cases, their common names, respective species, color, and seed source.

Cultivar	Common name	Species	Color	Source
Red Hybrid	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Red	Kitazawa Seed ^z
Green Spray	Mibuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Kitazawa Seed
Lime Streaks	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Kitazawa Seed
Central Red	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Red	Kitazawa Seed
Beni Houshi	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Red	Baker Creek
Red Streaks	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Red	Kitazawa Seed
Japanese Pink	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Baker Creek
605 Summer	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Baker Creek
Early	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Baker Creek
Microgreen	Mizuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Johnny's Selected Seeds
Open pollinated	Mibuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Kitazawa Seed
Early	Mibuna	<i>B. rapa</i> var. <i>nipposinica</i>	Green	Kitazawa Seed
Japanese Red Giant	Mustard	<i>B. juncea</i>	Red	Baker Creek
Red Splendor	Mustard	<i>B. juncea</i>	Red	Johnny's Selected Seeds
Ruby Streak	Mustard	<i>B. juncea</i>	Red	Kitazawa Seed
Miz America F1	Mustard	<i>B. juncea</i>	Red	Johnny's Selected Seeds
Scarlet Frill	Mustard	<i>B. juncea</i>	Red	Kitazawa Seed
Wasabina	Mustard	<i>B. juncea</i>	Green	Johnny's Selected Seeds
Golden Frills	Mustard	<i>B. juncea</i>	Green	Johnny's Selected Seeds
Red Kingdom F1	Mizuna	<i>B. rapa</i>	Red	Johnny's Selected Seeds
Garnet Giant	Mustard	<i>B. juncea</i>	Red	Johnny's Selected Seeds
Amara	Mustard	<i>B. carinata</i>	Green	Johnny's Selected Seeds

^zKitazawa Seed, Oakland, CA; Baker Creek, Mansfield, MO; Johnny's Selected Seeds, Fairfield, ME

the ISS, where the temperature ranges from 18 to 25 °C and the relative humidity is kept below 70%, and similar to ranges maintained in previous ground based testing by NASA (Burgner et al., 2020). Trays were placed under broad-spectrum light-emitting diodes (Scorpion Diablo, Horticulture Lighting Group; Maynardville, TN) providing 21:36:43 blue:green:red (%) radiation ratios, with a red:far-red ratio of 13:1, and a light intensity of $200 \pm 25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to the growing surface (~14% power, at 20 inches from the growing surface) for a 16-h photoperiod, which was monitored by quantum sensors (SQ-500S; Apogee, Logan, UT). CO₂ (Industrial Grade Carbon Dioxide CD 50S; Airgas, Knoxville, TN) was injected to provide $2,780 \pm 250 \mu\text{mol}\cdot\text{mol}^{-1}$, to match the $2,800 \mu\text{mol}\cdot\text{mol}^{-1}$ average aboard the ISS, and dosing was monitored and controlled by an infrared gas analyzer (LI-850; LI-COR Biosciences, Lincoln, NE) connected to a relay switch controlling an electronic regulator. CO₂ concentration was reported to a Raspberry Pi (Raspberry Pi 4 B; Raspberry Pi, Cambridge, England) every 20 seconds where it was recorded in a CSV file. Seeds were irrigated daily with reverse-osmosis water supplemented with 12N-1.8P-13.4K water-soluble fertilizer providing (mg·L⁻¹), 100 nitrogen (N), 15 phosphorus, 112 potassium, 58 calcium, 17 magnesium, 2 sulfur, 1.4 iron, 0.5 zinc, 0.4 copper and manganese, and 0.1 boron and molybdenum (RO Hydro FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (MgSO₄) to providing (mg·L⁻¹) 15 magnesium and 20 sulfur, and adjusted to a pH of 5.8 with H₂SO₄ or KCHO₃.

After fourteen days in the trays the seedlings were transplanted into 18-cm-deep by 61-cm-wide by 122-cm-long deep-water culture hydroponic systems (Premium Flood Table, Active Aqua; Petaluma, CA) with 61 by 122 cm sealed-surface foam rafts (36 ct lettuce raft; Beaver Plastics, Acheson, AB, Canada) and grown for 16 days. The environmental conditions were the same as the seedling stage. Eight systems were each filled with 108 L of reverse-osmosis water and supplemented with 12N-1.8P-13.4K water-soluble fertilizer (RO Hydro FeED; JR Peters, Inc.) and MgSO₄ providing twice the concentrations utilized during seedling production which were reported in the previous paragraph. Electrical conductivity (EC) and pH were measured (HI9813-6N Portable Waterproof pH/EC/TDS Meter; Hanna Instruments, Woonsocket, RI) and pH was adjusted to 5.8 using H₂SO₄ or KCHO₃. Air pumps (Active Aqua 110 L·min⁻¹ commercial air pump; Hydrofarm, Petaluma, CA) and air stones submerged in the hydroponic solution (Active Aqua air stone round 10 cm × 2.5 cm; Hydrofarm, Petaluma, CA) were used to provide dissolved oxygen.

Harvest, Morphological Data Collection, and Tissue Processing

Plants were harvested at the substrate surface and measurements of height (from the substrate surface to the tip of the tallest leaf), width (at the widest point and perpendicular to the widest point), the number of fully expanded leaves, and fresh mass were collected for 10 plants per cultivar, per replication. After weighing, fresh tissue of 5 plants was immediately placed in a plastic sample bag and flash frozen in liquid N. The plants were placed in a -80 °C freezer for storage. Samples were freeze dried (FreeZone 12+, LabConco; Kansas City, MO) for 4 days. After freeze drying, dry mass was recorded (Entris II Essential Line Milligram Balance; Sartorius, Göttingen, Germany), and individual plants were ground in liquid N with ceramic mortar and pestle. Once homogenized into a fine powder the tissue was divided into separate aliquots for future extraction procedures and stored in individual 15 mL centrifuge tubes in the -80 °C freezer. The remaining 5 plants were placed in a forced-air drying oven (130D, Precision Scientific; Chicago, Illinois) maintained at 65 °C for at least 3 d. These samples were homogenized in a single serving blender (magic bullet MB1001; nutribullet, Los Angeles, CA) in preparation for mineral analyses.

Anthocyanin Isolation and Quantification

Total anthocyanins were extracted and analyzed using a method derived from Islam et al. (2019). Extractions were performed under red light (peak wavelength: 654 nm) and samples were kept in a light blocking container filled with ice. One hundred mg of each sample was weighed into 15 mL polypropylene centrifuge tubes, then saturated with 5 mL of 95% ethanol/1.5 N HCl (85:15, v:v). Samples were placed on an orbital shaker at 200 rpm for 15 min, then stored in the dark at 4 °C for 24 hours. Sample contents were filtered into a 25 mL Erlenmeyer flask and a 200-μL aliquot of extractant was pipetted into a 96-well assay plate. Samples were analyzed using a Biotek PowerWave XS Microplate Reader (Agilent Technologies, Santa Clara, CA). The optical density was measured at 530 nm and total anthocyanin concentrations were calculated based on the calibration curve of cyanidin-3-o-glucoside chloride (MilliporeSigma, Burlington, MA).

Carotenoid Isolation and Quantification

The carotenoids β -carotene, lutein, and zeaxanthin were extracted and analyzed using a method derived from Kopsell et al. (2012). Extractions were performed under red light (peak wavelength: 654 nm) and samples were kept in a light blocking container filled with ice to prevent pigment degradation. One hundred mg of each homogenized sample were weighed into 15 mL polypropylene centrifuge tubes, then transferred to Potter–Elvehjem (Kontes, NJ) tissue grinding tubes. Eight hundred μ L of water purified by reverse osmosis, 800 μ L of ethyl- β -8'-apo-carotenoate (internal standard), and 2.5 mL of tetrahydrofuran (THF) were added to the tubes and samples were homogenized using a glass pestle attached to a homogenizer (Kinematica PT1035; Brinkmann, Westbury, NY). Homogenized samples were centrifuged at 4,000 rpm for 3 min, then the supernatant was transferred to a graduated culture tube. This process was repeated a second time, using only 2 mL of THF, and the resulting extractants were combined. Liquid extractants were dried down to 500 μ L with a N stream evaporator (N-EVAP 111; Organomation Inc., MA), diluted to 5 mL with acetone, and filtered. A 1-mL aliquot of filtered extractant was pipetted into a 2 mL amber crimp-top vial and analyzed using high-performance liquid chromatography (HPLC).

A 1200 Agilent Series HPLC unit, equipped with a reverse-phase ProntoSIL C30 (250 mm x 4.6 mm, 4 μ m) analytical column, and a 1200 Agilent Series diode array detector (Agilent Technologies, Santa Clara, CA) was used to identify and quantify pigments based on calibration curves of analytical reference standards (MilliporeSigma, Burlington, MA). The injection volume was 5 μ L per sample and the column temperature was thermostatically controlled at 30 °C. A mobile phase of 1% methyl tert-butyl ether (MTBE), 88.99% methanol, and 0.01% triethylamine (TEA) (v/v/v) was isocratically maintained at 1 mL $\cdot$ min⁻¹ for 62 minutes per sample. β -carotene, lutein, and zeaxanthin were detected at 453 nm.

Fat-Soluble Vitamin Isolation and Extraction

The fat-soluble vitamin phyloquinone (vitamin K₁) was extracted using a method derived from Pokkanta et al. (2019). Three hundred mg of each sample were weighed into 15 mL polypropylene centrifuge tubes and 5 mL of methanol was added to the tube. The tubes were subsequently placed on an orbital shaker for 5 minutes, in a hot water bath (40° C) and sonicated for 5 minutes, then centrifuged at 4,000 rpm for five minutes. The resulting supernatant was

dispensed into a glass dry down tube. The centrifuge tube was then filled with 5 mL of dichloromethane and the process of shaking, sonicating, and centrifuging was repeated. The resulting supernatant was added to the dry down tube. This entire process was repeated a final time with 5 mL of hexane, 0.8 mL of hydrochloric acid (0.1 M), and 2 mL of phosphate buffer (2.5 pH). The tubes were then spiked with 1 mL of vitamin K₂ as an internal standard. The liquid extractants were dried completely with a N stream evaporator (N-EVAP 111; Organomation Inc., MA), then brought up to 5 mL with dichloromethane. The tubes were then vortexed and filtered into a 25 mL Erlenmeyer flask. A 1-mL aliquot of filtered extractant was pipetted into a 2 mL amber crimp-top vial and analyzed using high-performance liquid chromatography (HPLC).

A 1200 Agilent Series HPLC unit, equipped with a reverse-phase ZORBAX Eclipse Plus C18 (250 mm x 4.6 mm, 4 μ m) analytical column and a 1200 Agilent Series diode array detector (Agilent Technologies, CA), was used to identify and quantify phyloquinone and tocopherol based on calibration curves of analytical reference standards (MilliporeSigma, MA). The injection volume was 10 μ L per sample and the column temperature was thermostatically controlled at 30 °C. The mobile phase consisted of methanol (A) and water (B) and the flow rate was maintained at 0.5 mL $\cdot$ min⁻¹. A linear gradient elution of 90% A was held for 13 min, increased to 95% A over 1 min, decreased to 85% A over 3 min, increased to 95% A over 5 min and held at 95% A for 20 min, then decreased and held at 90% A for a total run time of 60 minutes per sample. Phyloquinone and tocopherol were identified at 248 and 280 nm, respectively.

Water-Soluble Vitamin Isolation and Extraction

The water-soluble vitamins (WSVs) ascorbic acid (vitamin C) and thiamine (vitamin B₁) were extracted using a method derived from Seal and Chaudhuri (2018) and Sun et al. (2018). Extractions were performed under red light (peak wavelength: 654 nm) and samples were kept in a light blocking container filled with ice. Five hundred mg of each sample were weighed into 15 mL polypropylene centrifuge tubes, then hydrated with 5 mL of water purified by reverse osmosis, 1 mL of hydrochloric acid (0.1 M), and 5 mL of phosphate buffer (2.5 pH). Samples were vortexed for 15 seconds, centrifuged at 4,000 rpm for 5 min, and the supernatant was filtered into a 25 mL Erlenmeyer flask. This process was repeated once and the resulting

extractants were combined. A 1 mL aliquot of extractant was pipetted into a 2 mL amber crimp-top vial and analyzed by high-performance liquid chromatography (HPLC).

The WSVs were analyzed based on the method by Phillips et al. (2010) A 1200 Agilent Series HPLC unit, equipped with a reverse-phase ZORBAX Eclipse Plus C18 (250 mm x 4.6 mm, 4 μ m) analytical column and a 1200 Agilent Series diode array detector (Agilent Technologies, Santa Clara, CA), was used to identify and quantify WSVs based on calibration curves of analytical reference standards (MilliporeSigma, Burlington, MA). The injection volume was 20 μ L per sample and the column temperature was thermostatically controlled at 20 °C. The mobile phase (0.05% aq. formic acid) flow rate was isocratically maintained at 1 mL $\cdot$ min⁻¹ for 15 minutes per sample. Ascorbic acid and thiamine were detected at 255 nm.

Mineral Extraction

Mineral nutrient concentrations (Ca²⁺, Mg²⁺, K⁺, Fe³⁺) were quantified using methods derived from Hanlon et al. (1997) and Obreza and Morgan (2008). Homogenized tissue was rinsed in nitric acid (10 mL of HNO₃ at 15.6N) and microwave digested for 30 minutes (MARS 6 Microwaves; CEM Corp., Matthews, NC). Samples were then diluted with 50 mL of deionized water and vacuum-filtered through acid-washed paper (Laboratory Filtration Group; Houston, TX). Individual mineral concentrations were then quantified using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos EOP; Mahwah, NJ).

Statistics

Statistical analysis was performed in R (R Core Team, 2023). Five cultivars, ‘Central Red’, ‘Beni Houshi’ mizuna, ‘Japanese Red Giant’ mustard, ‘Miz America F1’ mustard, and ‘Red Kingdom F1’, were dropped from the analysis due to poor germination, insufficient mass for phytonutrient and mineral extractions, or a combination of both factors (Fig. 1.1). Data were organized, compiled, and visualized with the ‘tidyverse’ package (Wickham et al., 2019), and analysis of variance was performed with the ‘stats’ package on each metric of interest (R Core Team, 2023), after which a post-hoc Tukey’s Honest Significant Difference test was performed with the ‘emmeans’ package (Lenth, 2023). The exception to this process was the total anthocyanin concentration, for which red and green cultivars were first separated due to a large concentration difference between the two groups, then each group underwent separate analysis of

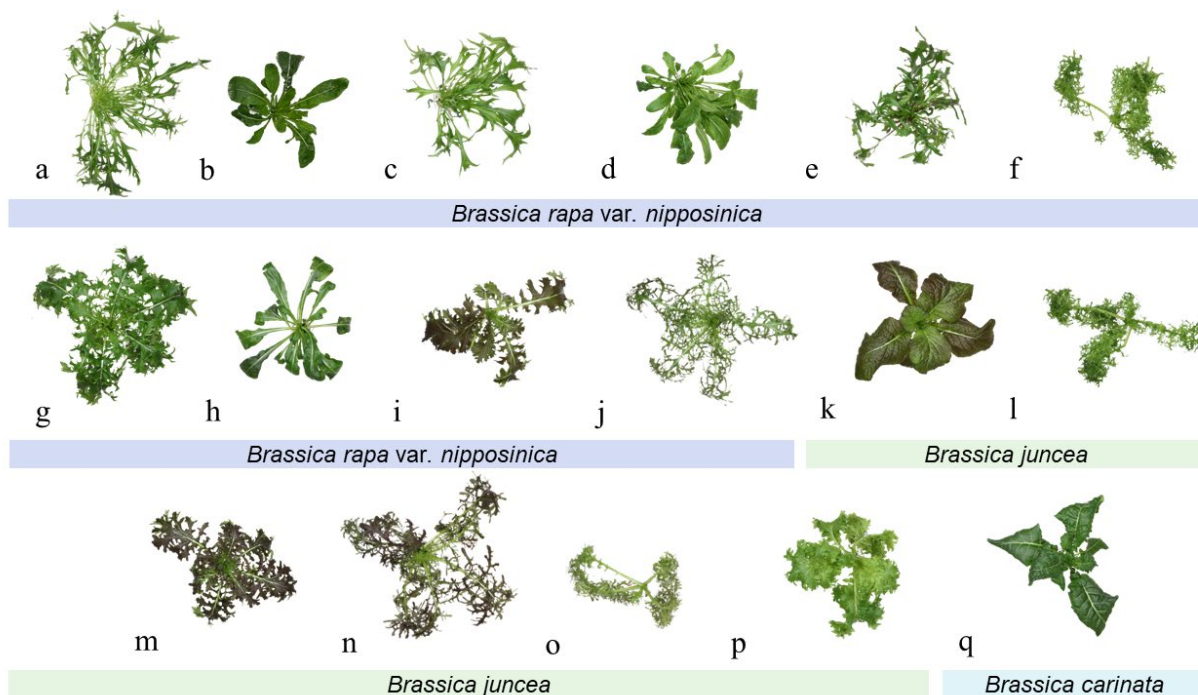


Figure 1.1. Photographs of the 17 cultivars of *Brassica* which germinated consistently under elevated CO₂ and produced sufficient biomass for phytonutrient evaluation. Nine cultivars of *Brassica rapa* var. *nipposinica* a) ‘605 Summer’, b) ‘Early’ mibuna, c) ‘Early’ mizuna, d) ‘Green Spray’, e) ‘Japanese Pink’, f) ‘Lime Streaks’, g) ‘Microgreen’ mizuna, h) open pollinated mibuna, i) ‘Red Hybrid’, and j) ‘Red Streaks’, six cultivars of *Brassica juncea* k) ‘Garnet Giant’, l) ‘Golden Frills’, m) ‘Red Splendor’, n) ‘Ruby Streak’, o) ‘Scarlet Frill’, p) ‘Wasabina’, and one cultivar of *Brassica carinata* q) ‘Amara’ are represented.

variance and Tukey's HSD. After determining mean separations, the individual parameter means were min-max normalized to allow for comparisons between parameters. The normalized means were then weighted according to importance multipliers (Table 1.2), based on those previously used by NASA (Massa et al., 2015). Weighted means were summed by cultivar to produce a final score for evaluation of use in supplementing the space diet.

Results

Morphological Characteristics

All three morphological parameters showed significant variation between cultivars at the $\alpha = 0.05$ level (Table 1.3). 'Green Spray' produced the highest average fresh mass (117.2 g) with the next largest cultivars producing masses of around 80 grams. There was much less separation among cultivars with the lowest fresh mass including 'Ruby Streak' (34.1 g), 'Amara' (36.8 g), 'Red Hybrid' (37.0 g), 'Red Streaks' (38.6 g), 'Garnet Giant' (38.7 g), 'Scarlet Frill' (40.6 g), 'Golden Frills' (47.7 g), open pollinated mibuna (48.0 g), and 'Red Splendor' (48.2 g) (Table 1.4). All percent moisture means fell between 92% and 95% (Table 1.4) with 'Red Hybrid', 'Scarlet Frill', 'Wasabina', and 'Garnet Giant' containing the highest percentage of water by mass while 11 cultivars have similarly lowest percent moisture (Table 1.4). Plant volume, or the cubic area occupied by a plant, was more varied. Cultivars 'Green Spray', 'Lime Streaks', 'Japanese Pink', '605 Summer', and 'Early' mibuna occupied the most production space while cultivars 'Red Streaks', 'Early' mizuna, 'Ruby Streak', 'Scarlet Frill', 'Wasabina', 'Garnet Giant', and 'Amara' occupied the least volume (Table 1.4).

Mineral Concentrations

Mean calcium concentrations ranged from 3.4 to 2.0 $\text{mg}\cdot\text{g}^{-1}$ of dry tissue, with 'Japanese Pink' (3.36 $\text{mg}\cdot\text{g}^{-1}$), '605 Summer' (3.13 $\text{mg}\cdot\text{g}^{-1}$), 'Early' mizuna (3.37 $\text{mg}\cdot\text{g}^{-1}$), and 'Microgreen' mizuna (3.19 $\text{mg}\cdot\text{g}^{-1}$) accumulating the highest calcium concentrations (Table 1.4). Mean iron concentrations did not separate as clearly, with 10 cultivars not significantly differing from any other cultivars. However, 'Green Spray' (109.3 $\mu\text{g}\cdot\text{g}^{-1}$) did accumulate more Fe^{3+} than 'Red Hybrid' (65.8 $\mu\text{g}\cdot\text{g}^{-1}$), 'Red Splendor' (73.4 $\mu\text{g}\cdot\text{g}^{-1}$), 'Ruby Streak' (77.4 $\mu\text{g}\cdot\text{g}^{-1}$), and 'Garnet Giant' (78.4 $\mu\text{g}\cdot\text{g}^{-1}$). Potassium concentration also showed significant overlap, with 9 cultivars not differing significantly from any others and all concentrations ranging from 6.9 $\text{mg}\cdot\text{g}^{-1}$ and

Table 1.2. A list of 14 metrics that are used to evaluate cultivar performance for use in the space diet. Metrics are grouped into five categories, and multipliers are provided to weigh final results according to importance. Table adapted from Massa et al.¹

Metric	Category	Multiplier	Justification
Fresh Mass	Morphological	× 2.5	Total phytonutrient content scales with this metric
Percent Moisture	Morphological	× -1.0	Higher dry mass proportion is desirable
Plant Volume	Morphological	× -1.5	Smaller plants are desirable due to limited space
Ca	Mineral	× 1.5	Mitigates bone loss in microgravity ⁴
K	Mineral	× 2.0	Currently deficient in space diet ¹
Mg	Mineral	× 1.0	Mitigates bone loss in microgravity
Fe	Mineral	× -1.5	Excessive iron can exacerbate bone loss in microgravity ¹
Ascorbic Acid	Water-Soluble Vitamin	× 1.5	Prevent scurvy; degrades quickly ^{2,6}
Thiamine	Water-Soluble Vitamin	× 1.5	Prevent beriberi; degrades quickly ^{2,6}
Phylloquinone	Fat-Soluble Vitamin	× 1.5	Promote proper blood coagulation; deficient in stored space diet ^{1,7}
β-carotene	Carotenoid	× 1.5	Vitamin A precursor; degrades quickly ^{2,8}
Lutein	Carotenoid	× 1.5	Preventing ocular photodamage ⁹
Zeaxanthin	Carotenoid	× 1.5	Preventing ocular photodamage ⁹
Total Anthocyanin	Anthocyanin	× 1.0	Potentially beneficial as an antioxidant

Table 1.3. ANOVA results from all metrics of interest for 17 mizuna cultivars, 5 cultivars were dropped before analysis due to poor germination or insufficient mass. Total anthocyanin results were split into two groups, red cultivars and green cultivars, before performing analysis of variance.

