Irradiance, total nitrogen, and nitrate-N : ammonium-N ratio requirements for optimal edible biomass production of basil

by

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GENERAL INTRODUCTION

Basil, *Ocimum basilicum* L., is a member of the Labiatae family, and it has been cultivated for hundreds of years in Asia. Now it is one of the most economically important herbs in developed countries (Garibaldi et al., 1997; Pistrick, 2006). Most global cultures have links to basil in their folklore, folk medicine, and culinary dishes, and therefore, basil can be considered a universal food product (Sharma et al., 1987).

The National Aeronautics and Space Administration (NASA) is developing recommendations for food-production systems on long-duration missions to outposts such as the International Space Station and lunar and Mars habitats. These habitats include space for the production of fresh produce, such as salad crops and herbs (Monje et al., 2002; Salisbury, 1991; Wheeler et al., 2008**)**. Plants also improve workplace environments in regards to reducing stress and cleaning the air (Bringslimark et al., 2007; Fjeld, 2000; Lohr et al., 1996). As long-duration missions become more common, it will become necessary that the environments of the vehicle and habitat facilitate the decrease of stress and promote clearer thinking for astronauts. Growing edible plants in an efficient and sustainable manner would be ideal in regards to reducing energy and monetary inputs for astronaut activities in long-term space-flight missions as well as lunar and Mars habitats (Salisbury, 1991).

Several decades of research in plant breeding for controlled environments and hydroponic technology have led to the development of dwarf cultivars of wheat, rice, and other staple crops (Tibbitts and Alford, 1982). Technical knowledge learned from these crops have evolved to work being done with several other food crops such as lettuce, potato, peanut, sweet potato, and in this case, basil (Hoff et al., 1982). These crops may be included in the daily diet of future inhabitants of long-duration missions, lunar stations, and Mars stations by being cultivated in controlled environment systems like that currently being used at the South Pole (Nelkin, 2006). The inclusion of culturally familiar food products such as basil could ease stresses inherent in long-duration missions (Kandiah et al., 2005; Kerwin and Seddon, 2002; Zellner et al., 2007).

Basil is a rapidly growing, edible herbaceous plant that produces leaves when cultivated using typical horticultural practices. Basil is an ideal candidate for easing psychological stresses on space missions in that astronauts will be able to experience hands-on time with plants (Bringslimark et al., 2007; Gupta et al., 2007). The freshness qualities of basil and other salad crops will enhance the daily diet of astronauts because they will provide an alternative to freeze-dried and otherwise prepackaged food products (Huntoon, 1999; Kerwin and Seddon, 2002). Astronauts generally prefer spicy foods to the typical bland food items provided while in flight and in orbit (Lane and Schoeller, 1999). Basil is considered a spicy food crop, and therefore it is an ideal candidate for inclusion in food production during space-flight missions and on extraterrestrial outposts.

Most plants require light to complete the seed-to-seed life cycle (Taiz and Zeiger, 2006). Space-flight missions cannot rely on the sun for lighting for plant growth, as is common on Earth, because the atmosphere of the Earth, which filters out many harmful wavelengths of light, is absent. Thus, missions into space must use appropriate irradiance supplied by artificial lighting systems (Salisbury, 1991). Research is needed to determine the amount of irradiance at which photosynthesis saturates in order to know the energy inputs needed for successful plant production (Frantz et al., 2004). Integrating the optimal irradiance along with optimal temperature, carbon dioxide enrichment, relative humidity, and mineral nutrition could make edible plant production in space quite efficient when energy inputs are compared with edible biomass output. It may be possible to develop a food-production system in spacecraft and outposts that utilizes minimal energy inputs for lighting and mineral nutrition and still provides adequate food supplies for astronauts.

Human body waste products produced by astronauts may contain unpredictable total nitrogen, nitrate, and ammonium content. The recycling and reprocessing of waste product materials generated by astronauts' human bodies could prove useful as ingredients in nutrient solutions for long-duration missions and extraterrestrial controlled-environment plant production. Commercial and hobby hydroponic growers utilize organic materials such as fish emulsion in nutrient solution formulations (Succop and Newman, 2004). Therefore, it may be possible to incorporate recycled and processed human waste products in controlled-environment, plant-production systems such as the International Space Station and lunar/Mars habitats. Knowing the total amount of nitrogen required and the nitrate : ammonium ratio tolerance for basil may benefit astronauts whose role it is to establish, maintain, and troubleshoot food production systems during the long-duration missions. This knowledge may be applied as a starting point for formulating nutrient solutions for a nearly sustainable controlled-environment food-production system.

Incorporation of these concepts of irradiance saturation, total nitrogen required, and the nitrate-N : ammonium-N ratio into a single controlled-environment system would be ideal. This potentially could result in a food-production system that uses the least amount of energy inputs for lighting and efficient reuse, after processing, of astronaut waste products for the nutrient solution. This may, in turn, prove to be a nearly self-sustaining system that would be ideal for lunar and Mars habitats.