Metric	P-Value	Significance
Fresh Mass	< 0.001	***
Percent Moisture	< 0.001	***
Plant Volume	< 0.001	***
Ca	< 0.001	***
K	< 0.001	***
Mg	< 0.001	***
Fe	< 0.001	***
Ascorbic Acid	0.047	*
Thiamine	< 0.001	***
Phylloquinone	0.298	NS
β-carotene	0.089	NS ^a
Lutein	< 0.001	***
Zeaxanthin	< 0.001	***
Total Anthocyanin (Red Cultivars)	0.063	NS
Total Anthocyanin (Green Cultivars)	0.006	*
^a ***, *, and NS indicate significant at $P \leq 0.001$, $P \leq 0.05$, or nonsignificant, respectively.		

Table 1.4. Mean separation (Tukey's HSD) results for morphological parameters and mineral concentrations by dry mass. Means that share the same letter designation did not differ at the $\alpha = 0.05$ level. Means without a letter designation were not subjected to post-hoc testing.

Cultivar	Fresh mass	Moisture	Volume	Ca	K	Mg	Fe
	(g)	(%)	(m ³)	mg·g ⁻¹			µg·g ⁻¹
Red Hybrid	37.0 f	94.1 abc	0.0183 efg	2.54 b	8.84 abc	0.578 c-f	65.8 c
Green Spray	117.2 a	93.6 b-e	0.0317 a	2.58 b	8.44 abc	0.627 cd	109.3 a
Lime Streaks	57.7 cde	92.9 ef	0.0262 abc	2.25 bc	8.89 ab	0.496 fg	100.8 abc
Red Streaks	38.6 ef	93.2 def	0.0185 d-h	2.52 bc	8.41 abc	0.500 d-g	77.3 abc
Japanese Pink	86.7 b	92.9 f	0.0259 a-d	3.36 a	6.92 c	0.820 a	96.5 abc
605 Summer	80.7 b	93.3 def	0.0276 ab	3.13 a	7.81 bc	0.775 a	82.3 abc
Early (mizuna)	58.1 cde	93.2 def	0.0180 e-h	3.37 a	7.57 bc	0.810 a	96.6 abc
Microgreen (mizuna)	83.0 b	93.5 c-f	0.0231 b-e	3.19 a	7.06 c	0.748 ab	106.0 ab
Open pollinated mibuna	48.0 c-f	92.9 ef	0.0190 c-g	2.58 b	8.54 abc	0.622 cde	97.9 abc
Early (mibuna)	72.8 bc	93.2 def	0.0264 a-d	2.42 bc	7.87 abc	0.567 c-g	101.0 abc
Red Splendor	48.2 def	93.8 bcd	0.0208 b-f	2.45 b	8.15 abc	0.592 c-f	73.4 bc
Ruby Streak	34.1 f	93.3 def	0.0179 e-h	2.41 bc	8.32 abc	0.520 d-g	77.4 bc
Scarlet Frill	40.6 ef	93.9 a-d	0.0162 fgh	2.41 bc	8.29 abc	0.526 d-g	98.8 abc

Table 1.4. Continued

Cultivar	Fresh mass	Moisture	Volume	Ca	K	Mg	Fe
	(g)	(%)	(m ³)	mg·g ⁻¹			μg·g ⁻¹
Wasabina	68.3 bcd	94.2 ab	0.0131 gh	2.44 b	9.66 a	0.516 efg	93.8 abc
Golden Frills	47.7 ef	93.0 ef	0.0194 c-g	2.01 c	8.29 abc	0.461 g	95.7 abc
Garnet Giant	38.7 ef	94.5 a	0.0113 h	2.53 b	7.33 bc	0.577 c-f	78.4 bc
Amara	36.8 f	93.3 def	0.0133 gh	2.66 b	7.46 bc	0.655 bc	104.9 ab

Table 1.5. Mean separation (Tukey's HSD) results for five phytonutrient concentrations by dry mass. Means that share the same letter designation did not differ at the $\alpha = 0.05$ level. Means without a letter designation were not subjected to post-hoc testing.

Cultivar	Ascorbic acid	Thiamine	β -carotene	Lutein	Zeaxanthin	Total Anthocyanins	Phylloquinone
	mg·g ⁻¹						
Red Hybrid	17.65 ab	0.1683 c-f	0.914	0.5580 a-d	0.0270 cd	8.038	1.680
Green Spray	18.91 ab	0.0329 fg	0.886	0.6699 a-d	0.0730 bcd	2.050 ab	2.037
Lime Streaks	16.55 ab	0.2002 b-e	0.841	0.6884 abc	0.0361 bcd	1.382 ab	1.691
Red Streaks	17.99 ab	0.1730 cde	0.824	0.5640 a-d	0.0202 cd	6.217	2.422
Japanese Pink	14.83 ab	0.0048 g	0.718	0.4814 d	0.0221 d	2.882 a	1.596
605 Summer	17.72 ab	0.0058 g	0.770	0.5045 d	0.0299 bcd	2.267 ab	1.687
Early (mizuna)	18.58 ab	0.0097 g	0.810	0.5218 cd	0.0491 bcd	1.413 b	1.809
Microgreen (mizuna)	19.02 ab	0.0321 g	0.808	0.6399 a-d	0.0881 b	1.940 ab	2.197
Open pollinated mibuna	23.43 ab	0.0025 g	0.817	0.7602 ab	0.1649 a	1.715 ab	1.804
Early (mibuna)	20.86 ab	0.0726 efg	0.806	0.7503 a	0.1710 a	2.563 ab	1.847
Red Splendor	24.97 ab	0.0984 d-g	0.840	0.5608 a-d	0.0196 d	10.317	1.885
Ruby Streak	24.64 ab	0.2884 abc	0.786	0.6452 a-d	0.0443 bcd	10.070	1.673
Scarlet Frill	18.32 ab	0.3351 ab	0.819	0.6647 a-d	0.0339 bcd	7.206	1.661

Table 1.5. Continued

Cultivar	Ascorbic acid	Thiamine	β-carotene	Lutein	Zeaxanthin	Total Anthocyanins	Phylloquinone
	$\text{mg}\cdot\text{g}^{-1}$						
Wasabina	20.91 ab	0.3058 abc	0.730	0.5463 bcd	0.0268 cd	2.469 ab	2.048
Golden Frills	19.16 ab	0.2783 abc	0.843	0.6950 abc	0.0754 bc	1.565 ab	2.116
Garnet Giant	11.68 b	0.2441 bcd	0.800	0.5493 a-d	0.0213 cd	11.714	2.244
Amara	27.85 a	0.4004 a	0.867	0.6473 a-d	0.0576 bcd	2.636 ab	2.563

9.6 mg·g⁻¹. ‘Wasabina’ and ‘Lime Streaks’ did, however, accumulate more potassium than ‘Japanese Pink’ and ‘Microgreen’ mizuna. The highest magnesium concentrations were accumulated by ‘Japanese Pink’ (0.820 mg·g⁻¹), ‘Early’ mizuna (0.810 mg·g⁻¹), ‘605 Summer’ (0.775 mg·g⁻¹), and ‘Microgreen’ mizuna (0.748 mg·g⁻¹). For comparison, the lowest magnesium accumulators contained concentrations ranging from 0.461 mg·g⁻¹ to 0.567 mg·g⁻¹ (Table 1.4).

Vitamin Concentrations

Only two cultivars had ascorbic acid (vitamin C) concentrations which differed from each other, with ‘Amara’ producing 27.85 mg·g⁻¹ and ‘Garnet Giant’ producing 11.68 mg·g⁻¹ (Table 1.5). Mean phyloquinone (vitamin K₁) concentrations were similar among cultivars (Table 1.5), with a p-value of 0.290 (Table 1.3). Mean thiamine (vitamin B₁) concentrations ranged from 0.0025 mg·g⁻¹ to 0.4004 mg·g⁻¹. Eight cultivars produced thiamine concentrations below 0.09 mg·g⁻¹ while ‘Amara’ (0.4004 mg·g⁻¹), ‘Scarlet Frill’ (0.3351 mg·g⁻¹), ‘Wasabina’ (0.3058 mg·g⁻¹), ‘Ruby Streak’ (0.2884 mg·g⁻¹), and ‘Golden Frills’ (0.2783 mg·g⁻¹), produced the highest concentrations (Table 1.5).

Carotenoid Concentrations

Cultivar β-carotene concentrations (Table 1.5) did not vary significantly with a p-value of 0.089 (Table 1.3). Lutein concentrations did show variance; however, 9 cultivars did not show significant separation from any others. ‘Early’ mibuna (0.7503 mg·g⁻¹) did produce higher lutein concentrations than cultivars ‘Japanese Pink’ (0.4814 mg·g⁻¹), ‘605 Summer’ (0.5045 mg·g⁻¹), ‘Early’ mizuna (0.5218 mg·g⁻¹), and ‘Wasabina’ (0.5463 mg·g⁻¹) (Table 1.5). Mean zeaxanthin concentrations ranged from 0.0202 to 0.1710 mg·g⁻¹, with open pollinated mibuna (0.1649 mg·g⁻¹) and ‘Early’ mibuna (0.1710 mg·g⁻¹) producing nearly double that of the next highest producing cultivar, ‘Microgreen’ mizuna (0.0881 mg·g⁻¹) (Table 1.5).

Total Anthocyanin Concentrations

Total anthocyanin means were separated first by color, as red cultivars contained significantly higher anthocyanin concentrations, with means ranging from 6.217 (‘Red Streaks’) to 11.714 mg·g⁻¹ (‘Garnet Giant’) and green cultivars containing concentrations ranging from 1.382 to 2.882 mg·g⁻¹ (Table 1.5). Red cultivars did not vary significantly from each other, while

green cultivars did (Table 1.3). ‘Japanese Pink’ produced more total anthocyanins per gram of dry weight, $2.882 \text{ mg}\cdot\text{g}^{-1}$, than ‘Early’ mizuna, which produced $1.3824 \text{ mg}\cdot\text{g}^{-1}$.

Weighted Scores

Final weighed scores, calculated by summing the normalized and weighted means of each parameter, indicate that ‘Amara’ mustard (*Brassica carinata*), with a score of 4.65 (Table 1.6) is the most promising candidate for use in supplementing the space diet, as it contains the best ratio of positive to negative traits once individual parameters are normalized and weighted (Fig. 1.2). Among *Brassica rapa* var. *nipposinica* the cultivars open pollinated mibuna, ‘Green Spray’, ‘Early’ mibuna, ‘Red Hybrid’, and ‘Microgreen’ mizuna had the top 5 scores, ranking second, fifth, sixth, seventh, and eighth when evaluated against all cultivars. Among *Brassica juncea*, ‘Ruby Streak’, ‘Wasabina’, ‘Red Splendor’, ‘Golden Frills’, and ‘Scarlet Frill’ were the top 5 cultivars, ranking third, fourth, ninth, tenth, and eleventh when compared across all cultivars.

Discussion

Of the twenty-two cultivars initially sown, five cultivars displayed poor germination or biomass production under the environmental conditions of the study. While the experimental design cannot indicate whether this was due to the elevated CO_2 , previous work has shown that some *Brassica* grow inconsistently under high concentrations (Burgner et al., 2019). The remaining seventeen cultivars consistently germinated and produced biomass when grown under the elevated CO_2 concentrations and would thus be better choices for space production than Chinese Cabbage (Burgner et al., 2020). NASA has previously worked with cultivars of *B. rapa* var. *nipposinica*, *B. juncea*, and *B. carinata*, and at least one cultivar of each has been grown aboard the ISS at some point (Johnson et al., 2021), but the results of this study provide a more comprehensive comparison of cultivars from these three species.

The strong ranking of ‘Amara’ is largely due to its high concentrations of water-soluble vitamins (Fig. 1.2), being among the top producers of both ascorbic acid and thiamine (Table 1.5), as well as its low moisture content and compact volume (Table 1.4). The high concentration of these water-soluble vitamins is promising, as the plant has grown well and accumulated low levels of microbial contamination in another ground-based experiment under ISS-like conditions (Bunchek et al., 2021). While it achieved the highest overall weighted score, there were undesirable aspects of its performance, such as low concentrations of anthocyanins when

Table 1.6. Evaluation scores for all 14 parameters, calculated by min-max normalizing the mean value and multiplying by the weighting multiplier in Table 1.2. Total score is calculated per cultivar by the sum of all parameter scores and is represented in bold on the right.

Cultivar	Parameter Score														Total Score
	Fresh Mass	Moisture	Volume	Ca	K	Mg	Fe	Ascorbic Acid	Thiamine	β -carotene	Lutein	Zeaxanthin	Anthocyanin	Phylloquinone	
Amara	0.39	- 0.58	- 0.38	0.44	0.766	0.46	- 0.48	0.80	0.79	0.75	0.65	0.22	0.08	0.74	4.65
Open pollinated mibuna	0.52	- 0.48	- 0.54	0.40	1.10	0.40	- 0.40	0.67	0.00	0.66	0.86	0.63	0.05	0.51	4.38
Ruby Streak	0.36	- 0.56	- 0.51	0.32	1.03	0.23	- 0.18	0.71	0.57	0.60	0.65	0.17	0.36	0.46	4.22
Wasabina	0.75	- 0.77	- 0.37	0.34	1.46	0.23	- 0.36	0.60	0.60	0.50	0.46	0.10	0.07	0.58	4.21
Green Spray	1.32	- 0.65	- 0.90	0.41	1.07	0.41	- 0.53	0.54	0.06	0.79	0.69	0.28	0.06	0.58	4.13
Early (mibuna)	0.81	- 0.56	- 0.75	0.33	0.89	0.31	- 0.44	0.60	0.14	0.64	0.84	0.65	0.08	0.52	4.06
Red Hybrid	0.39	- 0.74	- 0.52	0.39	1.20	0.33	- 0.05	0.50	0.33	0.84	0.48	0.10	0.29	0.47	4.00
Microgreen	0.92	- 0.61	- 0.66	0.69	0.63	0.61	- 0.49	0.55	0.06	0.65	0.64	0.34	0.05	0.63	4.00
Red Splendor	0.52	- 0.69	- 0.59	0.34	0.98	0.35	- 0.14	0.72	0.19	0.70	0.49	0.07	0.37	0.53	3.86
Golden Frills	0.52	- 0.51	- 0.55	0.14	1.02	0.14	- 0.38	0.55	0.55	0.71	0.74	0.29	0.04	0.60	3.85
Scarlet Frill	0.43	- 0.69	- 0.46	0.32	1.06	0.24	- 0.41	0.52	0.66	0.66	0.68	0.13	0.25	0.46	3.83
Red Streaks	0.41	- 0.56	- 0.52	0.38	0.79	0.20	- 0.18	0.52	0.34	0.67	0.49	0.08	0.22	0.70	3.80

Table 1.6. Continued

Cultivar	Parameter Score														Total Score
	Fresh Mass	Moisture	Volume	Ca	K	Mg	Fe	Ascorbic Acid	Thiamine	β -carotene	Lutein	Zeaxanthin	Anthocyanin	Phylloquinone	
Early (mizuna)	0.64	- 0.56	- 0.52	0.77	0.87	0.71	- 0.39	0.53	0.02	0.65	0.42	0.19	0.03	0.51	3.80
605 Summer	0.90	- 0.58	- 0.79	0.66	0.72	0.65	- 0.23	0.50	0.01	0.58	0.38	0.11	0.07	0.47	3.61
Garnet Giant	0.41	- 0.82	- 0.32	0.38	1.21	0.33	- 0.19	0.33	0.48	0.63	0.47	0.08	0.42	0.64	3.56
Lime Streaks	0.63	- 0.50	- 0.74	0.25	0.59	0.20	- 0.44	0.47	0.39	0.71	0.73	0.14	0.03	0.47	3.54
Japanese Pink	0.96	- 0.49	- 0.74	0.76	0.23	0.73	- 0.39	0.42	0.01	0.48	0.34	0.08	0.09	0.44	3.31

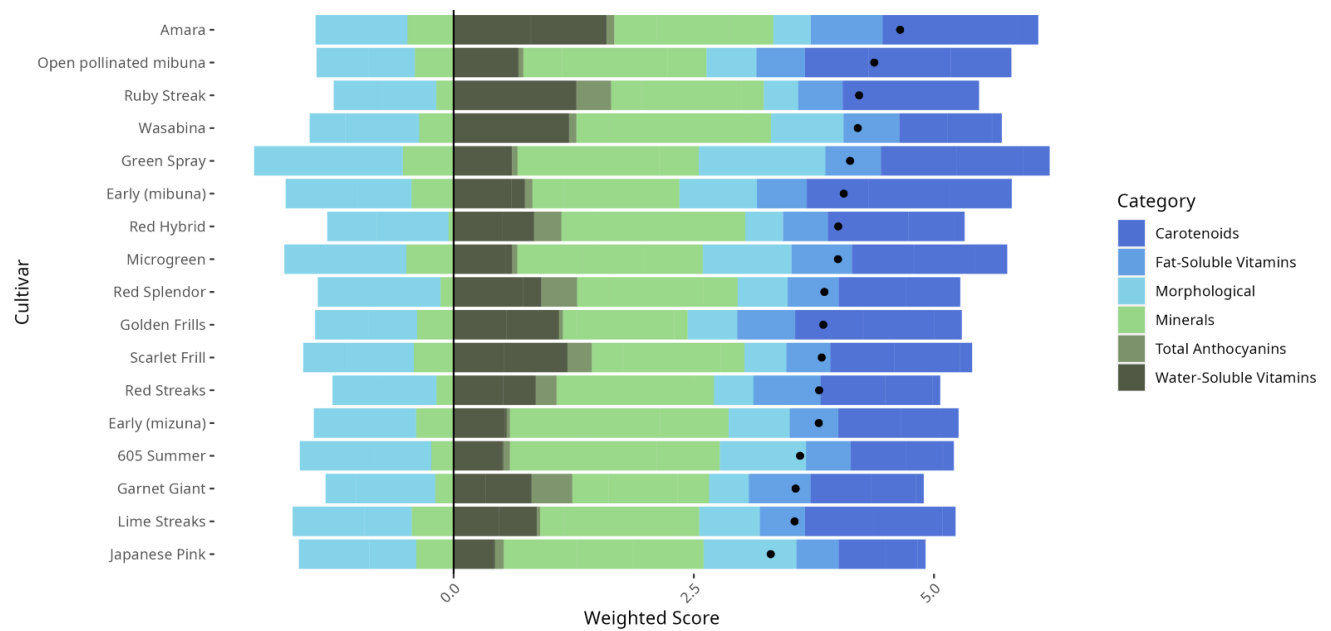


Figure 1.2. Summary scores, the sum of the normalized and weighted means of 14 metrics (Table 2) are displayed with a black dot. Portions of the bar to the left of 0 indicate negative components of the overall score (volume, Fe, and percent moisture), while bars right of 0 indicate positive components of the overall score (remaining metrics). Components are color coded by category as follows: carotenoids (β -carotene, lutein, zeaxanthin), fat-soluble vitamins (phyloquinone), morphological (fresh mass, percent moisture, volume), minerals (Ca, Fe, K, Mg), total anthocyanins, and water-soluble vitamins (ascorbic acid and thiamine).

compared to red cultivars, high concentrations of iron (Table 1.4), and one of the lowest fresh mass yields per plant (Table 1.4). Considering these limitations, especially its weak performance in the heavily weighted fresh mass parameter (Table 1.2), its position as the highest-ranking cultivar speaks to the degree to which its vitamin concentrations outperformed other cultivars.

As seen in the case of ‘Amara’, there are certain traits that tend to cancel out the effect of others. Due to the high correlation between plant volume and fresh mass, and the desirability of low volume and high mass, many cultivars lose advantages gained through one metric in poor performance of the other. Plants that escape this trend can gain an extra boost in their final score, as does ‘Wasabina’ with its relatively high fresh mass and low plant volume (Table 1.4). This is a desirable combination of traits, but a combination that is shared with ‘Amara’, which had higher vitamin and carotenoid concentrations. A similar effect is seen in mineral concentrations with cultivars that are high in Ca^{2+} and Mg^{2+} (‘Japanese Pink’, ‘Early’ mizuna, ‘605 Summer’, and ‘Microgreen’ mizuna) being among the lowest accumulators of K^{+} (Table 1.4). This is likely due to known antagonisms between these cation minerals (Elzam & Hodges, 1967), and its effect on cultivar ranking is more complex, as the score of plants high in calcium are both simultaneously boosted by their Mg^{2+} concentration and held back by their K^{+} concentration.

Carotenoid concentrations composed a significant part of the total weighted score, however, results for β -carotene showed little difference between cultivars and provided little advantage to any one cultivar in the final comparison. Conversely, zeaxanthin means ranged drastically, with open pollinated mibuna and ‘Early’ concentrations more than double the concentration of the next leading cultivar, and more than four-times that of many cultivars. These two cultivars also produced some of the highest lutein concentrations (Table 1.5). As previously mentioned, mibuna is a common name for cultivars of *B. rapa* var *nipposinica* that have smooth leaf edges, as opposed to the jagged edges of mizuna and was traditionally used for pickling due to its firmer texture (Kawakastu et al., 2021). The two highest concentrations of zeaxanthin being found in two of the three mibuna cultivars tested (the third being ‘Green Spray’, which also performed well) in this study is interesting, and further research into genetic differences between mizuna and mibuna cultivars within *B. rapa* var *nipposinica* may provide insights into these differences. Additionally, if future research is specifically concerned with increasing zeaxanthin

in the astronaut diet, the genotypes of these plants may hold clues on how to increase concentrations in other plants.

Much like zeaxanthin, thiamine concentrations showed distinct separation with some cultivars of *B. rapa* var. *nipposinica* ('Green Spray', 'Japanese Pink' mizuna, '605 Summer', 'Early' mizuna, 'Microgreen' mizuna, and open pollinated mibuna), producing low concentrations (Table 1.5). Only a single cultivar of *B. juncea*, 'Red Splendor', did not differ from these low values, with all other *B. juncea* cultivars producing significantly more thiamine. The single cultivar of *B. carinata*, 'Amara', produced higher concentrations of thiamine than did any of the *B. rapa* cultivars evaluated.