The overall purpose of this research was to determine specific values for controlled-environment parameters that control optimal edible biomass production of basil. The International Space Station served as our model controlled-environment. My work mainly focused on two areas: the irradiance saturation point for optimizing growth and edible biomass production in growth chambers, and mineral nutrition specific to the total amount of nitrogen needed and the optimal nitrate-N : ammonium-N ratio for maximum edible biomass production.

Thesis Organization

This thesis is divided into four chapters. The first chapter explains background information important to this research, and why this research was performed. Chapter two is a manuscript on irradiance saturation for overall basil growth and edible biomass production in controlled environments. Chapter three is a manuscript on the total nitrogen concentration requirement and the nitrate-N : ammonium-N ratio that produced the greatest edible biomass with nontoxic nitrate-N content for basil in silica-sand culture. Chapter four is an overall summary of the research that was conducted. All work contained in this thesis was conducted by Angela R. Beaman as part of her Master of Science work. The coauthors on the manuscripts worked in an advisory capacity only.

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BASIL REQUIRES AN IRRADIANCE OF 500 µMOL/M^2/S FOR GREATEST EDIBLE BIOMASS PRODUCTION

Abstract

Our objective was to determine the optimal irradiance for greatest edible biomass production of three cultivars of basil (*Basilicum ocimum* L.) in a controlled-environment production system. Preliminary experiments showed basil required a dark period, but the duration of the dark period was not resolved. There was no irradiance × cultivar interaction, but main effects of irradiance and cultivar were observed. Growth was least at 300 µmol/m^2/s and greatest at 500 or 600 µmol/m^2/s. In several cases, 400 µmol/m^2/s yielded an intermediate amount of growth or edible biomass. Within the main effect of cultivar, Italian Large Leaf produced greater edible biomass than Genovese, and Nufar yielded an intermediate amount of plant fresh weight and dry weight. Under our environmental conditions, photosynthesis saturated at 500 µmol/m^2/s, and no additional growth or accumulation of edible biomass occurred at 600 µmol/m^2/s. Based on our results, canopy-level irradiance should not exceed 500 µmol/m^2/s to conserve energy while enabling the greatest edible biomass production of basil in a controlled-environment production system.

Introduction

Long-term, extraterrestrial missions require accurate and precise use of area, volume, energy, and time for every task, particularly food production (Wheeler et al., 1996; 2008). Space-flight horticulturists typically do not have access to solar radiation, and artificial lighting becomes essential for crop production in extraterrestrial facilities such as the International Space Station, where energy generation and conservation is mandatory (Wheeler et al., 2008). A key factor for successful food-crop production in controlled life-support systems and future lunar and Mars missions is the capacity to produce sufficient edible biomass within the lowest possible area, volume, and energy inputs, such as irradiance.

Crops that are components of salads, such as lettuce (*Lactuca sativa* L.), have been selected for use in extraterrestrial food-production facilities and the advanced life-support program of the National Aeronautics and Space Administration (NASA) (Wheeler et al., 1996). Basil (*Basilicum ocimum* L.) is considered a counterpart crop to lettuce, and therefore, results from lettuce research might be applied directly to basil production (Morgan, 2005). Lettuce production quadrupled when plants were treated with a combination of high photosynthetic photon flux (PPF) (1000 μmol/m^2/s), high temperature, and elevated carbon dioxide concentrations (Frantz et al., 2004). However, lettuce exposed to a supraoptimal irradiance exhibited tip-burn damage (Seginer et al., 2006). In lettuce, biomass production is cultivar-dependent, and some lettuce cultivars exhibited decreased edible quality under constant illumination (Knight and Mitchell, 1983a; 1983b). In addition, supraoptimal irradiance and temperatures inhibit lettuce development and subsequent growth (He et al., 2001). Accumulation of dry matter in lettuce transplants was greater when the irradiance was less than 500 μmol/m^2/s for a longer period of time compared with transplants treated with irradiance greater than 500 μmol/m^2/s for shorter periods of time, and this indicated that daily light integral may be a key factor in edible biomass production of salad crops (Koontz and Prince, 1986; Oda et al., 1989).

Photosynthesis in most C_3 plants saturates at around 500 µmol/m^2/s (Taiz and Zeiger, 2006), and basil may follow a similar pattern. The growth responses of basil to different irradiances have not been studied, and development of a system that optimizes edible biomass production of basil will be required for use in extraterrestrial outposts. A principal component of this controlled-environment system will be the amount and type (source) of irradiance that will be required.

In addition, there may be health issues connected with the optimization of basil production. Essential oil content in basil increased with greater irradiance, whereas the concentration of the principal component of the essential oil, methyleugenol, decreased with UV-B treatment (Nitz and Schnitzler, 2004). Methyleugenol is carcinogenic in large amounts, and therefore, the reduction in the concentration of this compound with the increase in irradiance would be beneficial for human health (Nitz and Schnitzler, 2004). Our objective was to determine the optimal irradiance for the greatest edible biomass production of three cultivars of basil in a controlled-plant environment.