While final weighted scores may indicate the suitability of one cultivar over another, or even a best cultivar for *in-situ* supplementation of the astronaut diet, the advantage of a mixed-greens approach should not be ignored. As mentioned previously, 'Amara' provides high vitamin concentrations but does not produce high levels of anthocyanins. If anthocyanins are also desired, it may be advantageous to grow another cultivar alongside 'Amara'. In this case 'Ruby Streak' and 'Red Hybrid' are both promising candidates, due to their anthocyanin content but also due to their very low iron concentrations, as it would be undesirable to add additional iron to the already high concentrations of 'Amara' mustard.

CHAPTER 2:
LEVERAGING PHOTOPERIOD AND LIGHT INTENSITY INTERACTIONS TO IMPROVE
PHYTONUTRIENT PROFILE OF BRASSICA CARINATA AND BRASSICA RAPA VAR.
NIPPOSINICA FOR SPACE APPLICATIONS

Keywords: Controlled environment agriculture, mustard greens, mizuna, daily light integral, elevated CO₂, ascorbic acid, thiamine, phyloquinone, carotenoids

Abstract

Plant production on the International Space Station (ISS) is required for future mission success, due to critical nutritional deficiencies in the space diet, yet subject to environmental constraints, including limited space, elevated CO₂, and microgravity. A tool for increasing yield and phytonutrient concentrations is the manipulation of photoperiod and light intensity, and the objective of this study is to determine the optimal combination of these parameters when growing *Brassica carinata* ‘Amara’ greens and *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ at elevated CO₂ concentrations. This was determined by growing ‘Amara’ and ‘Red Hybrid’ under 200, 400, 600, and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensities in a factorial design with a 16- or 24-hr photoperiod for a total of 8 combinations. The plants were grown at an elevated CO₂ (2,800 $\mu\text{mol}\cdot\text{mol}^{-1}$) and a 23°C target air temperature to emulate ISS conditions, with a nutrient solution composed of a complete fertilizer (12.0N-1.7P-13.3K) supplemented with MgSO₄. After 13 days, seedlings were transplanted into raft hydroponic systems and harvested after 9 additional days. Growing was conducted twice over time and treatments were evaluated according to parameters important for future mission success, including fresh mass, plant volume, and concentrations of dry matter, Mg²⁺, Ca²⁺, K⁺, Fe³⁺, thiamine, ascorbic acid, phyloquinone, β -carotene, lutein, zeaxanthin, and total anthocyanins. After normalization and weighting, a combination of 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a 16-hr photoperiod produced the optimal profile of ‘Amara’ greens, providing, per 75 g serving, 98% of the recommended daily intake (RDI) of ascorbic acid, 29% RDI of thiamine, 100% RDI phyloquinone, and 90% RDI of vitamin A (β -carotene equivalency).

Introduction

The production of fresh vegetables aboard spacecraft has been identified as a key component of successful exploration-length space ventures (Perchonok et al., 2011). This is due to a need for fresh sources of vitamin C (ascorbic acid), vitamin B₁ (thiamine), and dietary carotenoids (β -carotene, lutein, zeaxanthin), as well as supplemental sources of vitamin K₁ (phyloquinone), potassium, magnesium, calcium, and antioxidants such as anthocyanins (Cooper et al., 2017). However, growing fresh produce in space presents many challenges, some of which

include limited space aboard craft, limited energy resources, and environmental constraints such as elevated CO₂ concentrations and microgravity (Burgner et al., 2020; Board and NRC, 2011).

Leafy greens are promising candidates for production aboard space craft, as they are more energy- and space-efficient than fruit or root crops. They also provide an advantage over dried supplements, such as pills, as accidental toxicities are much harder to incur (Tran & Demmig-Adams, 2007). Prior evaluation of leafy greens has shown species within the genus *Brassica* to be particularly promising, from a biomass, organoleptic, and phytonutrient perspective (Massa et al., 2015). More recent research has found *Brassica carinata* ‘Amara’ to contain high concentrations of desired phytonutrients while still performing well when grown under International Space Station (ISS)-like conditions (Buncheck et al., 2021; Chapter 1).

Additionally, plant tissue concentrations of many phytonutrients respond to environmental stimuli (Makus & Lester, 2002; Huang et al., 2021; Gerovac et al., 2016), and this may provide methods to increase concentrations of desirable compounds while maintaining or even increasing biomass yield. This is especially true of light, as compounds such as ascorbic acid (Smirnoff & Wheeler, 2000), secondary carotenoids (Demmig-Adams, 1990), and anthocyanins (Zhang et al., 2012) are involved in light-induced free-radical scavenging and absorption of excess light, while compounds such as phylloquinone (Kozuleva, et al., 2021) and primary carotenoids (Croce et al., 2002) are directly involved in the photosynthetic machinery. Thus, environmental control of light intensity and photoperiod is a potential tool to improve the quality of leafy greens for consumption as part of the space diet.

Light is also used more efficiently by plants at a lower intensity, and it can be more energy efficient to apply a lower intensity of light over a longer period of the day to achieve the same total photon exposure, or DLI (Daily Light Integral) (Palmer and van Iersel, 2020). This study aims to determine which combination of light intensity and photoperiod produces *Brassica* greens ‘Amara’ and ‘Red Hybrid’ mizuna with the highest phytonutrient concentrations when produced under an environment similar to that of the ISS, while still maintaining a compact morphology and plant vigor.

Materials and Methods

Experimental Design

The experiment was organized in a factorial design with 9 plants grown under each combination of light intensity (4) and photoperiod (2). The experiment was conducted twice over time. At harvest morphological data were collected on all 9 plants per replication. Phytonutrient and mineral data were collected on all 9 plants per replication, except for plants grown at a $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity and a 16-hr photoperiod, which did not provide enough tissue per plant for mineral nutrient extractions.

Plant Production

Seeds of *Brassica carinata* ‘Amara’ greens (Johnny’s Select Seeds, Fairfield, ME) and *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna (Kitazawa Seed, Oakland, CA) were sown in 162-cell bound coco-peat cubes (Preforma plugs; Jiffy Growing Solutions, Lorain, OH) and placed in a walk-in growth chamber (TC2, Environmental Grow Chambers; Chagrin Falls, OH) in a manner similar to Chapter 1. The temperature was $22.7 \pm 0.3^\circ \text{C}$, and the relative humidity was $61.6 \pm 7.3 \%$, both measured and logged every 10 minutes by a shielded temperature and relative humidity sensor (Hobo MX2300; OnSet, Bourne, MA). Trays were placed under broad-spectrum light-emitting diodes (Scorpion Diablo, Horticulture Lighting Group; Maynardville, TN) providing 21:36:43 blue:green:red (%) radiation ratios, with a red:far-red ratio of 13:1, and one of four light intensities applied at one of two photoperiods for a total of 8 treatments (Table 2.1), which were monitored by quantum sensors (SQ-500S; Apogee, Logan, UT). Leaf surface temperature was monitored with ultra-narrow field of view infrared radiometers (SI-131-SS; Apogee, Logan, UT), averaging $21.8 \pm 1.1^\circ \text{C}$ across all treatments (Table 2.1). CO_2 (Industrial Grade Carbon Dioxide CD 50S; Airgas, Knoxville, TN) was injected to provide $2,770 \pm 180 \mu\text{mol}\cdot\text{mol}^{-1}$. Dosing was monitored and controlled by an infrared gas analyzer (LI-850; LI-COR Biosciences, Lincoln, NE) connected to a relay switch controlling an electronic regulator. CO_2 concentration was reported to a Raspberry Pi (Raspberry Pi 4 B; Raspberry Pi, Cambridge, England) every 20 seconds where it was recorded in a CSV file. Seeds were irrigated daily with reverse-osmosis water supplemented with 12N-1.8P-13.4K water-soluble fertilizer providing ($\text{mg}\cdot\text{L}^{-1}$), 100 nitrogen, 15 phosphorus, 112 potassium, 58 calcium, 17 magnesium, 2 sulfur, 1.4

Table 2.1. Lighting treatments, their intensity, photoperiod, and daily light integral (DLI), as provided to the initial growing surface.

Target Light Intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Actual Light Intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Photoperiod (hrs)	Actual Daily Light Integral ($\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	Leaf Temperature (° C)
200	202 ± 10	16	11.6 ± 0.6	21.6 ± 0.8
400	391 ± 24	16	22.5 ± 1.4	21.4 ± 1.0
600	583 ± 40	16	33.6 ± 2.3	21.9 ± 1.2
800	780 ± 30	16	44.9 ± 1.7	22.1 ± 1.5
200	203 ± 11	24	17.5 ± 1.0	21.5 ± 0.6
400	396 ± 16	24	34.2 ± 1.4	22.1 ± 0.9
600	603 ± 25	24	52.1 ± 2.2	22.0 ± 1.0
800	782 ± 38	24	67.6 ± 3.3	22.4 ± 1.1

iron, 0.5 zinc, 0.4 copper and manganese, and 0.1 boron and molybdenum, (RO Hydro FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (MgSO_4) to provide ($\text{mg}\cdot\text{L}^{-1}$) 15 magnesium and 20 sulfur, and adjusted to a pH of 5.8 with H_2SO_4 or KCHO_3 .

After thirteen days the seedlings were transplanted into 18-cm-deep by 61-cm-wide by 122-cm-long deep-water culture hydroponic systems (Premium Flood Table, Active Aqua; Petaluma, CA) with 61 by 122 cm sealed-surface foam rafts (36 ct lettuce raft; Beaver Plastics, Acheson, AB, Canada) and grown for 9 days. The duration of the germination period as well as that of the finishing period were shorter than those seen in Chapter 1, as the higher light and longer photoperiod used in this experiment induced quicker growth, requiring action to prevent overcrowding. The environmental conditions were the same as the seedling stage. Eight systems were filled with 108 L of reverse-osmosis water and supplemented with 12N-1.8P-13.4K water-soluble fertilizer (RO Hydro FeED; JR Peters, Inc.) and MgSO_4 providing twice the concentrations reported during propagation. Electrical conductivity (EC) and pH were monitored (HI9813-6N Portable Waterproof pH/EC/TDS Meter; Hanna Instruments, Woonsocket, RI) and pH was adjusted to 5.8 using H_2SO_4 or KCHO_3 . Air pumps (Active Aqua 110 $\text{L}\cdot\text{min}^{-1}$ commercial air pump; Hydrofarm, Petaluma, CA) and air stones (Active Aqua air stone round 10 $\text{cm} \times 2.5 \text{ cm}$; Hydrofarm, Petaluma, CA) were used to provide dissolved oxygen to the nutrient solution.

Harvest, Morphological Data Collection, and Tissue Processing

Plants were harvested at substrate surface and morphological data were collected. Height from the substrate surface to the tip of the tallest leaf, width at the widest point and perpendicular to the widest point, the number of fully expanded leaves, and fresh mass were collected for 9 plants per replication. After weighing, fresh tissue of 9 plants per treatment was immediately placed in a plastic sample bag and flash frozen in liquid N. As described in Chapter 1, tissue was stored in a -80°C freezer, freeze dried, and homogenized in liquid N. Once homogenized, the tissue was divided into separate aliquots for future extraction procedures and stored in individual 15 mL centrifuge tubes in the -80°C freezer.

Anthocyanin Isolation and Quantification

Total anthocyanins were extracted and analyzed using the method outlined in Chapter 1 derived from Islam et al. (2019). However, 50 mg of tissue were used per sample, instead of the

100 mg used in Chapter 1. Under red light, samples were saturated with 5 mL of 95% ethanol/1.5 N HCl (85:15, v:v), placed on an orbital shaker for 15 minutes, and stored at 4 °C for 24 hrs. Samples were then filtered and analyzed with a microplate reader (Agilent Technologies, Santa Clara, CA).

Carotenoid Isolation and Quantification

The carotenoids β -carotene, lutein, and zeaxanthin were extracted and analyzed using the method described in Chapter 1 and derived from Kopsell et al. (2012). However, we used 50 mg of tissue instead of the 100 mg outlined in Chapter 1. Under red light, chilled samples were extracted in purified water and tetrahydrofuran with an internal carotenoid standard being used to quantify sample loss during homogenization. Samples were homogenized and tetrahydrofuran was used for a second extraction followed by homogenization. The resulting solution was centrifuged, and the eluent was dried down on a stream evaporator. After the addition of acetone, samples were filtered, and an aliquot was stored for subsequent identification and quantification on a 1200 Agilent Series HPLC unit equipped with a diode array detector. A reverse phase C30 column was used with a mobile phase composed of methyl tert-butyl ether, methanol, and triethylamine.

Fat Soluble Vitamin Isolation and Extraction

The fat-soluble vitamin phyloquinone (vitamin K₁) was extracted as outlined in Chapter 1 using a method derived from Pokkanta et al. (2019). Extractions were performed under red light (peak wavelength: 654 nm) and samples were saturated with a series of three solvents (methanol, dichloromethane, and hexane). After being saturated with a solvent, the sample underwent orbital shaking, sonication, and was finally centrifuged. After removing the supernatant, the next solvent in the series was added and the sample again underwent the three previously mentioned procedures. An internal standard was added, and the samples were dried completely, suspended in 5 mL dichloromethane, and filtered. A 1-mL aliquot was then collected for analysis with a 1200 Agilent Series HPLC equipped with a diode array detector, using a reverse phase C18 column and a mobile gradient composed of methanol and water. Phyloquinone was identified at 248 nm.

Mineral Extraction

Nitrogen concentration was quantified via combustion of 0.25 g of tissue and detection with a Thermal Conductivity Sensor (vario MAX cube; Elementar, Ronkonkoma, NY). Other minerals, Ca^{2+} , K^{+} , Mg^{2+} , and Fe^{3+} were extracted and quantified using a method derived from Jones (2001). A 0.5 g aliquot of tissue was dry ashed in a furnace at 500 °C, and then wet ashed by the addition of dilute acid. The resulting solution was then analyzed on an ICP-OES to determine individual mineral concentrations (SPECTROBLUE; Spectro Analytical Instruments Inc., Kleve, Germany). Due to limited tissue production under the $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by 16-hr photoperiod, mineral analysis was not able to be performed on samples produced under this treatment.

Water Soluble Vitamin Isolation and Extraction

The water-soluble vitamins (WSVs) ascorbic acid (vitamin C) and thiamine (vitamin B₁) were extracted as described in Chapter 1 using a method derived from Seal and Chaudhuri (2017) and Sun et al. (2018). However, 200 mg of tissue were used instead of 500 mg. Under red light, chilled samples were hydrated with purified water, hydrochloric acid, and a phosphate buffer. Samples were vortexed and supernatant was filtered. The process was repeated, and a 1 mL aliquot was collected for analysis on a 1200 Agilent Series HPLC equipped with a diode array detector. A reverse phase C18 column was used alongside an aqueous formic acid mobile phase. Both vitamins were detected at 255 nm.

Statistics

Statistical analysis was performed in R (R Core Team, 2013). Data were organized and compiled using the ‘tidyverse’ package (Wickham et al., 2019) and an initial two-way analysis of variance was performed with the ‘stats’ package to determine any interaction between the effect of light-intensity and photoperiod on the 14 parameters listed in Table 2.2 (R Core Team, 2013); when interactions were not present, data were pooled. Linear or quadratic regression were performed with the ‘stats’ package when light intensity or the interaction of light intensity and photoperiod was significantly different (R Core Team, 2013). For final scoring, treatment means of each parameter were calculated, min-max normalized, and multiplied by a weighting value, according to the values given in Table 2.2. Weighted means of each parameter were then added

Table 2.2. List of parameters of interest to NASA, their class, and the multiplier to be applied to the normalized mean. Multipliers were based on previous NASA research and reflect the importance of each parameter to astronaut health on future exploration-length missions (Massa et al, 2015). Negative values indicate undesired plant traits, while magnitude of value indicates importance.

Metric	Category	Multiplier	Justification
Fresh Mass	Morphological	× 2.5	Total phytonutrient content scales with this metric
Dry Matter	Morphological	× 1.0	Higher dry mass proportion is desirable
Plant Volume	Morphological	× -1.5	Smaller plants are desirable due to limited space
Ca	Minerals	× 1.5	Mitigates bone loss in microgravity ^a
K	Minerals	× 2.0	Currently deficient in space diet ^b
Mg	Minerals	× 1.0	Mitigates bone loss in microgravity
Fe	Minerals	× -1.5	Excessive iron can exacerbate bone loss in microgravity ^b
Ascorbic Acid	Water-Soluble Vitamins	× 1.5	Prevent scurvy; degrades quickly ^{c,d}
Thiamine	Water-Soluble Vitamins	× 1.5	Prevent beriberi; degrades quickly ^{c,d}
Phylloquinone	Fat-Soluble Vitamin	× 1.5	Promote proper blood coagulation; deficient in stored space diet ^{b,e}
β-carotene	Carotenoids	× 1.5	Vitamin A precursor; degrades quickly ^{c,f}
Lutein	Carotenoids	× 1.5	Preventing ocular photodamage ^g

Table 2.2. Continued

Metric	Category	Multiplier	Justification
Zeaxanthin	Carotenoids	× 1.5	Preventing ocular photodamage ^g
Total Anthocyanin	Anthocyanins	× 1.0	Potentially beneficial as an antioxidant

a) Flynn, A., The Role of Dietary Calcium in Bone Health. Proceedings of the Nutrition Society. 2003

b) Massa et al., Selection of Leafy Green Vegetable Varieties for a Pick-and- Eat Diet Supplement on ISS. 2015

c) Cooper et al., Initial Assessment of the Nutritional Quality of the Space Food System over Three Years of Ambient Storage. 2017

d) Jukes, T. H., The Prevention and Conquest of Scurvy, Beri-Beri, and Pellagra. 1989

e) Basset et al., Phylloquinone (Vitamin K1): Occurrence, Biosynthesis and Functions. 2017

f) Olson & Hayaishi, The Enzymatic Cleavage of Beta-Carotene into Vitamin A by Soluble Enzymes of Rat Liver and Intestine. 1965

g) Chyalck et al., NASA Study of Cataract in Astronauts (NASCA). Report 1: Cross-Sectional Study of the Relationship of Exposure to Space Radiation and Risk of Lens Opacity. 2009

together per treatment to produce a final score for comparison of total treatment value. Visualizations were created with ggplot (Wickham et al., 2019).

Results and Discussion

'Amara' Greens

Light intensity and photoperiod interacted to influence all parameters of interest besides total anthocyanins and β -carotene concentration (Table 2.3). Total anthocyanin concentrations were not influenced by light intensity nor by photoperiod (Table 2.3). However, β -carotene concentrations decreased linearly from 1.71 to 1.37 $\text{mg}\cdot\text{g}^{-1}$ of dry tissue as light intensity increased from 200 to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 2.1, Table 2.4). The response of both pigments is unexpected, as β -carotene is known to increase in response to increasing light to protect photosynthetic machinery (Kopsell et al., 2012, Frede et al., 2019), while anthocyanin concentrations are also associated with photoprotection (Zhang et al., 2012), and this would suggest an increase in concentrations with increasing light intensity.

The response of all three morphological characteristics to light intensity and photoperiod was interactive, however, the nature of this interaction varied by metric. Fresh mass increased linearly from 15.6 to 53.6 g at a 16-hr photoperiod (Fig. 2.2a, Table 2.4) as light increased from 200 to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This trend did not hold true at a 24-hr photoperiod, where mass initially increased from 26.6 to 65.5 g as light intensity increased from 200 to 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, then decreased to 50.4 g as light intensity increased to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plant volume showed a similar response, increasing from 0.0040 to 0.0060 m^3 at a 16-hr photoperiod as light intensity increased from 200 to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, but reached a peak around 0.0081 m^3 between 400 and 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at a 24-hr photoperiod, and decreased, albeit more sharply than fresh mass, to 0.0039 m^3 at 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 2.2c, Fig. 2.3, Table 2.4). These similarities are expected, as a larger plant will typically have more mass, but the sharper decrease in volume seen at 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is a favorable response for production in space, as it indicates more mass in a smaller production area. These trends are also accompanied by linear increases in dry matter percentage as light increased from 200 to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at both photoperiods, with the increase at 24-hrs, 6.9 to 16.3%, being more prominent than that at 16-hrs, 7.78 to 8.87% (Fig. 2.2e, Table 2.4).

Table 2.3. Two-way ANOVA results to determine presence of interactive effect of light intensity and photoperiod on fourteen parameters of interest in *Brassica carinata* ‘Amara’ greens.

Parameter	Light Intensity		Photoperiod		Light Intensity × Photoperiod	
	P-value	Signif.	P-value	Signif.	P-value	Signif.
Total anthocyanins	0.082	NS	0.202	NS	0.931	NS ^z
β-carotene	< 0.001	***	< 0.001	***	0.127	NS
Lutein	< 0.001	***	< 0.001	***	< 0.001	***
Zeaxanthin	< 0.001	***	< 0.001	***	0.013	*
Phylloquinone	0.124	NS	0.074	NS	0.004	*
Fresh mass	< 0.001	***	< 0.001	***	< 0.001	***
Percent moisture	< 0.001	***	< 0.001	***	< 0.001	***
Plant volume	0.003	*	< 0.001	***	< 0.001	***
Ca ²⁺	0.007	*	0.016	*	< 0.001	***
Fe ³⁺	< 0.001	***	< 0.001	***	< 0.001	***
K ⁺	< 0.001	***	0.002	*	< 0.001	***
Mg ²⁺	0.110	NS	0.952	NS	< 0.001	***
Ascorbic Acid	0.821	NS	0.296	NS	0.032	*
Thiamine	0.645	NS	0.045	*	< 0.001	***

^z***, *, and NS indicate significant at $P \leq 0.001$, $P \leq 0.05$, or nonsignificant, respectively.

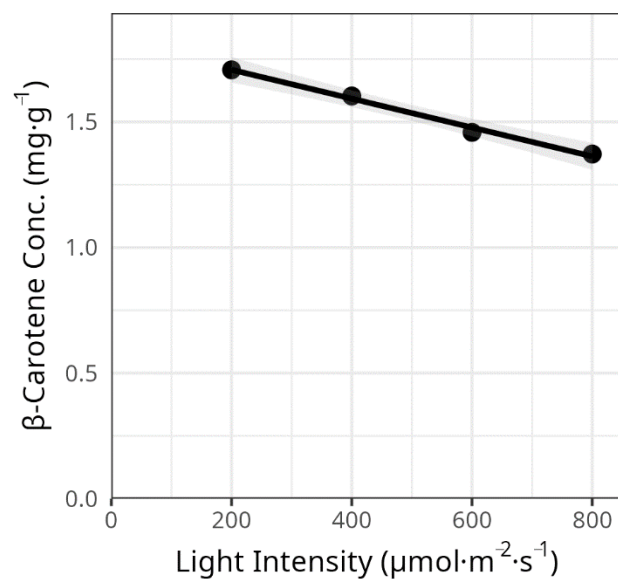


Figure 2.1. Light intensity effect on *Brassica carinata* ‘Amara’ greens β -carotene concentrations. Means are represented by black points and a vertical line represents the standard error. Lines represent regression predictions with a grey confidence interval, with the coefficients presented in Table 2.4.

Table 2.4. Regression equations describing the response of various aspects of *Brassica carinata* ‘Amara’ greens to light intensity. Parameters significant at the $\alpha = 0.05$ level are listed, along with the photoperiod, regression equation coefficients, and the r^2 .

Parameter	Photoperiod	a	b	c	R ² or r ²
β -carotene conc. (mg·g ⁻¹)	Combined	1.83 ^y (3.73E-2) ^z	-5.75E-4 (6.93E-5)		0.343
Fresh mass (g)	16-hr	4.63 (2.99)	5.64E-2 (5.47E-3)		0.604
Fresh mass (g)	24-hr	-1.99E1 (1.33E1)	2.68E-1 (5.59E-2)	-2.23E-4 (5.81E-5)	0.303
Ascorbic acid conc. (mg·g ⁻¹)	24-hr	5.39 (3.09)	3.25E-2 (1.43E-2)	-2.86E-5 (1.42E-5)	0.066
Dry matter (%)	16-hr	7.64 (6.01E-1)	3.49E-5 (1.09E-3)		0.123
Dry matter (%)	24-hr	3.15E-2 (9.12E-1)	1.52E-4 (1.65E-3)		0.551
Thiamine conc. (mg·g ⁻¹)	16-hr	8.27E-3 (1.38E-3)	-2.23E-5 (6.38E-6)	2.29E-8 (6.30E-9)	0.139
Thiamine conc. (mg·g ⁻¹)	24-hr	-6.19E-4 (1.53E-3)	2.85E-5 (7.01E-6)	-2.99E-8 (6.95E-9)	0.210
Plant volume (m ³)	16-hr	3.65E-3 (5.05E-4)	2.38E-6 (9.24E-7)		0.074
Plant volume (m ³)	24-hr	1.31E-3 (1.66E-3)	3.18E-5 (7.58E-6)	-3.55E-8 (7.49E-9)	0.285
Phylloquinone conc. (mg·g ⁻¹)	16-hr	5.22E-1 (1.97E-1)	1.28E-3 (3.52E-4)		0.161
Phylloquinone conc. (mg·g ⁻¹)	24-hr	2.01 (3.07E-1)	-1.19E-3 (5.77E-4)		0.506
Ca conc. (mg·g ⁻¹)	24-hr	2.15 (1.14E-1)	-7.16E-4 (2.11E-4)		0.166
Fe conc. (μg·g ⁻¹)	24-hr	1.10E2 (5.29)	-7.41E-2 (9.93E-3)		0.513

Table 2.4. Continued

Parameter	Photoperiod	a	b	c	R ² or r ²
K conc. (mg·g ⁻¹)	24-hr	6.73 (3.22E-1)	-4.74E-3 (5.98E-4)		0.539
Lutein conc. (mg·g ⁻¹)	16-hr	1.52 (3.82E-2)	-7.14E-4 (7.34E-5)		0.579
Lutein conc. (mg·g ⁻¹)	24-hr	1.52 (4.44E-2)	-1.08E-3 (7.85E-5)		0.740
Zeaxanthin conc. (mg·g ⁻¹)	16-hr	7.33E-1 (5.02E-2)	-3.30E-4 (9.52E-5)		0.149
Zeaxanthin conc. (mg·g ⁻¹)	24-hr	3.79E-1 (3.34E-2)	-2.37E-4 (5.92E-5)		0.188
^y Coefficients for model equations used to generate Figs. 2.1-2.5					
Linear: $y = a + bx$					
Quadratic: $y = a + bx + cx^2$					
^z (Standard Error)					

Vitamin concentrations also showed conflicting trends between a 16-hr and 24-hr photoperiod. Across a 24-hr photoperiod ascorbic acid increased quadratically from $10.8 \text{ mg}\cdot\text{g}^{-1}$ at $200 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to a peak concentration of around $14.9 \text{ mg}\cdot\text{g}^{-1}$ near $600 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while concentrations did not respond significantly to light intensity when applied across a 16-hr photoperiod (Fig. 2.2b, Table 2.4). Ascorbic acid plays a role in cell division and elongation (Arrigoni, 1994) and scavenging reactive oxygen species (ROS) (Smirnoff & Wheeler, 2000), both of which would be expected to increase with increasing light intensity, due to increased growth in larger plants and increased light stress, respectively. The fact that, when grown under a 24-hr photoperiod, ascorbic acid concentration tapers off in a trend similar to plant volume indicates that cell elongation and division may play a role in our observed ascorbic acid response.

Thiamine concentrations responded quadratically in response to increases in light intensity at both 16 and 24-hr photoperiods, with concentrations reaching a trough between 400 and $600 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ near $0.0035 \text{ mg}\cdot\text{g}^{-1}$ at a 16-hr photoperiod and reaching a peak near $0.0069 \text{ mg}\cdot\text{g}^{-1}$ at the 24-hr photoperiod within the same irradiation range (Fig. 2.2d, Table 2.4). Other studies have shown an increase in thiamine with increasing light intensity (Tunc-Ozdemir et al., 2009), and the more complex response seen in this study may have to do with the high CO_2 concentrations, which have previously been shown to induce very high starch concentrations (Levine et al., 2008), potentially diluting other metabolite concentrations. Phylloquinone concentrations increased linearly from 0.86 to $1.49 \text{ mg}\cdot\text{g}^{-1}$ at a 16-hr photoperiod, while they decreased linearly from 1.97 to $1.15 \text{ mg}\cdot\text{g}^{-1}$ when grown under a 24-hr photoperiod (Fig. 2.2e, Table 2.4). Phylloquinone serves as a cofactor in the electron transport chain of PSI (Kozuleva et al., 2021), and as such would be expected to decrease with increasing light intensity, as photosynthetic machinery is downregulated with excessive light. While this was observed under the 24-hr photoperiod, the opposite was observed under the 16-hr period, and this may indicate that the daily light levels were not yet sufficient to induce a reduction in photosynthetic apparatus.

The two carotenoids of interest (lutein and zeaxanthin) decreased linearly with increasing light intensity under both photoperiods, although they exhibited differing rates of decline (Fig. 2.4, Table 2.4). Lutein decreased from 1.37 to $0.97 \text{ mg}\cdot\text{g}^{-1}$ under a 16-hr photoperiod, while

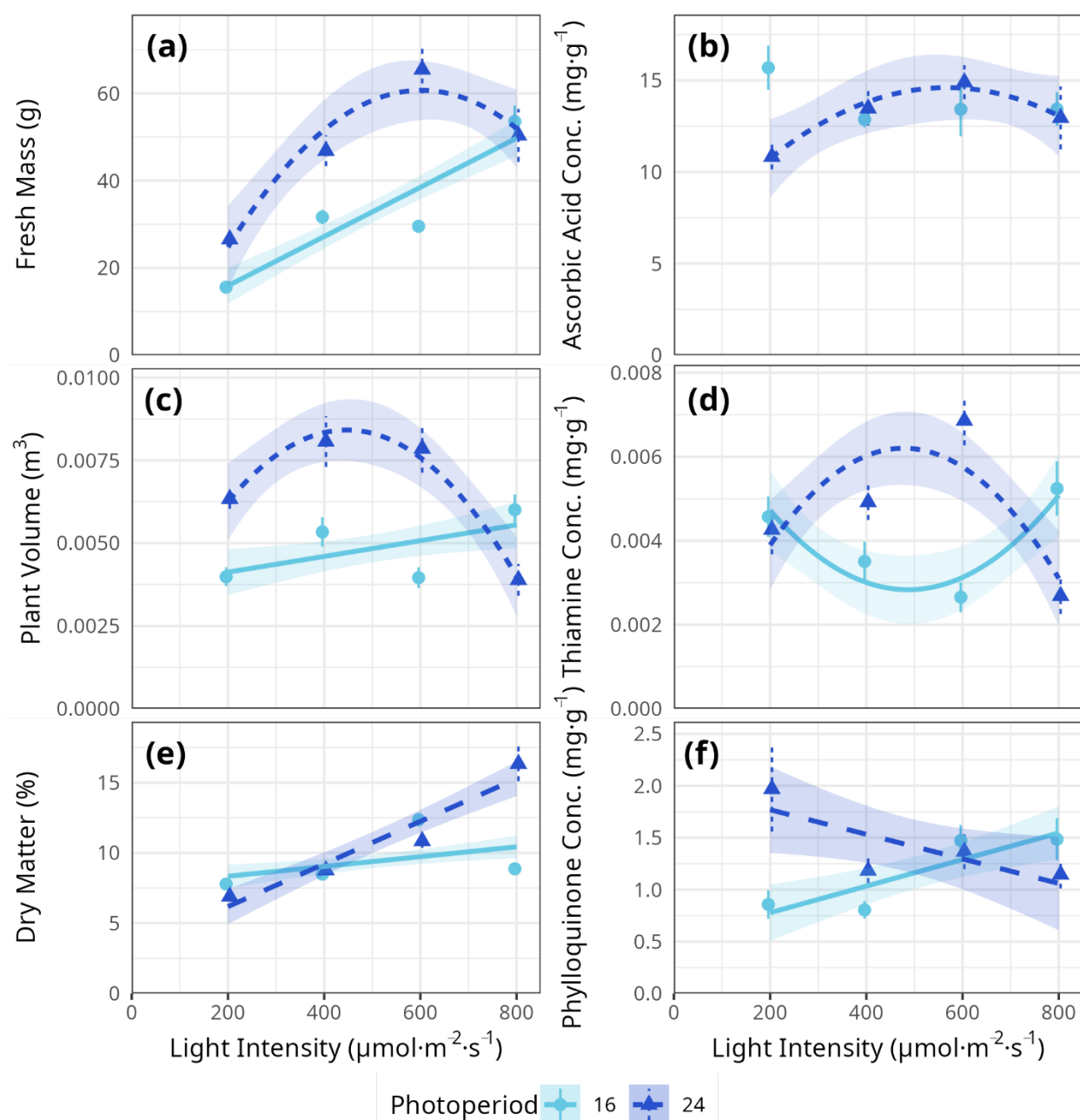


Figure 2.2. Light intensity and photoperiod effect on *Brassica carinata* 'Amara' greens morphological parameters and vitamin concentrations including a) fresh mass, b) ascorbic acid, c) plant volume, d) thiamine, e) dry matter percent, and f) phyloquinone. Means are represented by triangles (24-hr) or dots (16-hr) and a vertical line represents the standard error. Lines represent regression predictions with a shaded confidence interval, with coefficients presented in Table 2.4.

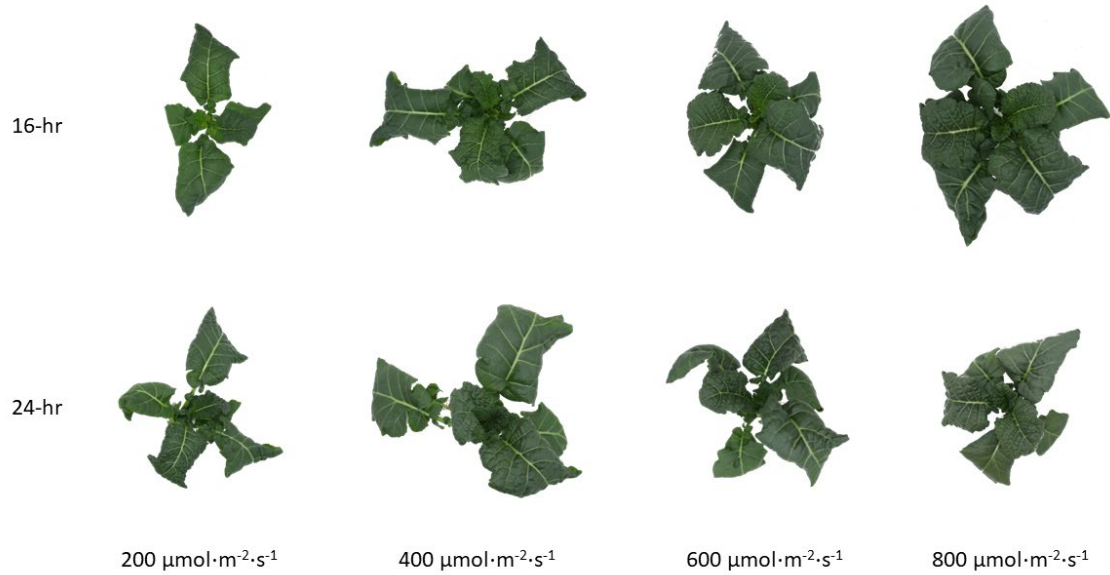


Figure 2.3. Photographs of representative *Brassica carinata* 'Amara' greens plants grown under a 16- or 24-hr photoperiod and light intensities of 200, 400, 600, or 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

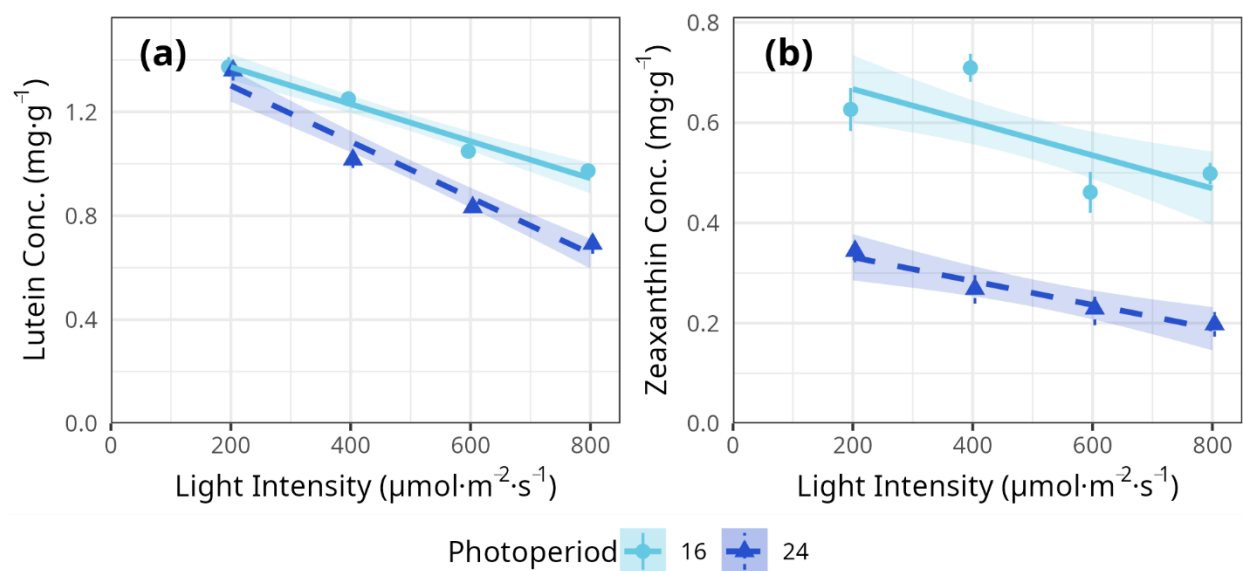


Figure 2.4. Light intensity and photoperiod effects on *Brassica carinata* 'Amara' greens a) lutein, and b) zeaxanthin. Means are represented by triangles (24-hr) or dots (16-hr) and a vertical line represents the standard error. Lines represent regression predictions with a shaded confidence interval, with coefficients presented in Table 2.4.

under a 24-hr photoperiod, it declined more sharply, falling from 1.36 to 0.69 mg·g⁻¹. On the other hand, zeaxanthin showed similar rates of decline at both photoperiods, but the 24-hr photoperiod produced lower concentrations at all light intensities. Both carotenoids serve a role in chlorophyll triplet quenching (Dall'Osto et al., 2006; Dall'Osto et al., 2012), the formation of which would increase at higher light intensities. The decrease in concentration of both pigments may point towards a diluting effect, similar to our thiamine concentration hypothesis described previously. This would also help explain the decrease in β -carotene concentration with increasing light intensity and could be supported by the increase in dry matter percentage, if that dry matter was composed increasingly of large molecular mass products of primary photosynthesis such as starches. Further study is needed to test this hypothesis.

Mineral data may provide more insight into the possibility of a dilution effect, as the uptake of Ca is largely passive, and is thus less impacted by confounding factors such as free radical presence or the up- and down-regulation of photosynthetic machinery, being driven primarily by transpiration rates. A linear decrease in Ca with increasing light intensity was observed under the 24-hr photoperiod, falling from 1.89 to 1.43 mg·g⁻¹ (Fig. 2.5a, Table 2.4). This decrease, alongside the increase in dry matter percentage under the same conditions (Fig. 2.2e), supports the idea that some of the declines in concentration seen under a 24-hr photoperiod are potentially due in part to a dilution effect and not entirely due to up- or down-regulation of the photosystems and their protective apparatus. The concentrations of Fe and K also decreased linearly under the 24-hr photoperiod with Fe decreasing from 98.5 to 50.7 μ g·g⁻¹ (Fig. 2.5b, Table 2.4) and K decreasing from 5.70 to 2.77 mg·g⁻¹ (Fig. 2.5c, Table 2.4). While the light intensity by photoperiod interaction was significant for Mg concentrations, there was no significant regression response.

Regardless of the mechanism behind these trends, the results still provide valuable insights into the optimal lighting conditions to produce *B. carinata* 'Amara' for dietary supplementation during space travel. Once mean parameters were normalized, weighted according to Table 2.2, and summed, the resulting scores indicate that the highest light intensity (800 μ mol·m⁻²·s⁻¹) applied for 16 hrs per day provides the best combination of traits for use in the space diet (Fig. 2.6). As supplemental lighting accounts for a large portion of the energy used in indoor production, alternative treatments may be desirable from an energy-use viewpoint, and

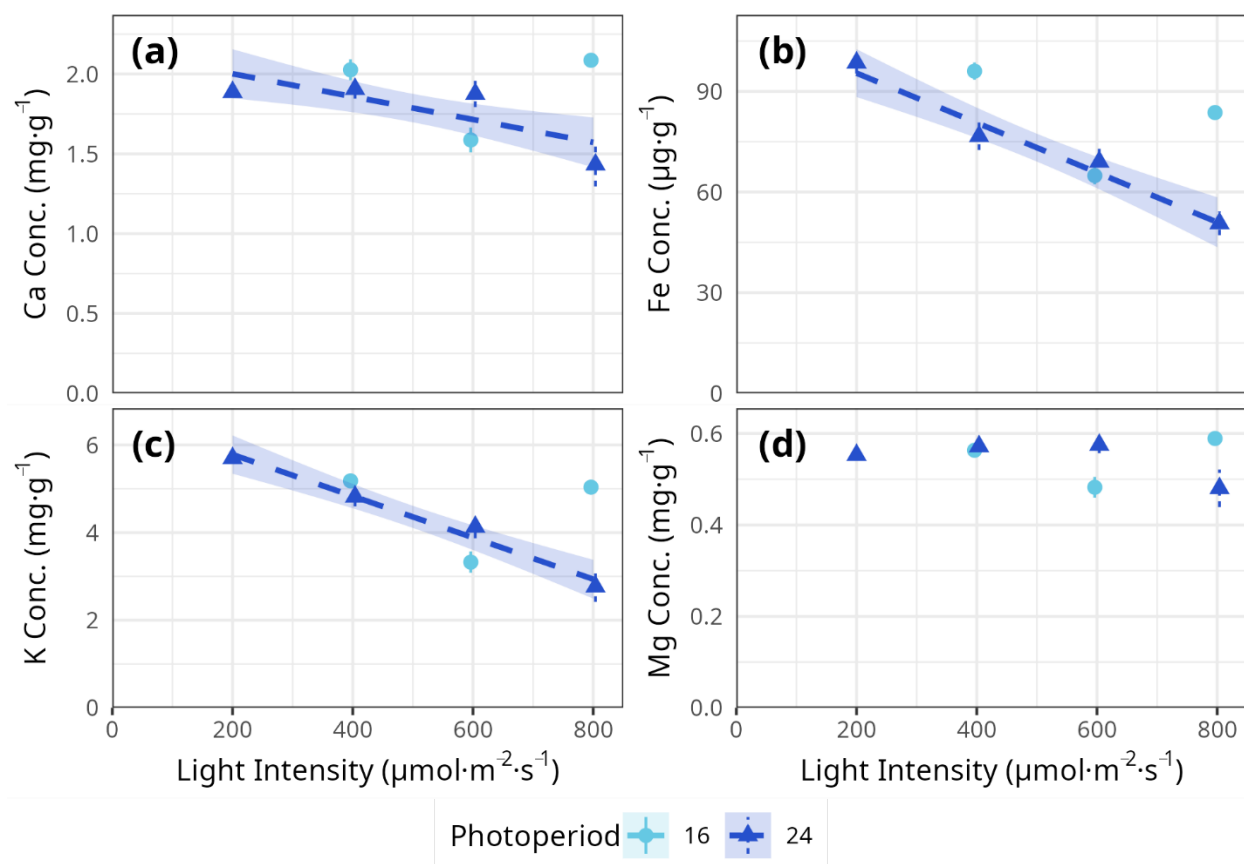


Figure 2.5. Light intensity and photoperiod effect on *Brassica carinata* 'Amara' greens a) calcium, b) iron, c) potassium, and d) magnesium. Means are represented by triangles (24-hr) or dots (16-hr) and a vertical line represents the standard error. Lines represent regression predictions with a shaded confidence interval, with coefficients presented in Table 2.4.