Materials and Methods

Plant growth. The basil cultivars Genovese, Italian Large Leaf, and Nufar (Johnny's Selected Seeds, Inc., Winslow, ME) were used. Seeds germinated in Oasis® LC-1 Horticubes® (Smithers-Oasis North America, Kent, OH) for 10 to 12 d in a growth chamber maintained at 25 ± 4 °C, with a 16-h photoperiod from cool-white fluorescent and incandescent lamps. During germination, irradiance at the canopy was 150 to 200 µmol/m^2/s. Seeds imbibed deionized water the first 24-h, and then they were fertigated with full-strength modified Hoagland's Solution No. 1 (Hoagland and Arnon, 1950) every 12-h until transplanting, which was done when the first two true leaves were ≥ 0.5 cm long. Hoagland's Solution No. 1 was modified by adjusting to pH 5.6 and using Sprint® 330 iron chelate (Becker Underwood, Inc., Ames, IA) at a diluted rate of 10 mg Fe/L fully chelated DTPA iron as the iron source in the final nutrient solution. Seedlings were selected for uniformity, and each LC-1 Horticube® with one plant was transplanted into a 12.5-cm top diameter standard pot filled with Sunshine® LC-1 soilless substrate (SunGro Horticulture, Bellevue, WA). Plants grew in Conviron® reach-in growth chambers (Model # E-15) maintained at 25 ± 4 °C. In a preliminary experiment, we grew plants for 15 d under continuous irradiation provided by a mixture of cool-white fluorescent and incandescent lamps. In subsequent experiments, four chambers were each set for a canopy irradiance of 300, 400, 500, or 600 µmol/m^2/s with a 16-h photoperiod provided by a mixture of cool-white fluorescent and incandescent lamps. Fifteen plants of each cultivar were placed into each chamber in a completely randomized design, and they grew for 29 d. Each chamber was assigned randomly to the specific irradiance treatment during each replication. Plants were fertigated to excess as needed with modified full-strength Hoagland's Solution No. 1. Plant height, plant canopy diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, and shoot fresh weight were recorded on day 29. Subsequently, individual shoots were placed into individual paper bags and placed into a 67 °C dryer for 7 d before shoot dry weight was determined.

Statistical analyses. The experiment was replicated three times over time. A completely randomized design was used with 12 treatments resulting from a factorial combination of four irradiances and three cultivars. Plant growth and edible biomass production parameters were plotted against irradiance, and the significance of linear or quadratic regression was analyzed by using ANOVA in SAS® ($P \leq 0.05$) (SAS Institute, 2003). Mean separation tests (Fisher's least significant difference tests) were completed by using PROC GLM in SAS® ($P \leq 0.05$) (SAS Institute, 2003) to determine the presence of interaction effects between irradiance and cultivar and main effects of irradiance and cultivar.

Results

Preliminary investigations showed basil required a dark period because plants grown for 15 d under 24-h irradiance exhibited stunting, chlorosis, and development of leaf necrosis, starting at the leaf tip and progressing to the leaf margin and then the midvein. The same plants also exhibited lignified stem tissue and an unusual dark green coloration on all shoot tissues.

There was no irradiance × cultivar interaction, but main effects of irradiance and cultivar were observed. Plants that received an irradiance of 500 or 600 µmol/m^2/s were taller than those that received 300 or 400 µmol/m^2/s (Fig. 1A). Plants that received 500 or 600 µmol/m^2/s had a greater canopy diameter than plants receiving 400 µmol/m^2/s, with 300 µmol/m^2/s leading to an intermediate value (Fig. 1B). Plants that received 500 or 600 µmol/m^2/s had more leaves $\geq$ 0.5 cm long than plants that received 300 µmol/m^2/s, with values at 400 and 600 µmol/m^2/s intermediate (Fig. 1C). Regression analyses indicated linear and quadratic relationships between irradiance and plant height ($P \leq 0.0001$, $P \leq 0.0001$, respectively), plant canopy diameter ($P = 0.003$, $P = 0.0074$, respectively), and number of leaves $\geq$ 0.5 cm long ($P \leq 0.0001$, $P \leq 0.0001$, respectively). Plant height, plant diameter, and number of leaves that were $\geq$ 0.5 cm long correlated with irradiance between 300 and 600 µmol/m^2/s (Fig. 1A – 1C).

Leaf fresh weight, plant fresh weight, and plant dry weight were greatest at 500 and 600 µmol/m^2/s, lowest at 300 µmol/m^2/s, and intermediate values were obtained at 400 µmol/m^2/s (Fig. 2A – 2C). Regression analyses showed linear and quadratic relationships between irradiance and leaf fresh weight ($P = 0.0004$, $P = 0.0004$, respectively), plant fresh weight ($P \leq 0.0001$, $P \leq 0.0001$, respectively), and plant dry weight ($P \leq 0.0001$, $P \leq 0.0001$, respectively). Edible biomass yield correlated with irradiance between 300 and 600 µmol/m^2/s (Fig. 2A – 2C).

There were cultivar main effects for plant height, plant diameter, leaf fresh weight, plant fresh weight, and plant dry weight (Table 1). ‘Italian Large Leaf’ and ‘Nufar’ were shorter plants with a greater diameter than plants of ‘Genovese’ (Table 1). ‘Italian Large Leaf’ had more edible biomass (leaf fresh weight, plant fresh weight, and plant dry weight) than ‘Genovese’, and ‘Nufar’ exhibited an intermediate amount of edible biomass (Table 1).

Discussion

Our results suggest the photosynthetic capacity of basil saturated at an irradiance of about 500 µmol/m^2/s, and little, if any, additional growth occurred in plants that received 600 µmol/m^2/s. The irradiance saturation point for photosynthesis determined in this research is consistent with research on tropical conditions that inhibit lettuce growth (He et al., 2001) and C_3 plants in general (Taiz and Zeiger, 2006). We recommend lighting that does not exceed 500 µmol/m^2/s to conserve energy while enabling the greatest edible biomass production of basil in a controlled-environment facility. This research investigated only instantaneous irradiance during a photoperiod of 16-hr, not continuous irradiance, because preliminary results showed basil suffered stem and foliar injury during a continuous photoperiod. The length of light and dark periods that maximize edible biomass production of basil in controlled-environment production requires further investigation. This is the first report of basil requiring a dark period. Also, if environmental parameters such as temperature, relative humidity, and carbon dioxide concentration differ from our experimental conditions, the optimal irradiance for optimal edible biomass production of basil may differ, and this also requires further investigation.

The production of edible biomass in herbaceous plants such as basil requires maintenance of vegetative tissues and preventing the development of reproductive tissues. Therefore, control of flowering in basil is important. The actual metabolic and biochemical mechanisms are still unknown for what causes the change from vegetative growth to reproductive growth (i.e. flowering) in basil. However, the concept of daily light integral has proven useful in determining how to time flowering in other plants. Determination of the role of daily light integral in orchid (*Phalaenopsis*) production has removed much of the uncertainty associated with synchronizing orchids to flower in response to market demand (Wang, 1995). Similarly, work on ornamental annual species has shown great promise for manipulation of plant growth and control of flowering (Moccaldi and Runkle, 2007; Warner and Erwin, 2005). Use of daily light integral, along with temperature and relative humidity regimens used for lettuce production, may be the key to understanding how to prevent basil from flowering and becoming determinate, thereby preserving qualities that make the basil more palatable for a longer time (Nitz and Schnitzler, 2004).

Keeping basil vegetative also may optimize food safety, flavor, and aroma. Two basil cultivars contained elevated nitrate content when grown under 50% irradiance reduction, whereas plants grown under 0% light reduction had a nitrate content acceptable for human consumption (Raimondi et al., 2006). Because manipulation of irradiance and photoperiod of bedding plants can increase chlorophyll concentration (Langton et al., 2003), it is plausible that optimal irradiance during production of salad crops such as lettuce and basil also may increase carotenoid concentration as well (Mou, 2005).

Commercial basil growers select cultivars based on their growth habit, production of biomass, and disease resistance qualities (Morales and Simon, 1997; Morgan, 2005; Raimondi et al., 2006; Reuven et al., 1998). ‘Italian Large Leaf’ and ‘Nufar’ would be good candidates for long-term space missions because they produce more biomass in less area and volume than ‘Genovese’. Compared with ‘Genovese’, ‘Italian Large Leaf’ and ‘Nufar’ produced shorter plants with greater diameter. This would be important for long-term missions where area and volume for food production are limited and must be used efficiently (Wheeler et al., 2008).

Production of edible biomass of basil is optimized at an irradiance of 500 µmol/m^2/s using our environmental conditions of 25 ± 4 °C, controlled relative humidity, no carbon dioxide enrichment, and mineral nutrition using a modified Hoagland's solution. Certainly, other combinations of environmental parameters such as temperature, relative humidity, carbon dioxide concentration, and mineral nutrition may produce a similar amount of basil in a unit of area, volume, or time, and these combinations need to be researched and explored. As it is with the production of any type of plant material, greater amounts of one or more environmental parameter may be used to increase productivity, but the efficiency of energy utilization may not be optimized appropriately for the particular species. Further research is also needed for investigating the food safety factors of nitrate and methyleugenol accumulation acceptable for human consumption within the edible tissues of basil when grown in controlled-environments.

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Table 1

Plant height, plant diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, plant fresh weight, and plant dry weight of three cultivars of basil grown at four irradiances. Plants grew in chambers for 29 d with a 16-h photoperiod at 25 ± 4 °C, and they were fertigated with full-strength modified Hoagland's Nutrient Solution No. 1. Data are means over all four irradiances.