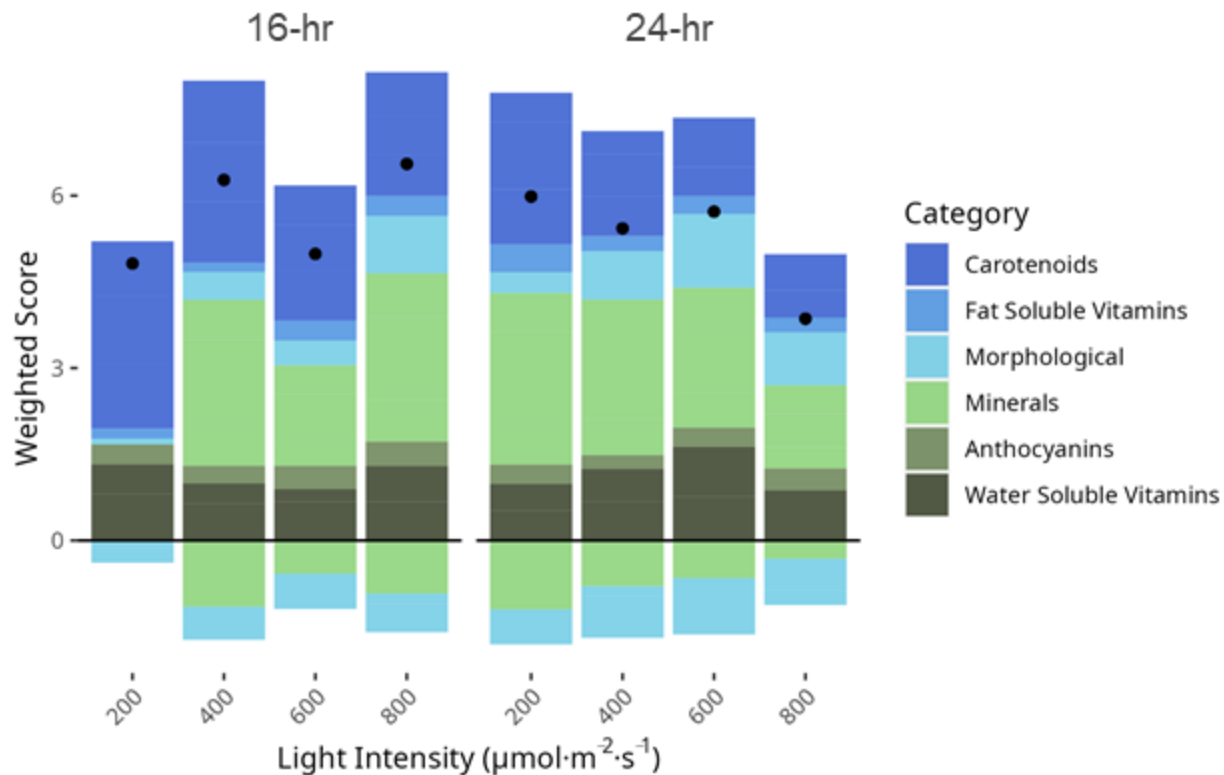


Figure 2.6. Final scores for each treatment combination, calculated by normalizing the means of each parameter of interest for *Brassica carinata* ‘Amara’ greens, multiplying by the weighting factor in Table 2.2, and summing the results from all 14-parameters into a total score.

Components of the score visualized under the 0-line are negatively weighted while those above the line are positive. The black dot represents the total score, or the negative components subtracted from the positive.

the performance of a $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity under a 24-hr photoperiod is also strong, while utilizing less energy. Additionally, due to a lack of sufficient tissue produced for mineral extractions at a $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity under a 16-hr photoperiod, this treatment ranks quite poorly, but it ranks highest for ascorbic acid, lutein, and β -carotene concentrations, and second highest for zeaxanthin concentrations. If energy use is a concern, a $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity can still be utilized to produce nutritious ‘Amara’ plants.

Providing a 16-hr photoperiod and $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light, the average ‘Amara’ plant provided 53.6 g of fresh mass, requiring 1.4 plants, in 0.006 m^3 of production space per plant for 22 days, to provide the daily serving of 75 g fresh mass. This combination of light intensity and photoperiod provides a resulting crop of ‘Amara’ which, per serving (75 g fresh mass) produces 98% of the recommended daily intake (RDI) of ascorbic acid, 29% RDI of thiamine, all the recommended phyloquinone, and 90% RDI of vitamin A (calculated at a 12:1 β -carotene:retinol equivalency) (Haskell, 2012). The mineral supplementation is less impressive, providing per serving: 1.4% RDI Ca, 1.1% RDI K, and 0.9% RDI Mg, which are welcome, albeit minimal additions to the current freeze-dried diet. However, this is not a weakness of the treatment effect, rather a limitation of utilizing ‘Amara’ greens for mineral supplementation.

‘Red Hybrid’ Mizuna

While many of the parameters tested displayed an interactive effect between light intensity and photoperiod, ascorbic acid, total anthocyanin, phyloquinone, and magnesium concentrations did not (Table 2.5). Additionally, while total anthocyanin concentrations decreased linearly from 11.9 to $7.2 \text{ mg}\cdot\text{g}^{-1}$ with increasing light intensity (Fig. 2.7a, Table 2.6), ascorbic acid, phyloquinone, and magnesium concentrations did not respond significantly to changes in light intensity or photoperiod (Table 2.5). Anthocyanins are known to help attenuate the negative effects of increasing light concentrations, and thus plants are expected to produce more anthocyanins under high irradiance (Chalker & Scott, 1999). The decrease in concentration seen in this study may be due to the previously described dilution effect, in which primary photosynthetic products (sugars, starches, cellulose, etc.) are produced in higher amounts due to the elevated CO_2 , accounting for a higher percentage of the dry mass than under normal conditions. Additionally, elevated CO_2 allows the photosynthetic light response curve to plateau

Table 2.5. Two-way ANOVA results to determine presence of interactive effect of light intensity and photoperiod on fourteen parameters of interest in *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna.

Parameter	Intensity		Photoperiod		Intensity × Photoperiod	
	P-value	Signif.	P-value	Signif.	P-value	Signif.
Total anthocyanins	< 0.001	***	< 0.001	***	0.198	NS ^z
β-carotene	< 0.001	***	< 0.001	***	0.032	*
Lutein	< 0.001	***	< 0.001	***	0.002	*
Zeaxanthin	< 0.001	***	0.172	NS	< 0.001	***
Phylloquinone	0.155	NS	0.112	NS	0.075	NS
Fresh mass	< 0.001	***	< 0.001	***	< 0.001	***
Percent moisture	< 0.001	***	0.001	***	< 0.001	***
Plant volume	0.048	*	< 0.001	***	< 0.001	***
Ca ²⁺	< 0.001	***	< 0.001	***	< 0.001	***
Fe ³⁺	< 0.001	***	< 0.001	***	0.022	*
K ⁺	< 0.001	***	0.042	*	< 0.001	***
Mg ²⁺	0.110	NS	0.356	NS	0.072	NS
Ascorbic Acid	0.508	NS	0.501	NS	0.242	NS
Thiamine	0.987	NS	0.196	NS	0.001	***
^z ***, *, and NS indicate significant at $P \leq 0.001$, $P \leq 0.05$, or nonsignificant, respectively.						

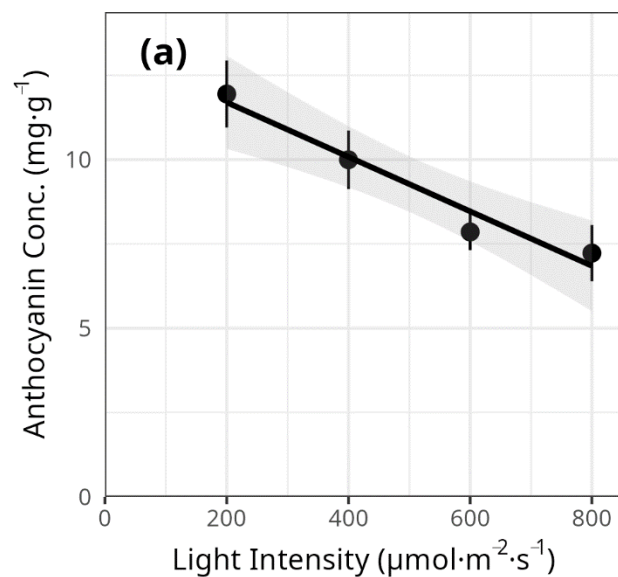


Figure 2.7. Light intensity effect on *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna total anthocyanin concentration. Means are represented by black points and a vertical line represents the standard error. Lines represent regression predictions with a grey confidence interval, with the coefficients presented in Table 2.6.

Table 2.6. Regression equations describing the response of various aspects of *B. rapa* var. *nipposinica* ‘Red Hybrid’ greens to light intensity. Parameters significant at the $\alpha = 0.05$ level are listed, along with the photoperiod, regression equation coefficients, and the r^2 .

Parameter	Photoperiod	a	b	c	R ²
Total anthocyanin	Combined	13.31 ^y (1.01) ^z	-8.08E-03 (1.82E-03)		0.122
Fresh mass	16-hr	6.63 (2.12)	5.73E-02 (3.88E-03)		0.756
Fresh mass	24-hr	-6.56 (1.02E+01)	2.26E-01 (4.63E-02)	-1.91E-04 (4.56E-05)	0.321
Ca ²⁺	24-hr	2.03 (7.29E-02)	-5.57E-04 (1.39E-04)		0.236
Dry matter (%)	16-hr	6.99 (3.21E-01)	3.41E-03 (5.83E-04)		0.325
Dry matter (%)	24-hr	4.41 (6.24E-01)	1.02E-02 (1.13E-03)		0.545
K ⁺	24-hr	6.92 (2.88E-01)	-4.09E-03 (5.42E-04)		0.523
Plant volume	16-hr	4.94E-03 (5.44E-04)	3.51 (9.95E-07)		0.140
Plant volume	24-hr	1.15E-02 (9.24E-04)	-5.02E-06 (1.67E-06)		0.104
Fe ³⁺	24-hr	71.74 (2.91)	-3.53E-02 (5.70E-03)		0.438
Thiamine	24-hr	6.66E-04 (1.39E-03)	1.57E-05 (6.35E-06)	-1.60E-08 (6.25E-09)	0.066
β-carotene	16-hr	1.72 (4.47E-02)	-4.01E-04 (8.05E-05)		0.278
β-carotene	24-hr	1.66 (6.68E-02)	-5.34E-04 (1.26E-04)		0.211
Lutein	16-hr	1.21 (3.46E-02)	-6.14E-04 (6.31E-05)		0.595
Lutein	24-hr	1.12 (3.99E-02)	-6.80E-04 (7.24E-05)		0.565
Zeaxanthin	24-hr	-2.20 (9.36E-02)	9.07E-04 (1.65E-04)		0.325

^y Coefficients for model equations used to generate Figs. 2.7-2.9.

Linear: $y = a + bx$

Quadratic: $y = a + bx + cx^2$

^z(Standard Error)

at a higher light intensity than what would be expected under atmospheric CO₂ conditions, producing more primary metabolites than would otherwise be possible at these higher light intensities. A similar effect has been seen with the dilution of chlorophyll by increased primary metabolites, both structural and non-structural, in *Brassica juncea* when grown under elevated CO₂ concentrations (Upreti & Mahalaxmi, 2000). However, further research would be required to test this hypothesis against plants grown under atmospheric CO₂, as all treatments in the current study occurred at elevated concentrations. Ascorbic acid concentrations were approximately 9 mg·g⁻¹ across all treatments, which was lower than the concentrations found in *Brassica carinata* ‘Amara’ greens, where ascorbic acid concentrations were all above 10 mg·g⁻¹ and were as high as 15 mg·g⁻¹. (Fig. 2.2b). Magnesium concentrations did not respond to differing light intensities, which was also seen in the response of ‘Amara’ greens (Fig. 2.5d, Table 2.3).

Other minerals, as well as all morphological traits quantified, exhibited interactions in their response to light intensity and photoperiod. Fresh mass (Fig. 2.8a, Table 2.6) increased in response to increasing light intensity at both photoperiods, although this increase tapered off after reaching 60 g at 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and decreased to 53 g at 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, similar to the mass of 52 g produced at 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. At each tested light intensity, a 24-hr photoperiod produced higher fresh mass than that of a 16-hr photoperiod, with the exception of the highest light intensity where the 16-hr photoperiod produced more mass. Dry matter percentage increased linearly with increasing light at both photoperiods, although the response was stronger at the 24-hr photoperiod (Fig. 2.8c, Table 2.6). Plant volume also increased linearly with increasing light intensity at a 16-hr photoperiod but decreased linearly when grown at a 24-hr photoperiod (Fig. 2.8e, Fig 2.9, Table 2.6). The increase in mass despite a decrease of required production space is a favorable combination of factors for production in space, and this combination of features is attainable with a high light intensity applied across a 24-hr photoperiod.

With the exception of the previously mentioned magnesium, all minerals show a linear decrease in concentration with increasing light intensity when light is applied at a 24-hr photoperiod. This was most apparent in the response of potassium concentration (Fig. 2.8b, Table 4) which decreased by 39% when light was increased from 200 to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

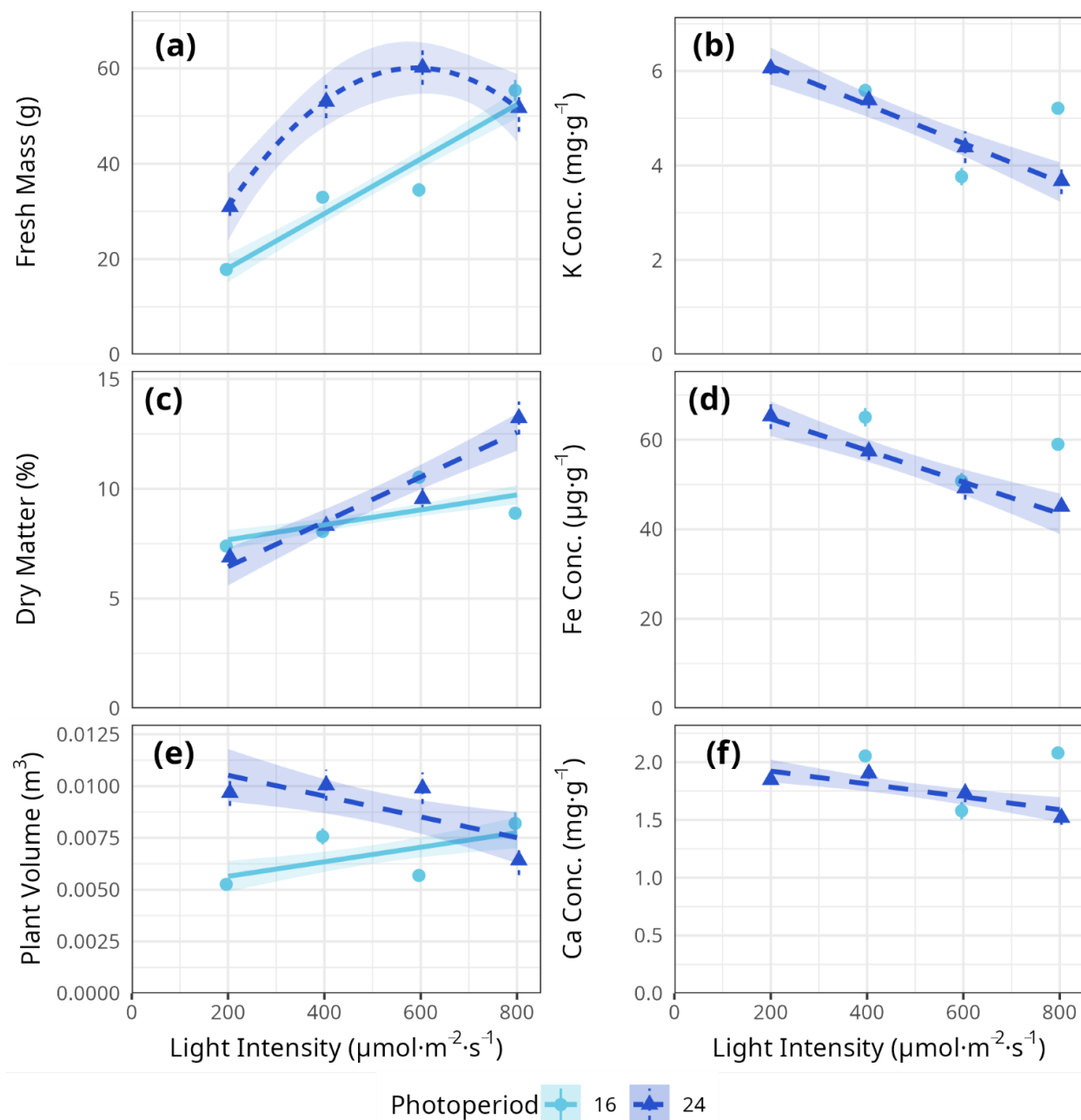


Figure 2.8. Light intensity and photoperiod effect on morphological parameters and mineral concentrations of *Brassica rapa* var. *nipposinica* 'Red Hybrid' mizuna including a) fresh mass, b) calcium, c) dry matter, d) potassium, e) plant volume, and f) iron. Means are represented by triangles (24-hr) or dots (16-hr) and a vertical line represents the standard error. Lines represent regression predictions with a shaded confidence interval, with coefficients presented in Table 2.6.

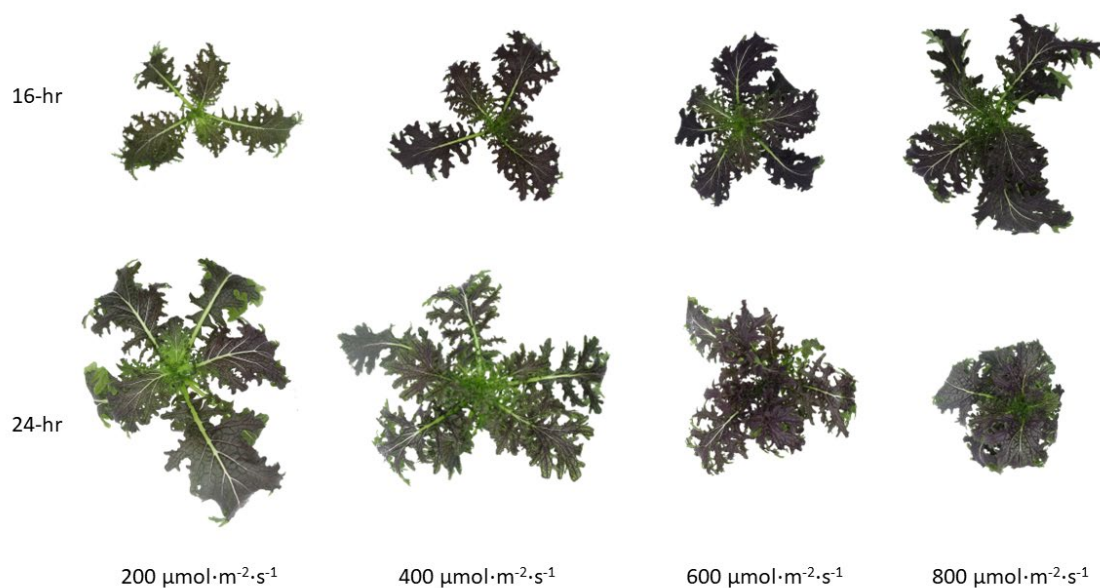


Figure 2.9. Photographs of representative *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna greens plants grown under a 16- or 24-hr photoperiod and light intensities of 200, 400, 600, or 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Across the same lighting range iron concentrations decreased by 31% (Figure 2.8d, Table 2.6) and calcium decreased by 18% (Fig. 2.8f, Table 2.6). The general decrease in mineral concentrations with increasing light could be explained by the same dilution effect mentioned regarding anthocyanin pigments (Upreti & Mahalaxmi, 2000), however, all four minerals quantified in this study are at least partially incorporated into the plant by passive uptake. As passive uptake is driven by transpiration which occurs prominently during the photoperiod, it would be logical to assume that plants grown at a 24-hr photoperiod would contain higher concentrations of passively incorporated minerals. This was not seen (Fig. 2.8bdf) and the opposite was true at the highest light intensity. Previous studies have found that super-elevated CO₂ concentrations can prevent nighttime stomatal closure (Levine et al., 2008) and this could partially explain the failure of the 24-hr treatment to produce plants with higher mineral concentrations, although it does not explain why the 16-hr treatment did not experience a similar decline at higher light intensities.

Thiamine (vitamin B₁) responses increased from 0.003 to 0.005 mg·g⁻¹ as light increased from 200 to 600 μmol·m⁻²·s⁻¹ but decreased back to 0.003 mg·g⁻¹ as light increased further (Fig. 2.10a, Table 2.6). Thiamine helps plants to respond to oxidative stress (Tunc-Odzemir et al., 2009) and an upregulation in antioxidant compounds is typically seen in response to increasing light intensity. The decrease in thiamine after 600 μmol·m⁻²·s⁻¹ could be due to a variety of factors, including metabolic burnout under high daily light integral and high CO₂ (Reuvini & Bugbee, 1997) or dilution due to starch accumulation.