Cultivar	Plant height (cm)	Plant diameter (cm)	Number of leaves ≥ 0.5 cm long	Leaf fresh weight (g)	Plant fresh weight (g)	Plant dry weight (g)
Genovese	19.7 a[z]	13.9 b	41 a	6.3 b	8.8 b	0.71 b
Italian Large Leaf	16.9 b	16.8 a	43 a	7.9 a	10.7 a	0.84 a
Nufar	13.9 c	16.8 a	37 a	7.4 a	9.9 ab	0.79 ab

[z] Means within each column followed by the same letter are not different at $P \leq 0.05$ according to Fisher’s least significant difference test. N = 180.

Figure Captions

Fig. 1. Plant height (A), plant diameter (B), and number of leaves $\geq$ 0.5 cm long (C) of 'Genovese', 'Italian Large Leaf', and 'Nufar' basil grown for 29 d in soilless medium in growth chambers maintained at irradiances of 300, 400, 500, and 600 μmol/m^2/s provided by a mixture of cool-white fluorescent and incandescent lamps on a 16-h photoperiod at 25 ± 4 °C D/N. No cultivar × irradiance interaction was found, and therefore, data for the three cultivars were combined, and the values reflect means of 3 replicates with 60 observations per replicate.

Fig. 2. Leaf fresh weight (A), plant fresh weight (B), and plant dry weight (C) of 'Genovese', 'Italian Large Leaf', and 'Nufar' basil grown for 29 d in soilless medium in growth chambers maintained at irradiances of 300, 400, 500, and 600 μmol/m^2/s provided by a mixture of cool-white fluorescent and incandescent lamps on a 16-h photoperiod at 25 ± 4 °C D/N. No cultivar × irradiance interaction was found, and therefore, data for the three cultivars were combined, and the values reflect means of 3 replicates with 60 observations per replicate.