Carotenoids are also utilized in plant response to high light stress (secondary function), in addition to participating in the photosynthetic machinery (primary function). β-carotene (Fig. 2.10b, Table 2.6) and lutein (Figure 2.10c, Table 2.6) both decreased linearly in response to increasing light and produced lower concentrations at a 24-hr photoperiod. β-carotene (Croce et al., 2002) and lutein (Dall'Osto et al., 2006; Croce et al., 2002) are both required for proper function of the antennae complexes of higher plants, both in gathering light and mitigating the impact of excessive photon exposure, and in leaf tissue are primarily concentrated in PSI and PSII. Zeaxanthin, while also a part of the photosystem, is also found in a secondary role in the lipid bilayer of the photosynthetic membrane (Havaux & Garcia-Plazaola, 2014). This secondary location and function of zeaxanthin could explain its sharp increase at high light at a 24-hr

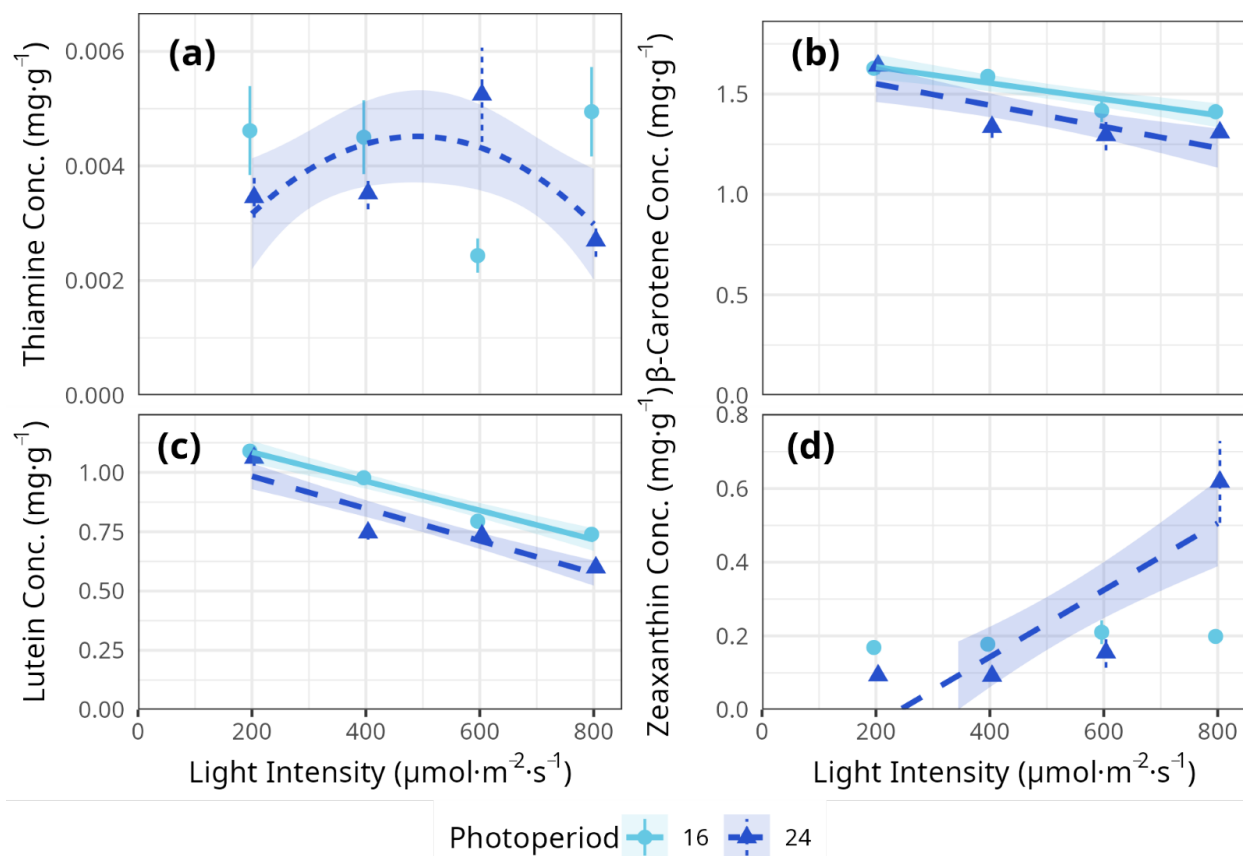


Figure 2.10. Light intensity and photoperiod effect on phytonutrient concentrations of *Brassica rapa* var. *nipposinica* 'Red Hybrid' mizuna including a) thiamine, b) β -carotene, c) lutein, and d) zeaxanthin. Means are represented by triangles (24-hr) or dots (16-hr) and a vertical line represents the standard error. Lines represent regression predictions with a shaded confidence interval, with coefficients presented in Table 2.6.

photoperiod (Figure 2.10d, Table 2.6), an increase that did not occur in carotenoids which are primarily tied to the photosystems in leaf tissue.

Again, parameter means were normalized and weighted according to Table 2.2, and the resulting weighted means were summed by treatment to determine the optimal conditions for producing ‘Red Hybrid’ under ISS-like conditions. As in the case of ‘Amara’, we found that plants grown at a light intensity of $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity at a 16-hr photoperiod provided the best combination of traits for supplementing the astronaut diet (Fig. 2.11). As mentioned previously, energy constraints may make other treatments preferable, particularly the application of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity at a 24-hr photoperiod. While this treatment does not produce ‘Red Hybrid’ plants with as high of fresh mass, it still maintains high carotenoid and mineral concentrations.

The average ‘Red Hybrid’ plant grown at $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity at a 16-hr photoperiod requires 1.4 plants grown for 22 days in 0.008 m^3 of production space per plant to produce 75 g of fresh biomass yield. A 75 g serving of ‘Red Hybrid’ produced under these conditions shows similar trends to that of ‘Amara’, providing all the necessary phyloquinone, 63, 2.7, and 97% of ascorbic acid, thiamine, and vitamin A precursor respectively. Again, mineral nutrients are represented, but minimal, providing 1.1, 1.3, 0.9, and 4.9% of Ca, K, Mg, and Fe (RDI).

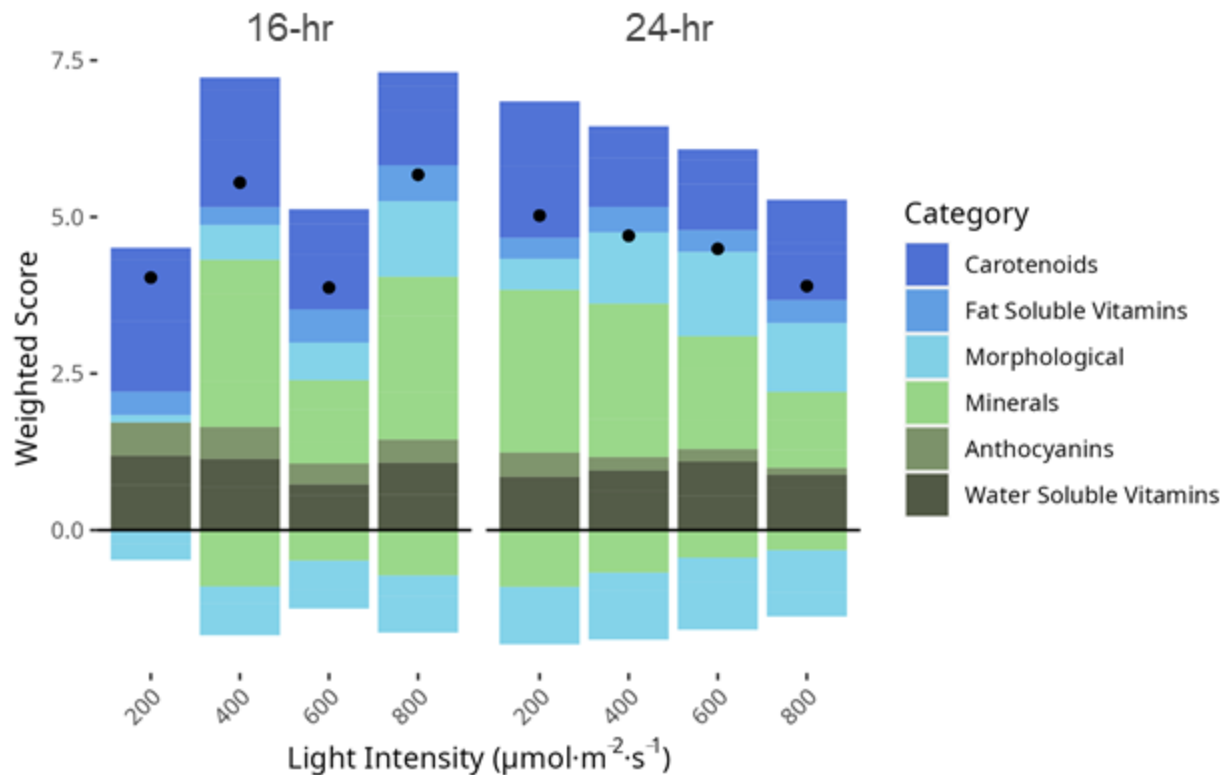


Figure 2.11. Final scores for each treatment combination, calculated by normalizing the means of each parameter of interest for *Brassica rapa* var. *nipposinica* ‘Red Hybrid’, multiplying by the weighting factor in Table 2.2, and summing the results from all 14-parameters into a total score. Components of the score visualized under the 0-line are negatively weighted while those above the line are positive. The black dot represents the total score, or the negative components subtracted from the positive.

CHAPTER 3:

TEMPORAL CHANGES IN BRASSICA RAPA VAR. NIPPOSINICA PHYTONUTRIENT CONCENTRATION AND BIOMASS YIELD DUE TO SUCCESSIVE HARVEST METHOD

Abstract

Astronaut health and subsequent mission success depend on access to a full range of vitamins, something lacking in the current space diet. As sustainable food production systems are developed for use during long space flights, the utilization of continuous harvest (cut-and-come-again) methods can play a role in efficient use of limited space and resources. However, it is unknown how successive harvests will affect the desired phytonutrient profile over time. This study was designed to determine the optimal indicator of harvest readiness (time vs. development) for peak phytonutrient production, and to determine the effect of 5 consecutive harvests on phytonutrient concentrations and biomass yield of *Brassica rapa* var. *nipposinica* ‘Red Hybrid’. Seeds were sown in a growth chamber and transplanted after 14 days into raft deep-water culture systems. A target air temperature of 23°C and a CO₂ concentration of 2,800 $\mu\text{mol}\cdot\text{mol}^{-1}$ were set to emulate space-station conditions, and 200 or 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light were applied at a 16-hr photoperiod. Nutrient solution was composed of a 12.0N-1.7P-13.3K complete fertilizer supplemented with MgSO₄, and provided 100 or 200 $\text{mg}\cdot\text{L}^{-1}$ N during propagation and raft production, respectively. After 10 days (time) or 8 leaves were produced (development), leaves that extended 3.5-cm above the substrate surface were harvested. Harvests were repeated cyclically to achieve 5 harvests per plant. This was conducted twice in time. Tissue morphology and phytonutrient concentration were evaluated. Harvest interval (time or development) was compared to determine optimal crop-cycle length and harvest methodology for consistent nutritious vegetable production.

Introduction

Plants have been recognized as a critical component of future exploration-length space missions, both aboard spacecraft and during planetary colonization, due to their utility in recycling waste CO₂, producing O₂, and providing steady source of food (Galston, 1992). More recently, as nutritional deficits have been recognized in the current freeze-dried space diet (Cooper et al., 2017), plants again offer a solution by providing a fresh source of non-shelf-stable vitamins, ascorbic acid (vitamin C) and thiamine (vitamin B₁). Additionally, plants provide beneficial phytonutrients including carotenoids and anthocyanins, which have been associated with protection against radiation-induced cell damage (Krinsky et al., 2003, Kuntz et al., 2014).

Leafy greens have been studied for use in space more than fruiting crops (Johnson et al., 2021), likely due to their compact size, quick production cycle, and high proportion of edible biomass, however, their efficiency can be further improved with a ‘cut-and-come-again’, or continuous harvest, production method. In contrast to a typical harvest in which the entire plant is removed from the substrate, a cut-and-come-again approach involves removing only a portion of the plant and allowing for regrowth of new tissue, taking advantage of the linear growth phase and avoiding the slow growth of a new plant during germination (Richards, 1959). This method would allow for more efficient use of space, time, resources, and energy. NASA has conducted research on this method of production, with results showing higher biomass yield over time in *B. carinata* ‘Amara’ greens and *Glebionis coronaria* ‘Shungiku’ greens as well as higher microbial growth, particularly in the case of *G. coronaria* (Bunchek et al., 2021). However, it is unknown how phytonutrient concentrations, a primary reason for growing these plants in space, change with each subsequent harvest when grown under the conditions of the International Space Station (ISS).

Brassica rapa var. *nipposinica* (mizuna) is another leafy green that NASA has tested in their Veggie system, as well as in earth-based studies. It performs well in the environment of the ISS and provides a favorable nutritional and organoleptic profile (Massa et al., 2015). However, it is likely that concentrations of these nutritional compounds will change with subsequent harvests, whether as part of a plant herbivory response, a general stress response, or simply due to different proportions of tissue after regrowth. It is also possible that plant response will be dependent on environmental conditions, as the stress of multiple harvests could be compounded by an already stressful environment, or mediated by one that is supportive. Antioxidant compounds such as ascorbic acid, thiamine, carotenoids, and anthocyanins are particularly sensitive to increased plant stress and are likely to change over sequential harvests. This study aims to determine how biomass yield and phytonutrient concentrations of *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna change over 5 sequential harvests, and to determine the effect harvest method and environmental light intensity have on this response.

Materials and Methods

Experimental Design

The experiment was organized in a factorial design with 9 plants of each cultivar grown under one of four combinations of light intensity (200 and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and harvest methodology (harvesting every 10 days or harvesting when a plant had produced 8 expanded leaves). Five harvests were attempted per plant irrespective of treatment. The experiment was conducted twice over time. At harvest morphological tissue data were collected on all 9 samples per replication. Phytonutrient data were collected on all 9 plants per replication.

Plant Production

Seeds of *B. rapa* var. *nipposinica* ‘Red Hybrid’ (Kitizawa Seed, Oakland, CA) were sown in 162-cell bound coco-peat cubes (Preforma plugs; Jiffy Growing Solutions, Lorain, OH) and placed in a walk-in growth chamber (TC2; Environmental Grow Chambers, Chagrin Falls, OH). Similar to Chapters 1 and 2, the temperature was $21.8 \pm 1.0^\circ\text{C}$ and the relative humidity $67.2 \pm 7.7\%$, both setpoints measured and logged every 10 minutes by a shielded temperature and relative humidity sensor (Hobo MX2300; OnSet, Bourne, MA). Trays were placed under broad-spectrum light-emitting diodes (Scorpion Diablo, Horticulture Lighting Group; Maynardville, TN) providing one of two light intensities across a 16-hr photoperiod, monitored by quantum sensors maintained at canopy height (SQ-500S; Apogee, Logan, UT). CO_2 (Industrial Grade Carbon Dioxide CD 50S; Airgas, Knoxville, TN) was injected to provide $2,780 \pm 200 \mu\text{mol}\cdot\text{mol}^{-1}$, dosing was monitored and controlled by an infrared gas analyzer (LI-850; LI-COR Biosciences, Lincoln, NE) connected to a relay switch controlling an electronic regulator. CO_2 concentration was reported to a Raspberry Pi (Raspberry Pi 4 B; Raspberry Pi, Cambridge, England) every 20 seconds where it was recorded in a CSV file. Seeds were irrigated daily with reverse-osmosis water supplemented with 12N-1.8P-13.4K water-soluble fertilizer providing ($\text{mg}\cdot\text{L}^{-1}$), 100 nitrogen, 15 phosphorus, 112 potassium, 58 calcium, 17 magnesium, 2 sulfur, 1.4 iron, 0.5 zinc, 0.4 copper and manganese, and 0.1 boron and molybdenum (RO Hydro FeED; JR Peters, Inc., Allentown, PA) and magnesium sulfate (MgSO_4) to provide ($\text{mg}\cdot\text{L}^{-1}$) 15 magnesium and 20 sulfur, and adjusted to a pH of 5.8 with H_2SO_4 or KCHO_3 .

After fourteen days in the trays the seedlings were transplanted into 18-cm-deep by 61-cm-wide by 122-cm-long deep-water culture hydroponic systems (Premium Flood Table, Active

Aqua; Petaluma, CA) with 61 by 122 cm sealed-surface foam rafts (36 ct lettuce raft; Beaver Plastics, Acheson, AB, Canada) and grown for 14 days. The environmental conditions were the same as the seedling stage. Eight systems were filled with 108 L of reverse-osmosis water and supplemented with 12N-1.8P-13.4K water-soluble fertilizer (RO Hydro FeED; JR Peters, Inc.) and MgSO_4 providing twice the concentrations reported previously. Water was topped off to 108 liters every 10 days. Electrical conductivity (EC) and pH were monitored (HI9813-6N Portable Waterproof pH/EC/TDS Meter; Hanna Instruments, Woonsocket, RI) and pH was adjusted to 5.8 using H_2SO_4 or KCHO_3 , while EC was brought back up to 1.80 after each water refill. Air pumps (Active Aqua 110 $\text{L}\cdot\text{min}^{-1}$ commercial air pump; Hydrofarm, Petaluma, CA) and air stones (Active Aqua air stone round 10 cm $\times$ 2.5 cm; Hydrofarm, Petaluma, CA) were used to provide dissolved oxygen.

Harvest, Morphological Data Collection, and Tissue Processing

Two harvest duration methods were deployed, a temporally-based and a developmentally-based method. For the temporal method, plants were harvested every 10 days after transplant. For the developmentally-based harvest schedule, plants were harvested once they produced 8 fully expanded leaves. For both harvest treatments, all leaves that extended higher than 3.5 cm above the substrate surface were removed during the harvest. Care was taken to leave some meristematic tissue during each harvest event. This was repeated until 5 harvests had been collected from each plant, or until the plant stopped producing tissue. The fresh mass of the harvested tissue was recorded and then placed in a plastic sample bag and flash frozen in liquid N. Samples were placed in a -80°C freezer for storage. Samples were freeze dried (FreeZone 12+; LabConco, Kansas City, MO) for 4 days. After freeze drying, dry mass was recorded (Entris II Essential Line Milligram Balance; Sartorius, Göttingen, Germany), and individual samples were ground in liquid N with ceramic mortar and pestle. Once homogenized into a fine powder the tissue was divided into separate aliquots for future extraction procedures and stored in individual 15 mL centrifuge tubes in a -80°C freezer.

Anthocyanin Isolation and Quantification

Similar to Chapters 1 and 2, total anthocyanins were extracted and analyzed using a method derived from Islam et al. (2019). However, 50 mg of tissue were used per sample, instead of the 100 mg used in Chapter 1. Under red light, samples were saturated with 5 mL of

95% ethanol/1.5 N HCl (85:15, v:v), placed on an orbital shaker for 15 minutes, and stored at 4 °C for 24 hrs. Samples were then filtered and analyzed with a microplate reader (Agilent Technologies, Santa Clara, CA).

Carotenoid Isolation and Quantification

Similar to Chapters 1 and 2, carotenoids β -carotene, lutein, and zeaxanthin were extracted and analyzed using a method derived from Kopsell et al. (2012). However, we used 50 mg of tissue instead of the 100 mg outlined in Chapter 1. Under red light, chilled samples were extracted in purified water and tetrahydrofuran with an internal carotenoid standard being used to quantify sample loss during homogenization. Samples were homogenized and tetrahydrofuran was used for a second extraction followed by homogenization. The resulting solution was centrifuged, and the eluent was dried down on a stream evaporator. After the addition of acetone, samples were filtered, and an aliquot was stored for subsequent identification and quantification on a 1200 Agilent Series HPLC unit equipped with a diode array detector. A reverse phase C30 column was used with a mobile phase composed of methyl tert-butyl ether, methanol, and triethylamine.

Water Soluble Vitamin Isolation and Extraction

The water-soluble vitamins (WSVs) ascorbic acid (vitamin C) and thiamine (vitamin B₁) were extracted using a method as described in Chapter 2 and derived from Seal and Chaudhuri (2017) and Sun et al. (2018). However, 200 mg of tissue were used instead of 500 mg. Under red light, chilled samples were hydrated with purified water, hydrochloric acid, and a phosphate buffer. Samples were vortexed and supernatant was filtered. The process was repeated, and a 1 mL aliquot was collected for analysis on a 1200 Agilent Series HPLC equipped with a diode array detector. A reverse phase C18 column was used alongside an aqueous formic acid mobile phase. Both vitamins were detected at 255 nm.

Statistics

Statistical analysis was performed in R (R Core Team, 2023). Data were organized, compiled, and visualized with the ‘tidyverse’ package (Wickham et al., 2019). Data were initially separated into two groups based on harvest methodology (time vs. development), and a two-way analysis of variance (ANOVA) was performed on each group to determine the effect of light intensity and consecutive harvest on each parameter of interest (R Core Team, 2023), after

which a post-hoc Tukey's Honest Significant Difference test was performed with the 'emmeans' package (Lenth, 2023). Groupings for the Tukey's HSD were based on ANOVA results, with parameters that showed significant interaction being grouped by individual harvest and light intensity, while those parameters that showed no interaction but significant variation across harvests were grouped only by harvest. When lighting treatment or harvest interval were not significant, data were pooled. Individual parameter means were then cumulatively summed across harvests and divided by the number of days required to reach that harvest, producing a per diem yield to determine how efficiency changed over consecutive harvests. Finally, these numbers were divided by the number of megajoules used per day by each lighting treatment to visualize how the return in biomass and phytonutrient yields changed over consecutive harvests from an energy use perspective.

Results and Discussion

Temporally-Based Harvest

When harvested on a 10-day schedule 'Red Hybrid' mizuna was able to produce five consecutive harvests. Across these five harvests the fresh mass, dry matter percent, total anthocyanin concentration and β -carotene concentration all showed interactions in their response to harvest event and light intensity (Table 3.1). The carotenoids lutein and zeaxanthin, and the water-soluble vitamins ascorbic acid and thiamine did not show variation in concentrations in response to harvest event but did respond differently when plants were produced under 200 vs. 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light. A lack of variation due to harvest event is not undesirable for space applications, as a steady supply across multiple harvests would be better than a single harvest from the plant. However, a decrease in fresh biomass yield or phytonutrient concentrations with subsequent harvests would be undesirable.

Fresh mass yield was higher across all five harvests when plants were grown under 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light, and were highest during the first three harvests (>60 g), and lowest during the final harvest (49 g) (Fig. 3.1a). When grown under 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light, plants produced between 21 and 27 g per harvest, but did not show significant variation from harvest to harvest. These results indicate that from a biomass yield perspective it may be better to sow new plants after a fourth consecutive harvest when growing at a higher light intensity. Dry matter percent trends were similar, with the plants grown under 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light producing a higher

Table 3.1. Results of analysis of variance performed on *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ metrics of plants harvested 5 times at a 10-day interval between each harvest.

Metric	Harvest		Light Intensity		Harvest × Light Intensity	
	P-value	Signif.	P-value	Signif.	P-value	Signif.
Fresh Mass (g)	< 0.001	***	< 0.001	***	0.013	*
Dry Matter Percent (%)	< 0.001	***	< 0.001	***	< 0.001	***
Anthocyanin Conc. (mg·g ⁻¹)	0.003	*	0.015	*	0.002	*
β-Carotene Conc. (mg·g ⁻¹)	< 0.001	***	< 0.001	***	< 0.001	***
Lutein Conc. (mg·g ⁻¹)	0.353	NS	< 0.001	***	0.221	NS
Zeaxanthin Conc. (mg·g ⁻¹)	0.838	NS	< 0.001	***	0.088	NS
Ascorbic Acid Conc. (mg·g ⁻¹)	0.424	NS	0.001	***	0.142	NS
Thiamine Conc. (mg·g ⁻¹)	0.059	NS	< 0.001	***	0.473	NS
***, *, and NS indicate significant at $P \leq 0.001$, $P \leq 0.05$, or nonsignificant, respectively.						