Figure 1

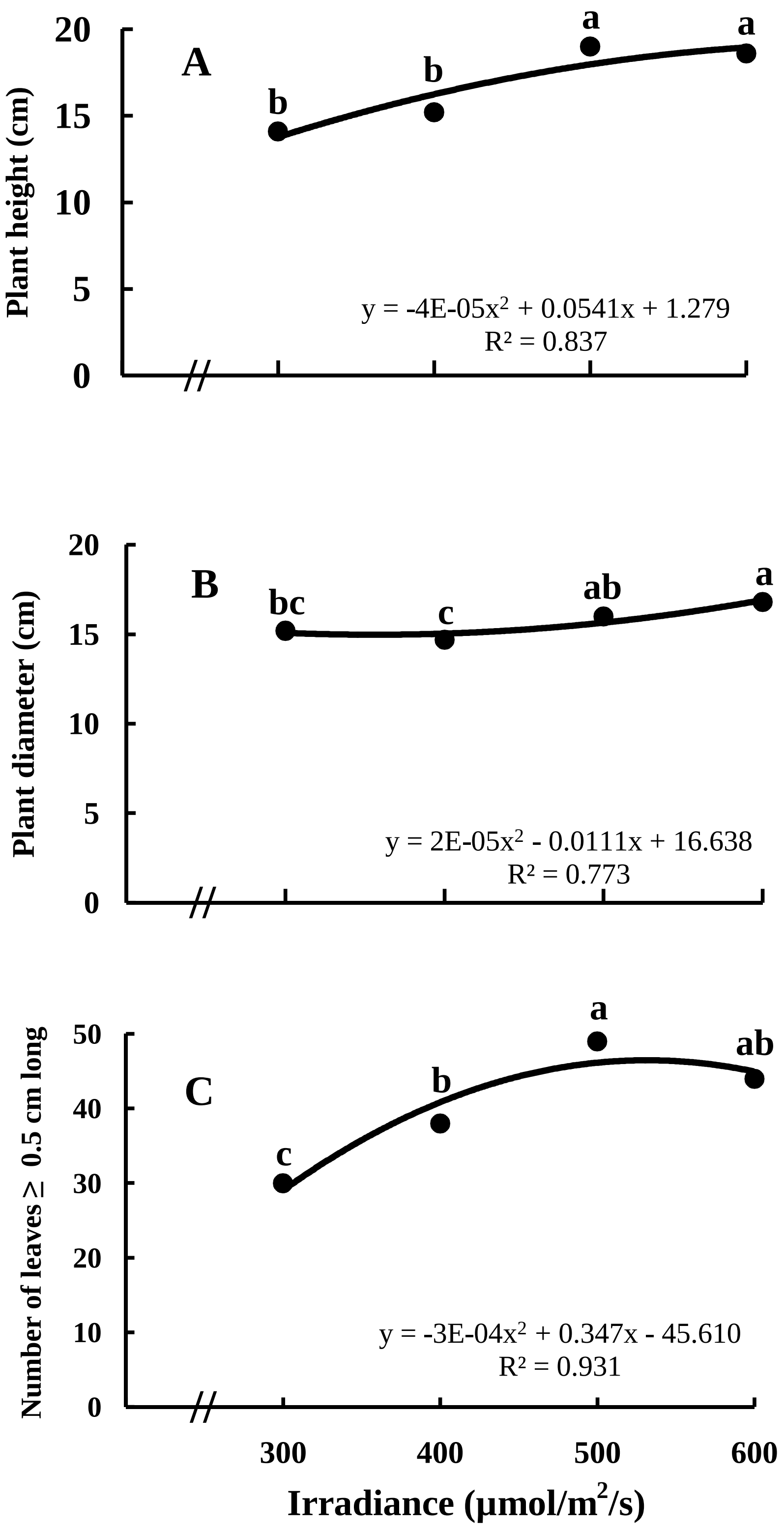
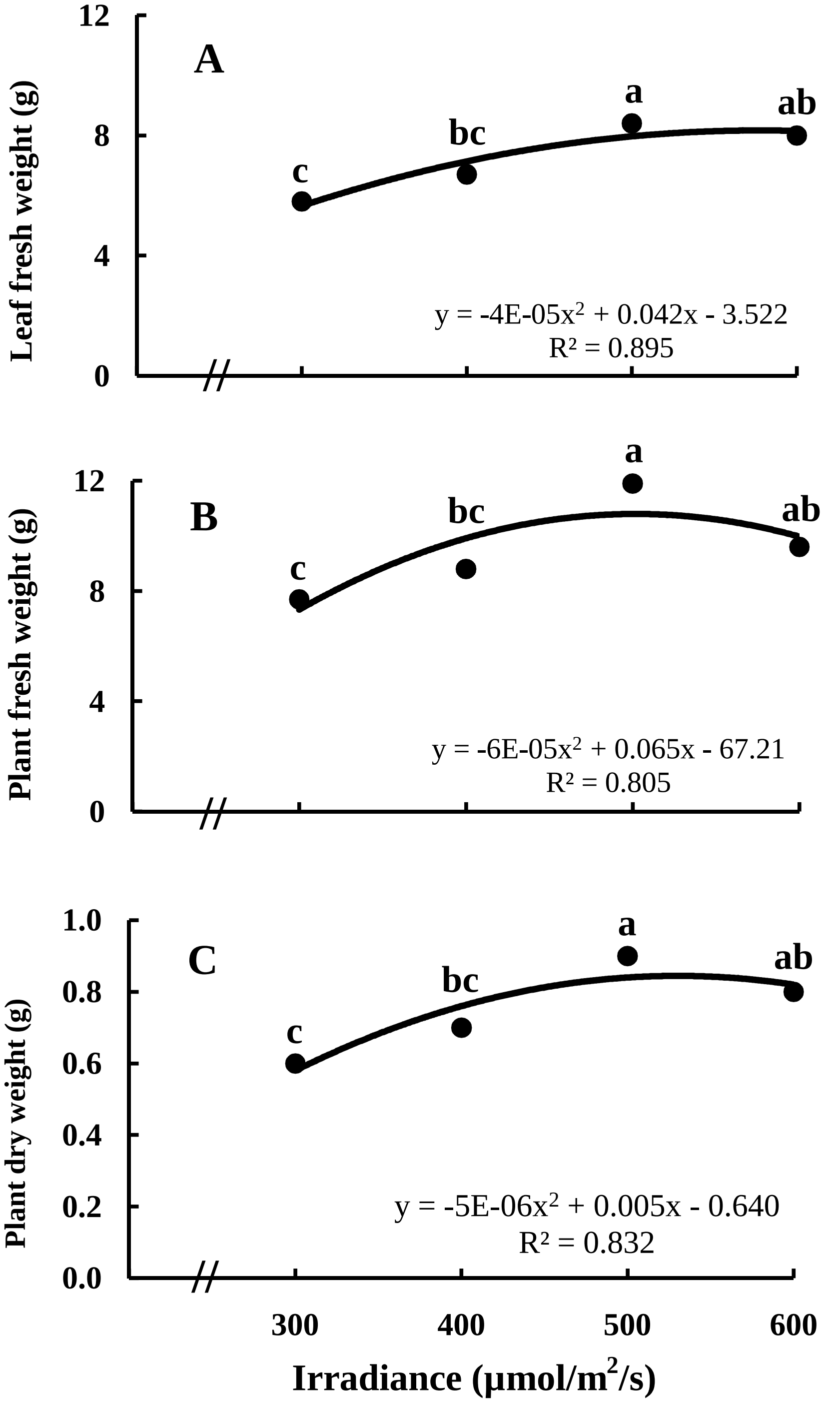

A
Plant height (cm)
20
15
10
5
0
b
b
a
a
y = -4E-05x² + 0.0541x + 1.279
R² = 0.837
B
Plant diameter (cm)
20
15
10
5
0
bc
c
ab
a
y = 2E-05x² - 0.0111x + 16.638
R² = 0.773
C
Number of leaves ≥ 0.5 cm long
50
40
30
20
10
0
c
b
a
ab
y = -3E-04x² + 0.347x - 45.610
R² = 0.931
300
400
500
600
Irradiance (µmol/m²/s)

Figure 2


12
A
a
ab
8
bc
c
4
y = -4E-05x² + 0.042x - 3.522
R² = 0.895
0
Leaf fresh weight (g)
a
12
B
bc
ab
c
8
4
y = -6E-05x² + 0.065x - 67.21
R² = 0.805
0
Plant fresh weight (g)
1.0
a
C
ab
0.8
bc
c
0.6
0.4
0.2
y = -5E-06x² + 0.005x - 0.640
R² = 0.832
0.0
Plant dry weight (g)
300
400
500
600
Irradiance (µmol/m²/s)

TOTAL N REQUIREMENT AND NITRATE-N : AMMONIUM-N RATIO FOR OPTIMAL EDIBLE BIOMASS PRODUCTION OF BASIL

Abstract

Our objectives were to determine the total nitrogen (N) concentration requirement and the best ratio of nitrate-N : ammonium-N for the greatest edible biomass production of two cultivars of basil (*Basilicum ocimum* L.). Optimal edible biomass production occurred at 100 to 250 mg N/L at a ratio of 50:50 nitrate-N : ammonium-N. There was no interaction effect between cultivar and total N concentration or cultivar and nitrate-N : ammonium-N ratio, but there were main effects of cultivar, total N concentration, and nitrate-N : ammonium-N ratio. All plants died in the 100% ammonium-N treatment. Regression models that allow analysis for optimum conditions suggest a nitrate-N : ammonium-N ratio of 50:50 to 75:25 yielded the greatest biomass of basil.

Introduction

Long-term space missions require accurate, precise, and efficient use of space, energy, and time for every task, including those associated with food production (Wheeler et al., 1996). Salad crops such as lettuce have been selected for use in edible biomass production facilities and the advanced life-support program of the National Aeronautics and Space Administration (NASA) (Wheeler et al., 2006; 2008). Basil has been included in the choices for salad crop production, as it can be used in place of lettuce or as an additional component of salads. In Earth-based commercial production, growers typically switch from lettuce to basil when the ambient temperature becomes too hot for effective lettuce production (Morgan, 2005). Thus, lettuce is considered a counterpart crop with basil, and therefore, results from lettuce research may be applied to basil production in some instances.

Space-flight horticulturists typically do not have access to an easily obtainable supply of minerals for fertilizers and nutrient solutions for edible biomass (food) production. Therefore, processing and recycling of astronaut human body waste products may be essential for successful food production in controlled-environment outposts such as the International Space Station or lunar or Mars habitats.

The amount of absorption and utilization of N by plants is greater than the absorption and utilization of all other essential elements combined (Epstein and Bloom, 2005). Nitrogen is the key element responsible for protein manufacture and several edible biomass qualities including leaf fresh weight, plant fresh weight, plant size, and chlorophyll content (Bar-Tal et al., 2001a, 2001b; Dorais et al., 2001). Nitrogen is available to plants in either the nitrate form or the ammonium form, and different plant species respond differently to the two forms of N in nutrient solution (Epstein and Bloom, 2005). Most plants grow best in a particular combination of these two forms of N, and the specific ratio of the forms of N may be critical to successful production of a given crop. For example, food crops such as bell pepper (Marti and Mills, 1991), cucumber (Heuer, 1991), taro (Osorio et al., 2003), tomato (Errebhi and Wilcox, 1990; Pivot et al., 1997), and maize (Xu et al., 1992) seem to grow best if they are provided a combination of nitrate-N and ammonium-N. In addition, the World Health Organization (WHO) and the European Union have established standards for the nitrate and nitrite content of food products, and we used these standards to determine what nitrate-N:

ammonium-N ratio might be safe for consumption by astronauts (WHO, 2007; European Communities, 2008).

Our primary objectives were to determine the optimal total N concentration for the greatest edible biomass production of basil and to determine the optimal nitrate-N : ammonium-N ratio for the greatest edible biomass production of basil. Secondary objectives were to determine whether there are interaction effects between the total concentration of N applied and the cultivars of basil, and the nitrate-N: ammonium-N ratio and cultivar of basil. On a preliminary basis, we wanted to determine whether the basil produced would have a nitrate content that would be safe for human consumption, based on the standards established by the World Health Organization and the European Union (WHO, 2007; European Communities, 2008).