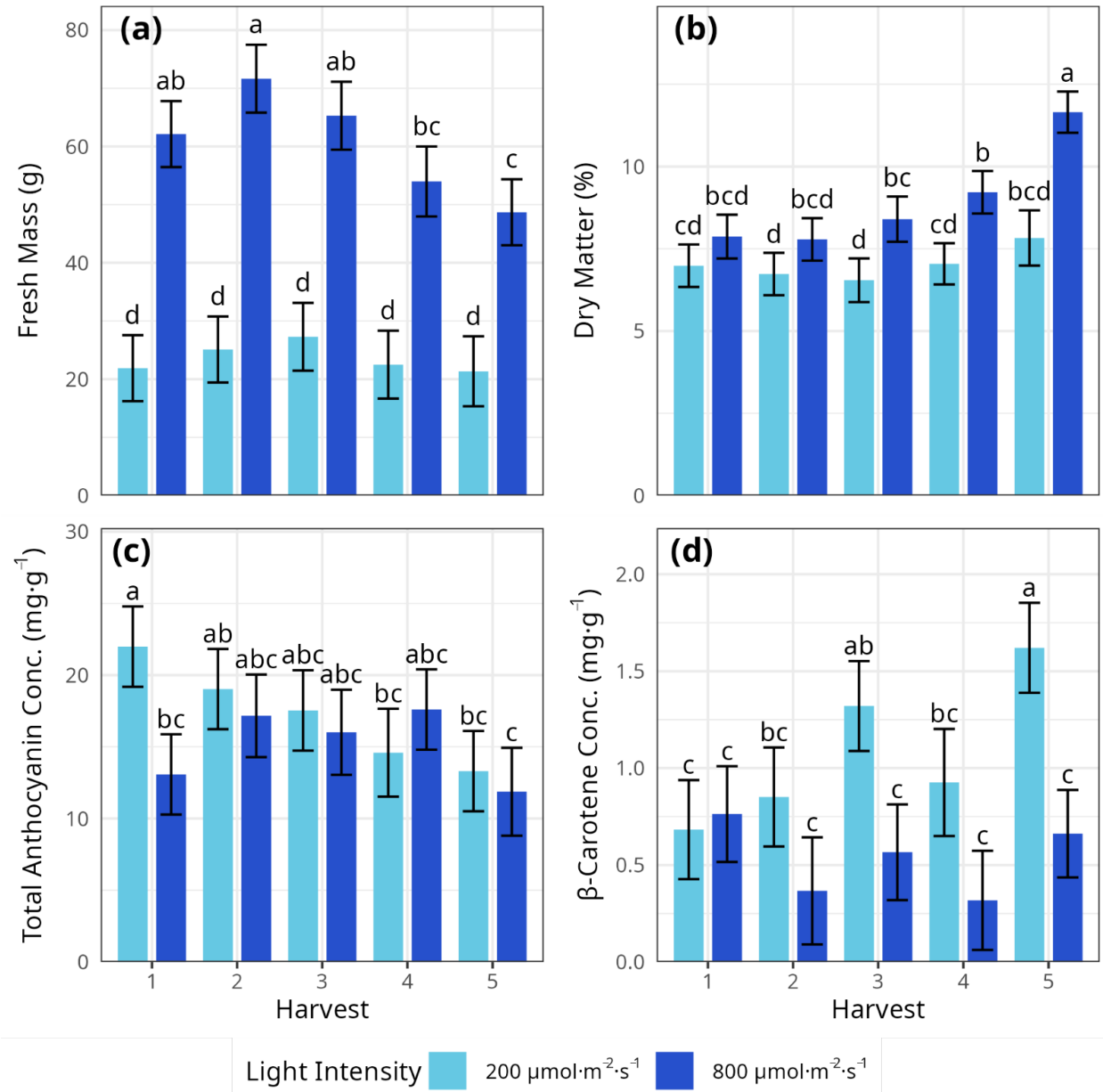


Figure 3.1. Effect of light intensity and subsequent harvest event on *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna plants harvested every ten days. a) Fresh mass, b) dry matter percent, c) total anthocyanin concentration, and d) β -carotene concentration are represented by blue bars. Brackets represent confidence intervals, while bars that share letters do not differ significantly at the 0.05 α level.

proportion of dry matter (Fig. 3.1b). The fifth consecutive harvest from plants grown at 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produce more dry matter (11.7%), and the fourth harvest produced more dry biomass (9.2%) than the initial four harvests grown at the lower light intensity, which averaged 6.8%. While the dry matter percent is a positive indicator of plant fitness for the space diet, as seen in Chapters 1 and 2, it may be more indicative of primary metabolite content, rather than a predictor of phytonutrient content.

The interactive effect of light intensity and harvest event on total anthocyanin concentration was primarily seen in the first harvest, where plants grown under a higher light intensity had lower anthocyanin concentrations (13.1 $\text{mg}\cdot\text{g}^{-1}$) than those grown at a lower light intensity (22.0 $\text{mg}\cdot\text{g}^{-1}$) (Fig. 3.1c). This difference was lost with consecutive harvests, where plants grown under high and low intensity lighting were comparable to each other. However, by the fifth harvest, both treatments were producing anthocyanin concentrations lower than that of the initial 22.0 $\text{mg}\cdot\text{g}^{-1}$ produced by the low light treatment. As anthocyanins are a beneficial, but not required, phytonutrient in the human diet, the lower performance of consecutive harvests when grown at low light intensity likely does not warrant resowing seeds after a single harvest.

Conversely, β -carotene concentrations were similar among lighting conditions at the first harvest event, but concentrations produced by the plants grown at a lower light intensity tended to increase with subsequent harvests (Fig. 3.1d), producing higher concentrations than their high light counterparts on the third (1.32 vs. 0.57 $\text{mg}\cdot\text{g}^{-1}$) and fifth harvests (1.62 vs. 0.66 $\text{mg}\cdot\text{g}^{-1}$). β -carotene was the only one of the three carotenoids quantified in this study to show an interactive effect between harvest event and light intensity when harvested on a time-based interval. However, lutein and zeaxanthin concentrations were both higher when plants were produced at a lower light intensity. Lutein concentrations averaged 0.85 and 0.64 $\text{mg}\cdot\text{g}^{-1}$ when grown at 200 and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light, respectively, while mean zeaxanthin concentrations were 0.56 and 0.46 $\text{mg}\cdot\text{g}^{-1}$ (Figs. 3.2a and 3.2b).

The water-soluble vitamins were both produced at higher concentrations at the higher light intensity, with ascorbic acid (vitamin C) concentrations rising from 26.6 to 38.9 $\text{mg}\cdot\text{g}^{-1}$ (Fig. 3.2c) and thiamine (vitamin B₁) rising from 0.04 to 0.05 $\text{mg}\cdot\text{g}^{-1}$ (Fig. 3.2d). The differing

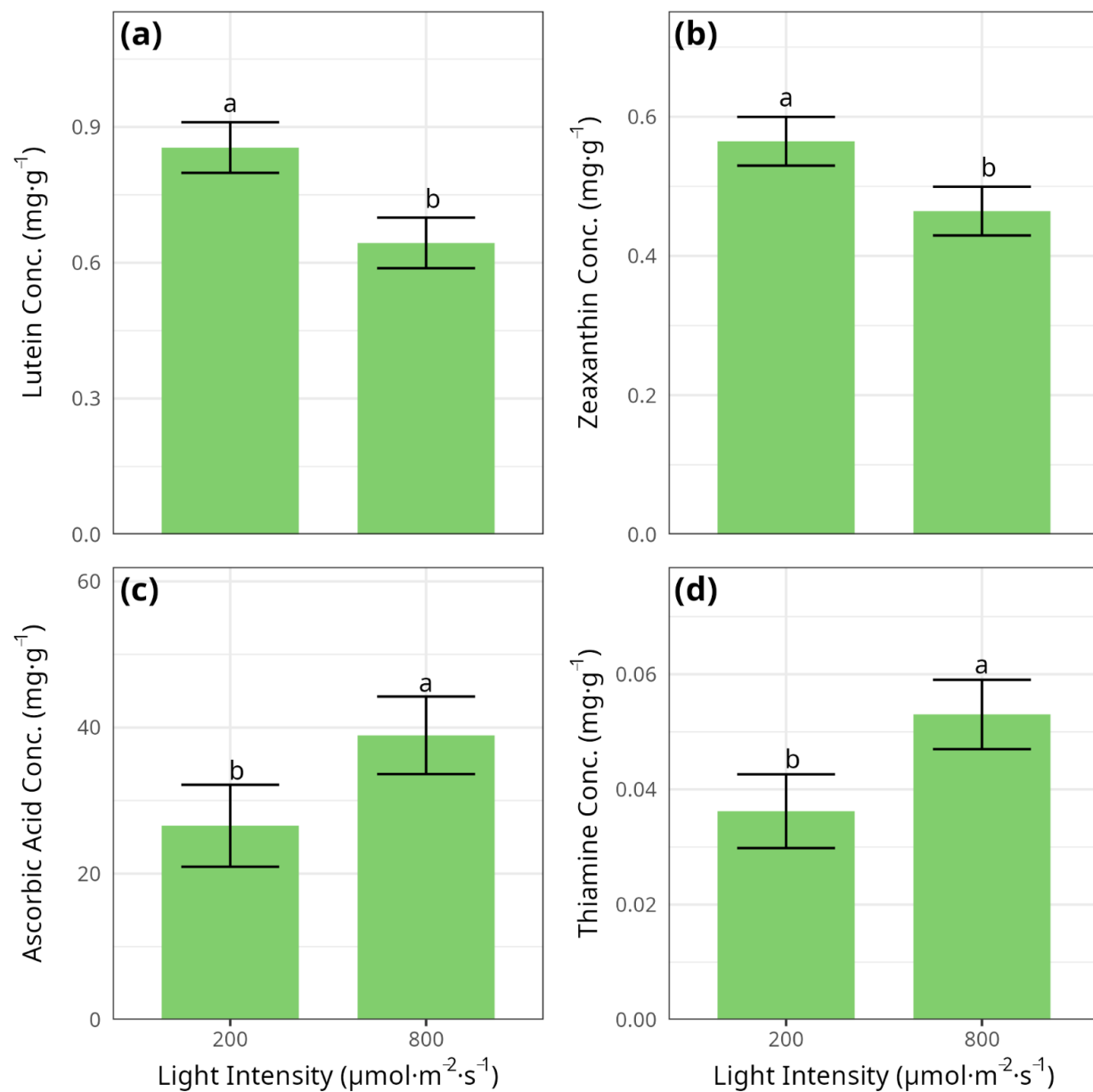


Figure 3.2. Effect of light intensity on *Brassica rapa* var. *nipposinica* 'Red Hybrid' mizuna plants harvested every ten days. a) Lutein, b) zeaxanthin, c) ascorbic acid, and d) thiamine concentrations are represented by green bars. Brackets represent confidence intervals, while bars that share letters do not differ significantly at the 0.05 α level.

responses of the water-soluble vitamins and carotenoids to light intensity may seem difficult to resolve, however, as they were not impacted by consecutive harvest events, they do not play a role in determining an ideal cultivation length for optimal use of resources and time.

Developmentally-Based Harvest

When ‘Red Hybrid’ plants were harvested after producing 8 fully expanded leaves, 4 consecutive harvests were achieved per plant. The lack of a fifth harvest when utilizing this method was primarily due to the longer interval between harvests allowing for the development of reproductive structures. Once plants bolted, they produced very little harvestable tissue for consecutive harvests. Across these 4 harvests, only dry matter percent, total anthocyanin concentration, and thiamine concentration responded to the interactive effect of harvest event and light intensity (Table 3.2). Fresh mass and ascorbic acid (vitamin C) concentrations did vary over consecutive harvests, while all three carotenoids varied in concentration between high and low light intensity (Table 3.2).

Dry matter percentages followed a similar trend to those seen when plants were harvested every ten days, with all 4 harvest events produced at the lower light intensity producing among the lowest dry matter percentages, averaging 7.9% (Fig. 3.3a). The higher light intensity produced its highest percentages on the third and final harvest, averaging 13.9%. Despite fewer harvests, the trends were very similar, with a low light intensity producing a flatter response over consecutive harvests. Total anthocyanin concentrations also followed a similar trend across both harvest methods, with a low light treatment producing a significantly lower concentration by the fourth harvest, producing $16.3 \text{ mg}\cdot\text{g}^{-1}$ on the initial harvest and $5.11 \text{ mg}\cdot\text{g}^{-1}$ at the final harvest (Fig. 3.3b). A high light treatment produced a flat response here with no significant differences between harvests. Finally, thiamine concentrations increased on the third and fourth harvests, producing $0.07 \text{ mg}\cdot\text{g}^{-1}$, almost double that of the initial harvest (Fig. 3.3c). Again, harvest event seemed to affect the response less at a higher light intensity, with no differences in thiamine concentration between harvests when grown at $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Fresh mass did not respond to light intensity when developmentally-based harvests were deployed, something not seen previously during Chapter 2 of this thesis or during the time-based portion of the current Chapter. This is probably largely due to the nature of a developmentally

Table 3.2. Results of analysis of variance performed on *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ metrics of plants harvested 4 times, with each harvest occurring when the plant had produced 8 mature leaves.

Metric	Harvest		Light Intensity		Harvest × Light Intensity	
	P-value	Signif.	P-value	Signif.	P-value	Signif.
Fresh Mass (g)	< 0.001	***	0.129	NS	0.567	NS
Dry Matter Percent (%)	< 0.001	***	< 0.001	***	0.036	*
Anthocyanin Conc. (mg·g ⁻¹)	< 0.001	***	0.618	NS	0.009	*
β-Carotene Conc. (mg·g ⁻¹)	0.546	NS	< 0.001	***	0.489	NS
Lutein Conc. (mg·g ⁻¹)	0.324	NS	< 0.001	***	0.533	NS
Zeaxanthin Conc. (mg·g ⁻¹)	0.156	NS	< 0.001	***	0.668	NS
Ascorbic Acid Conc. (mg·g ⁻¹)	< 0.001	***	0.425	NS	0.575	NS
Thiamine Conc. (mg·g ⁻¹)	< 0.001	***	0.542	NS	0.040	*
***, *, and NS indicate significant at $P \leq 0.001$, $P \leq 0.05$, or nonsignificant, respectively.						

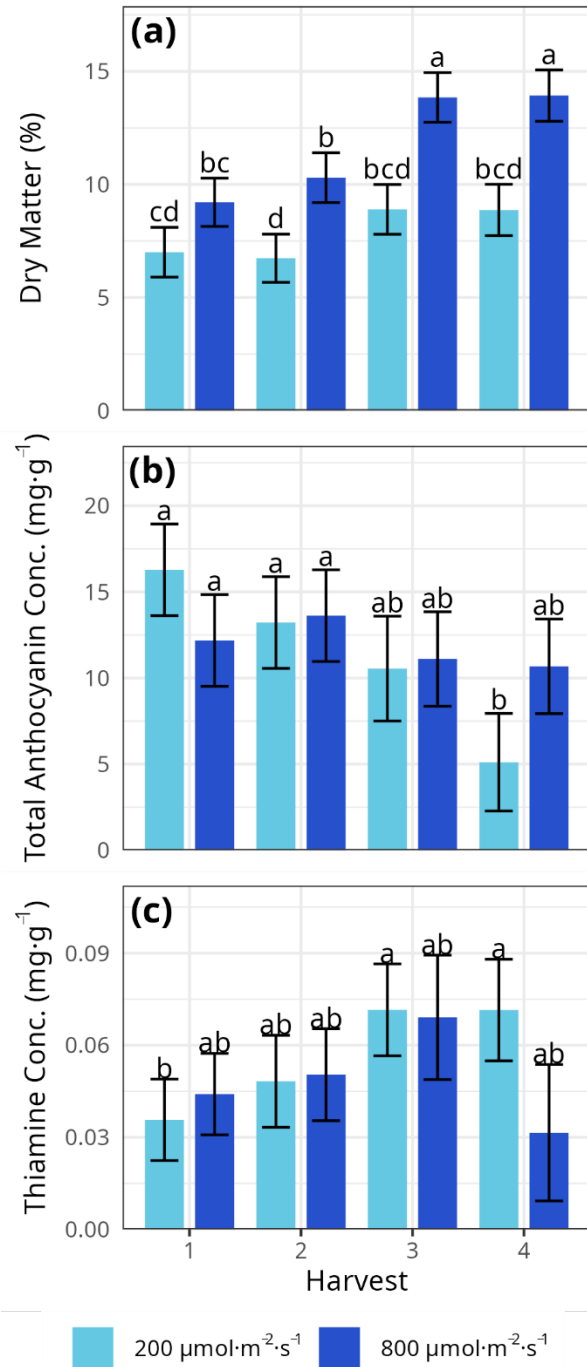


Figure 3.3. Effect of light intensity and subsequent harvest event on *Brassica rapa* var. *nipposinica* 'Red Hybrid' mizuna plants harvested after the production of 8 fully expanded leaves. a) Dry matter percent, b) total anthocyanin, and c) thiamine concentration are represented by blue bars. Brackets represent confidence intervals, while bars that share letters do not differ significantly at the 0.05 α level.

based harvest, which allows the crop, regardless of other treatments, to reach a relatively standard size. However, the initial harvest was smaller (60 g) than the subsequent three (>85 g), likely due to the production of additional tissue from axillary meristems as a result of harvesting (Fig 3.4a). While plants were not harvested until 8 leaves had fully expanded, this did not mean that only eight leaves were harvested, as many younger leaves could have begun to emerge in addition to the eight expanded leaves. Ascorbic acid also did not vary with light intensity during a developmentally based harvest schedule. It did show variation between harvests, with the first and final harvests containing significantly less ascorbic acid per gram of dry tissue (Fig. 3.4b). Both of these trends, in addition to those seen in thiamine, anthocyanin, and dry matter response, seem to indicate that at least three harvests can safely be attained from plants harvested on a developmental basis without decreases in nutritional value.

The three carotenoids did not respond to harvest event, and therefore do not affect the evaluation of whether or not to terminate a crop before the third harvest. However, they did vary between light intensity, with plants producing higher concentrations of all three carotenoids when produced at the lower light intensity (Fig. 3.5abc). β -carotene decreased most drastically as light intensity increased from 200 to 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light, dropping from 1.08 to 0.52 $\text{mg}\cdot\text{g}^{-1}$ of dry tissue, while lutein and zeaxanthin dropped from 0.74 to 0.53 $\text{mg}\cdot\text{g}^{-1}$ and 0.47 to 0.39 $\text{mg}\cdot\text{g}^{-1}$ respectively. These responses, in hand with the higher thiamine concentrations produced at later harvests at low light intensity (Fig. 3.3a), support a production system that utilizes a lower light intensity.

Per Diem Comparison Across Harvest Methods

While both production systems have their advantages, a comparison of total yield, both of fresh biomass and of each phytonutrient, on a per diem basis provides more insight into the preferred method of production, as well as the most efficient length of time before a final harvest and resow. When compared per diem, the advantages of a ‘cut-and-come again’ method, regardless of harvest methodology, are most evident during the second harvest event (Table 3.3, Table 3.4, Fig. 3.6, Fig. 3.7) and remain evident until the ultimate or penultimate harvest. Even in those cases where per diem yield dips on the final harvest, as seen in anthocyanin and ascorbic acid yields in plants produced under 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light and harvested developmentally, yield is still higher per diem than at the first harvest (Table 3.4, Fig. 3.6). These gains in per diem

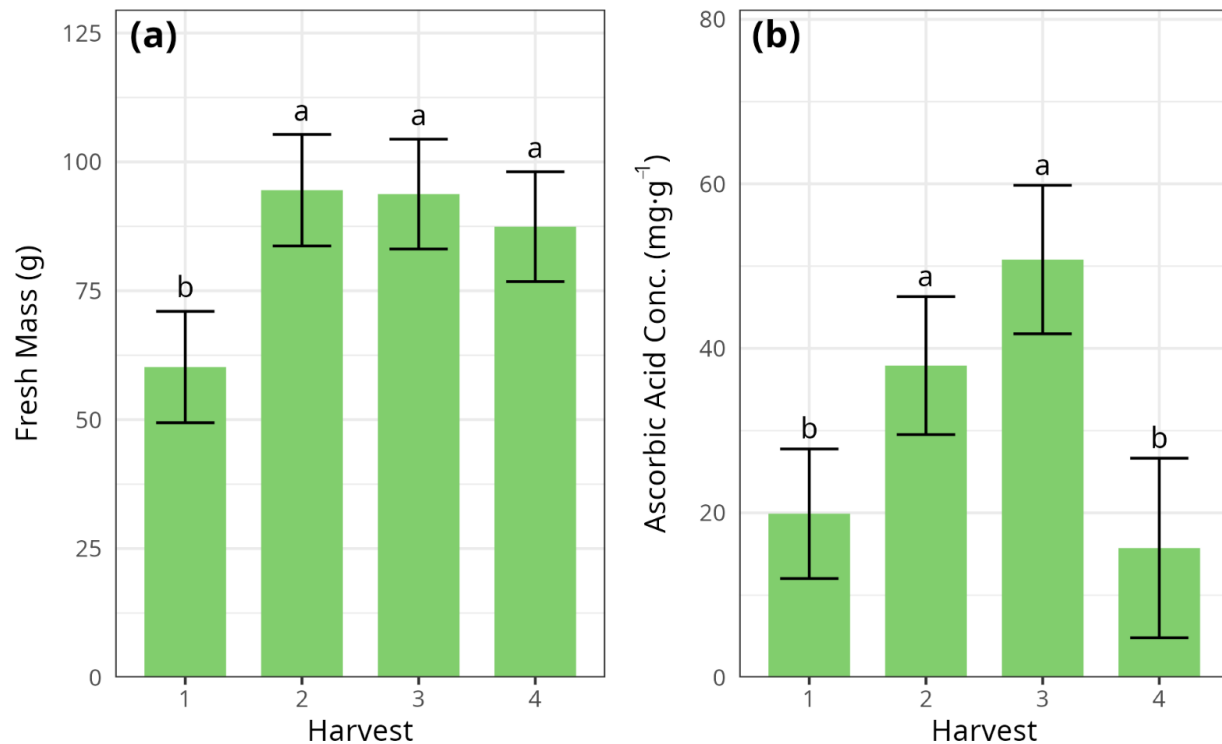


Figure 3.4. Effect of harvest event on *Brassica rapa* var. *nipposinica* 'Red Hybrid' mizuna plants harvested after the production of 8 fully expanded leaves. a) Fresh mass and b) ascorbic acid, concentrations are represented by green bars. Brackets represent confidence intervals, while bars that share letters do not differ significantly at the 0.05 α level.

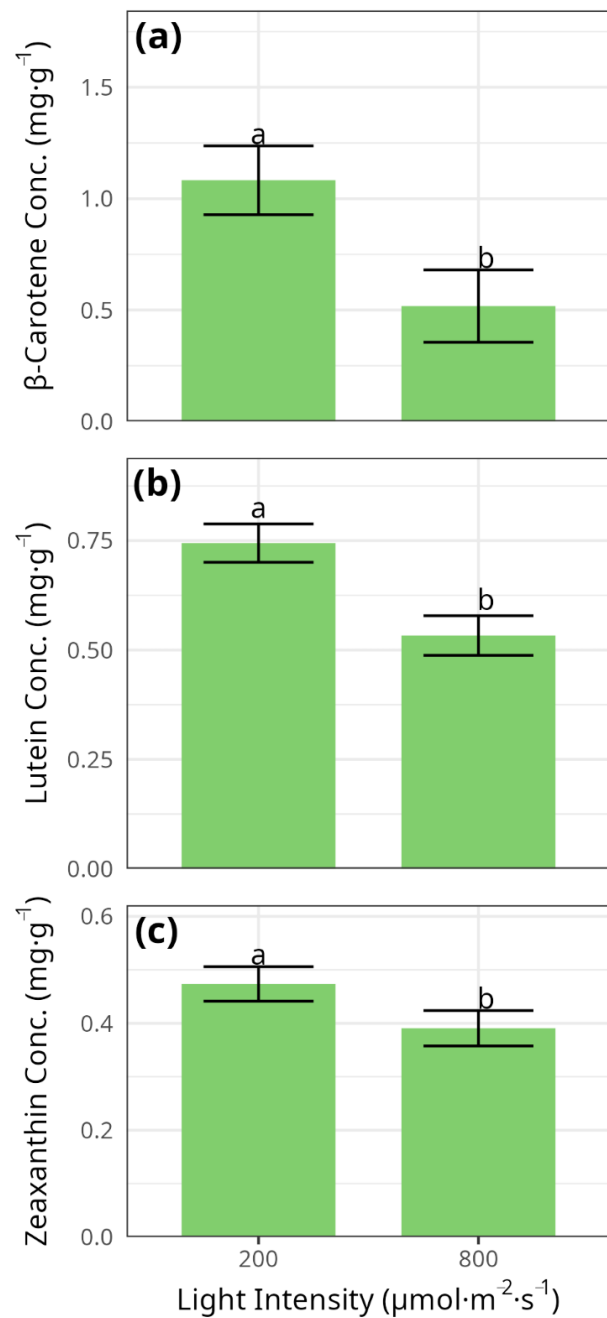


Figure 3.5. Effect of light intensity on *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna plants harvested after the production of 8 fully expanded leaves. a) β -carotene, b) lutein, and c) zeaxanthin, concentrations are represented by green bars. Brackets represent confidence intervals, while bars that share letters do not differ significantly at the 0.05 α level.

Table 3.3. Per diem calculations per treatment combination for fresh biomass and phytonutrient yield per plant in temporally harvested (ten-day intervals after transplant) *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna. Numbers in bold indicate the most efficient per diem yield per parameter.

Parameter	Light Intensity	Sequential Harvest Event – Time Based				
		1	2	3	4	5
		(24 days)	(34 days)	(44 days)	(54 days)	(64 days)
Fresh Mass	200	0.91	1.38	1.69	1.79	1.84
(g·d ⁻¹)	800	2.59	3.93	4.52	4.69	4.71
Anthocyanin	200	1.40	1.93	2.21	2.23	2.23
(mg·d ⁻¹)	800	2.66	4.70	5.62	6.20	6.29
β-Carotene	200	0.04	0.07	0.11	0.12	0.14
(mg·d ⁻¹)	800	0.16	0.17	0.20	0.19	0.22
Lutein	200	0.06	0.08	0.10	0.11	0.11
(mg·d ⁻¹)	800	0.15	0.19	0.24	0.25	0.26
Zeaxanthin	200	0.04	0.06	0.06	0.07	0.07
(mg·d ⁻¹)	800	0.09	0.14	0.17	0.18	0.19
Ascorbic Acid	200	2.00	2.54	3.04	3.27	3.45
(mg·d ⁻¹)	800	6.09	11.61	15.59	15.84	16.57
Thiamine	200	0.002	0.004	0.004	0.005	0.005
(mg·d ⁻¹)	800	0.01	0.02	0.02	0.02	0.02

Table 3.4. Per diem calculations per treatment combination for fresh biomass and phytonutrient yield per plant in developmentally harvested (eight expanded leaves) *Brassica rapa* var. *nipposinica* ‘Red Hybrid’ mizuna. Numbers in bold indicate the most efficient per diem yield per parameter.

Parameter	Sequential Harvest Event – Development Based							
	200				800			
	1	2	3	4	1	2	3	4
	27 days	44 days	62 days	83 days	26 days	39 days	55 days	71 days
Fresh Mass (g·d ⁻¹)	1.86	3.29	3.81	3.85	2.78	4.24	4.83	4.97
Anthocyanin (mg·d ⁻¹)	2.12	3.21	3.66	3.18	3.12	5.44	6.66	6.98
β-Carotene (mg·d ⁻¹)	0.15	0.27	0.31	0.33	0.12	0.19	0.24	0.31
Lutein (mg·d ⁻¹)	0.10	0.18	0.22	0.23	0.15	0.22	0.30	0.31
Zeaxanthin (mg·d ⁻¹)	0.07	0.10	0.14	0.14	0.11	0.16	0.22	0.23
Ascorbic Acid (mg·d ⁻¹)	2.61	6.82	10.69	9.51	5.06	13.23	24.14	20.84
Thiamine (mg·d ⁻¹)	0.005	0.010	0.016	0.018	0.011	0.020	0.031	0.030

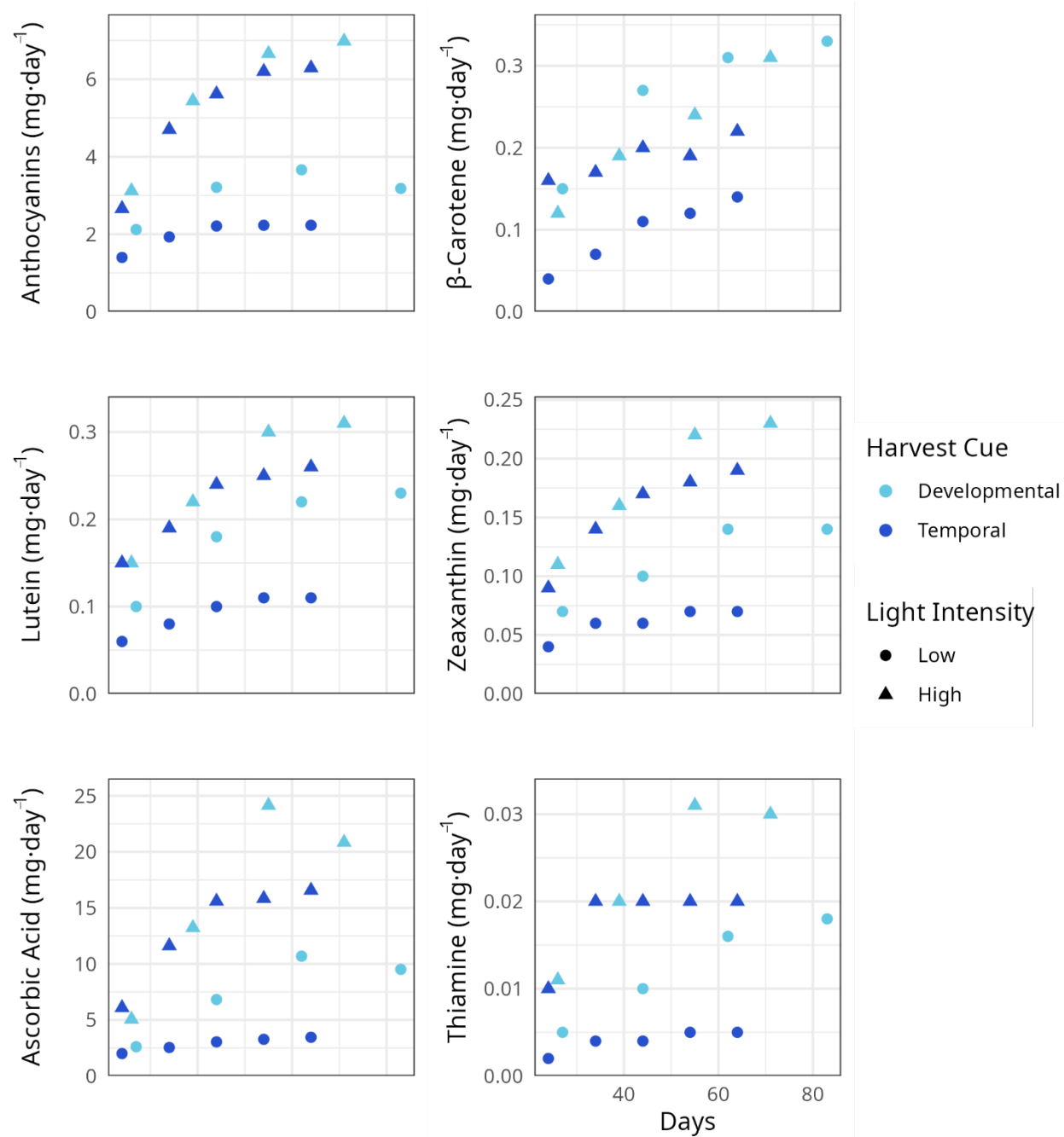


Figure 3.6. Per diem phytonutrient yields are plotted across multiple harvests, with averages calculated by harvest cue (developmental or temporal) and light intensity (low or high). Temporal harvest cue was every ten days, while developmental cue was every 8 expanded leaves. Low and high light were respectively 200 and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

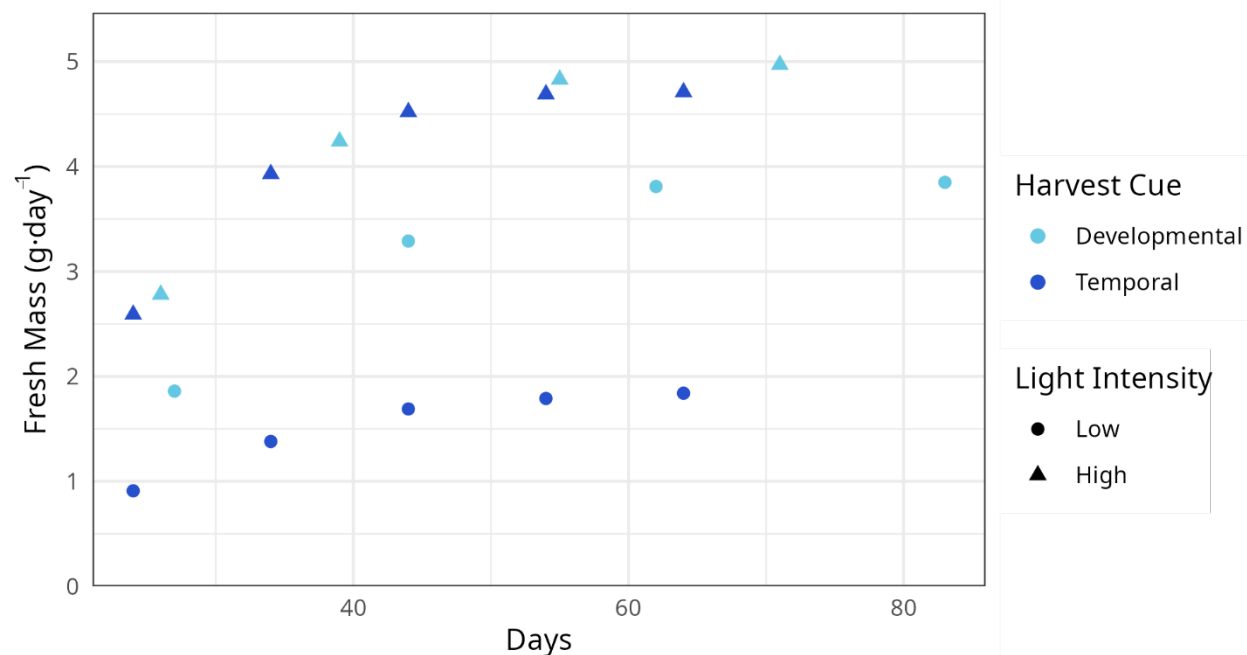


Figure 3.7. Per diem biomass yields are plotted across multiple harvests, with averages calculated by harvest cue (developmental or temporal) and light intensity (low or high). Temporal harvest cue was every ten days, while developmental cue was every 8 expanded leaves. Low and high light were respectively 200 and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

yields with subsequent harvests are largely due to the avoidance of an additional germination period, which requires fourteen days and produces very little biomass. In the example of ‘Red Hybrid’ mizuna plants harvested every ten days after the initial harvest, three harvests are achieved within 44 days, while 48 days (24 x 2) would be required to achieve two harvests under a traditional single harvest system.

A decrease of per diem phytonutrient yields on a subsequent harvest only occurred in the developmentally harvested mizuna, and further study would be required to determine if this was due to the method of harvest itself, or due to the longer time required for this methodology. A fifth harvest of the temporally harvested plants was achieved on the sixty-fourth day, while a developmentally harvested plant required 71 days to achieve a *fourth* harvest. While this may seem less efficient, a closer look reveals that at the sixty-fourth day of production under $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, plants harvested with a temporal method had produced $4.71 \text{ g}\cdot\text{d}^{-1}$ (Table 3.3, Fig. 3.7), while plants harvested with a developmental method had surpassed this, producing $4.83 \text{ g}\cdot\text{d}^{-1}$, by the fifty-fifth day (Table 3.4, Fig. 3.7). This indicates a higher efficiency in biomass yield when allowing plants to go longer between harvests, despite the lack of a fifth harvest.

Energy Use Comparison Across Harvest Methods and Light Intensities

Finally, when considering the efficiency of production aboard the ISS or other space-based applications, energy-use cannot be underestimated. The final lens through which to view the data produced by this study is through that of yield per megajoule (MJ). This is a complex question to answer, as the type of LED fixtures utilized, the efficiency of the plant photosystems at varied light intensities and spectra, and the off-target effects of higher light intensity (such as increased heat output and evapotranspiration, altering the energy required to maintain temperature setpoints), could all play into the equation. However, for exploratory and demonstrative purposes, the LED energy use per diem was calculated and applied to the numbers in Tables 3.3 and 3.4, allowing for a visualization of energy use efficiency over time on a per plant basis, which could also be conceptualized as per plant-site or per unit of production area. This visualization clearly illustrates the advantage provided by a lower light intensity when efficient energy use is a priority, with every single phytonutrient (Fig. 3.8) as well as fresh mass (Fig. 3.9) showing higher yields per megajoule per plant. Additionally, the trends seen in the

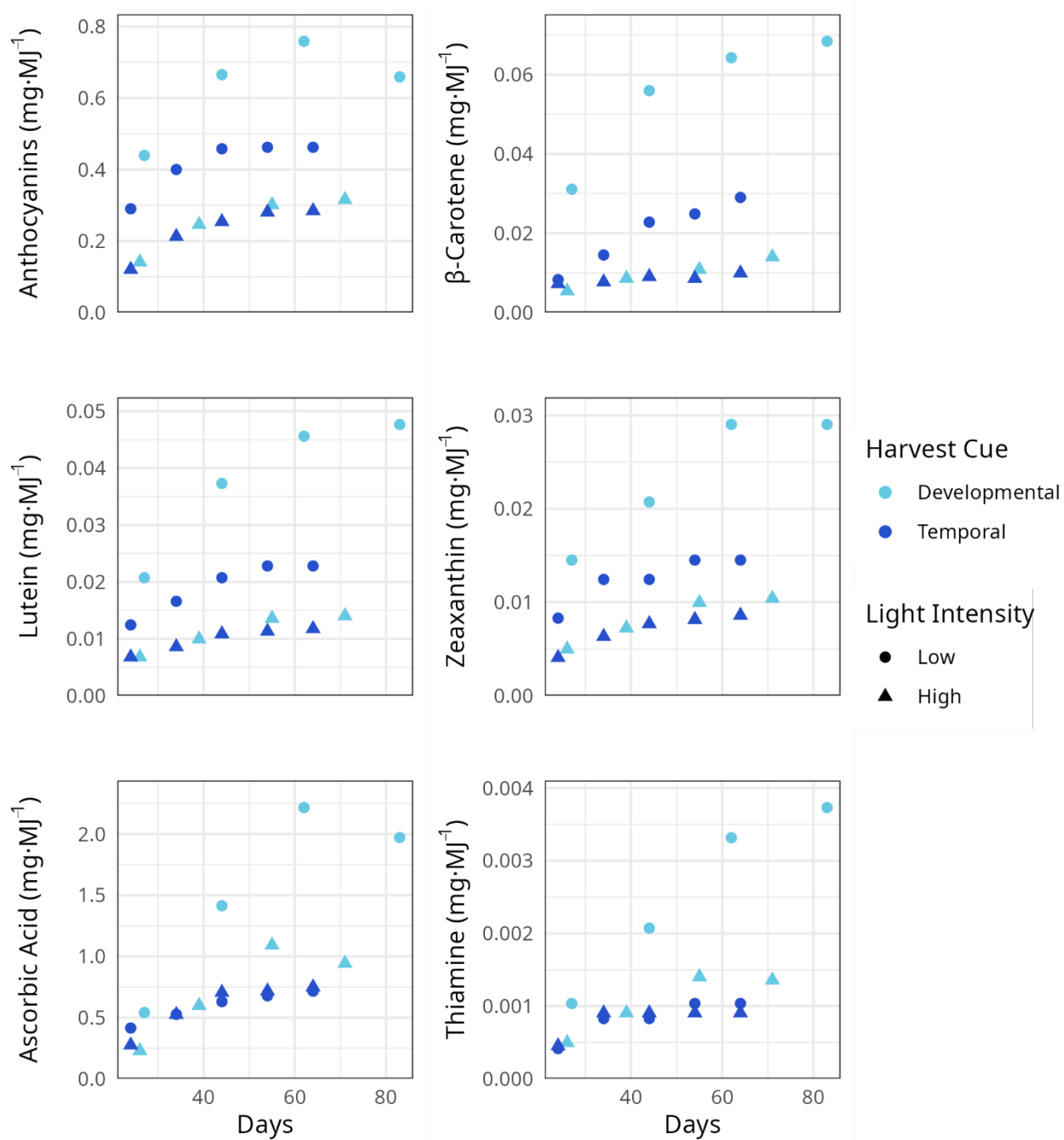


Figure 3.8. Per megajoule phytonutrient yields are plotted across multiple harvests, with averages calculated by harvest cue (developmental or temporal) and light intensity (low or high). Temporal harvest cue was every ten days, while developmental cue was every 8 expanded leaves. Low and high light were respectively 200 and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

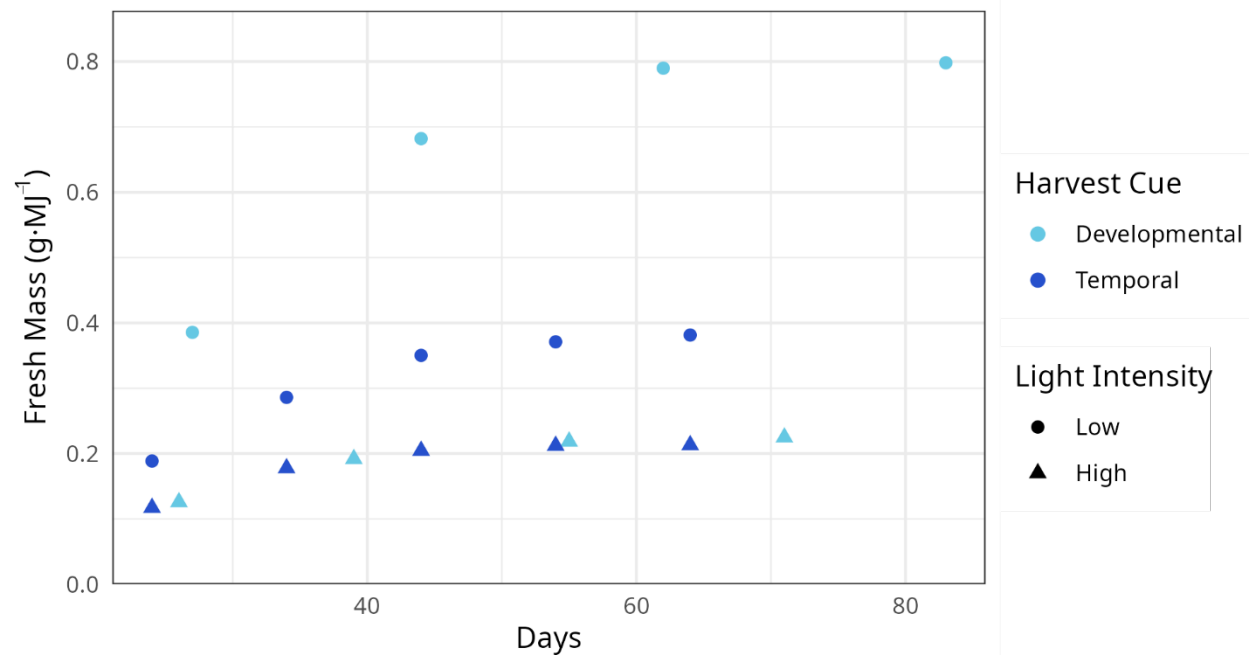


Figure 3.9. Per megajoule biomass yields are plotted across multiple harvests, with averages calculated by harvest cue (developmental or temporal) and light intensity (low or high).

Temporal harvest cue was every ten days, while developmental cue was every 8 expanded leaves. Low and high light were respectively 200 and 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

previous per diem calculations remain present here, with returns per megajoule increasing with subsequent harvests, especially in the case of the plants produced under low light intensity and harvested on a developmental cue.

These findings indicate that for overall maximum yields, a high light intensity combined with either harvest indicator provides the best results. However, if energy use is a concern the phytonutrient and biomass return per megajoule is better at the lower light intensity, and can be increased further by allowing the plants to develop more between harvests. That said, a developmental harvest cue can be less efficient in human-hours, as a leaf count would need to be performed each day to determine which plants are ready for harvest. Instead, a solution could be to harvest the plants based on the average time required to develop 8 expanded leaves (or a different developmental cue, if a better one is determined). At the lower light intensity in this study, the average time required to develop 8 expanded leaves shifted as plants underwent each consecutive harvest, starting at 17 days, then 18, then 21. More research could be conducted to find an ideal time between harvests to allow for optimal development, while limiting time used to determine harvest readiness.

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VITA

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