Materials and Methods

Plant growth and handling

Total N concentration experiment. *Ocimum basilicum* L. 'Italian Large Leaf' and 'Nufar' (Johnny's Selected Seeds, Inc., Winslow, ME) were used. Seeds germinated in Oasis® LC-1 Horticubes® (Smithers-Oasis North America, Kent, OH) for 10 to12 d in a growth chamber maintained at 25 ± 3 °C, with a 16-h photoperiod provided by cool-white fluorescent and incandescent lamps. Seeds imbibed deionized water for the first 24-h, and they were then fertigated with modified full-strength Hoagland's Solution No. 1 (Hoagland and Arnon, 1950), with pH adjusted with KOH to 6.0 to 6.5, every 12-h until transplanting when the first two true leaves were ≥ 0.5 cm long. Hoagland's Solution No. 1 was modified by using Sprint® 330 iron chelate (Becker Underwood, Inc., Ames, IA) at a diluted rate of 10 mg Fe/L. During germination and early growth, canopy PAR averaged 150 to 200 μmol/m^2/s (Li-Cor® LI-185A; LI-COR Inc., Lincoln, NE). Each LC-1 Horticube® with one plant was transplanted into a 12.5 cm standard pot filled with silica-sand (Unimin® #2040, Unimin® Corporation, New Canaan, CT), placed on a greenhouse bench, and fertigated to excess daily with 250-ml of the respective total N concentration treatment for 16 d. Total N concentrations were based on a modified Hoagland's solution that included five total N concentrations (50, 100, 150, 200, and 250 mg N/L) with the N supplied in a 50:50 ratio of nitrate-N : ammonium-N (Romero et al., 2006), with exception of pH adjustment of the nutrient solution to 6.0 to 6.5 by using 1M KOH at approximately 0.07 ml/L of nutrient solution. The greenhouse was maintained at 25 ± 5 °C. Supplementary light provided by high-pressure sodium lamps (SunSystemIII, Sunlight Supply Inc., Vancouver, WA) was maintained at a canopy irradiance of 400 to 550 μmol/m^2/s in addition to natural irradiance. Fifteen plants of each cultivar were arranged in a completely randomized design and grew for 16 d. Plants were fertigated daily to excess with the randomly assigned total N concentration treatments from day of transplanting to day 27. Plant height, plant diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, and plant fresh weight were recorded on day 27. The entire plant shoot then was placed in a 67 °C dryer for seven days, and total shoot dry weight was determined.

Nitrate-N: ammonium-N ratio experiment. The same protocols for seed germination, controlled-environment specifications, treatment administration, and plant measurement at day 27 were used in this experiment as were done for the total N concentration experiment. 'Italian Large Leaf' and 'Nufar' also were used in the nitrate-N: ammonium-N ratio experiment. The five nitrate-N: ammonium-N ratios used were 0:100, 25:75, 50:50, 75:25, and 100:0, using a modified Hoagland's solution that included a total N concentration of 200 mg N/L (Romero et al., 2006). The pH adjustment of the nutrient solution to 6.0 to 6.5 was done by using 1M KOH at approximately 0.07 ml/L of nutrient solution. The differences in K concentration among treatments were considered insignificant as a confounding effect. After drying, and dry weight determination, the entire plant shoot tissue from one replication was analyzed in duplicate for inorganic nitrate content by the cadmium-reduction method at the Department of Agronomy Soil and Plant Testing Laboratory at Iowa State University (Missouri Ag. Expt. Stn. Pub. 1998).

Statistical analyses

Total N experiment. This experiment was replicated three times over time in a completely randomized design with five total N concentrations in factorial combination with two basil cultivars. Five samples from each cultivar and each treatment were measured at day 27. The experiment was replicated three times over time, and a total of 75 samples were recorded in this experiment. Linear and quadratic trends and analysis of variance were conducted by using ANOVA in PROC REG in SAS ($P \leq 0.05$) (SAS Institute, 2003). Mean separation tests were completed by using PROC GLM in SAS® (*P*

≤ 0.05) (SAS Institute, 2003) to determine interactions between treatments and the main effects of total N concentration and cultivar.

Nitrate-N: ammonium-N ratio experiment. This experiment was replicated three times over time in a completely randomized design with five nitrate-N: ammonium-N treatment ratios in factorial combination with two cultivars. Five samples from each cultivar and each treatment were measured at day 27. The experiment was replicated three times over time, and a total of 75 samples were recorded in this experiment. Linear and quadratic trends and analysis of variance were conducted by using ANOVA in PROC REG in SAS ($P \leq 0.05$) (SAS Institute, 2003). Mean separation tests were completed by using PROC GLM in SAS® ($P \leq 0.05$) (SAS Institute, 2003) to determine interactions between treatments and the main effects of nitrate-N : ammonium-N ratio and cultivar. Analysis of variance (ANOVA) was performed to quantify the effects of cultivar, nitrate-N : ammonium-N ratio, and cultivar by nitrate-N : ammonium-N ratio interaction on plant height, plant diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, plant fresh weight, and plant dry weight. Fisher's Least Significant Difference test (LSD) was performed to establish mean separations among individual cultivar and nitrate-N : ammonium-N ratio treatments. Linear and quadratic regression analyses were performed to summarize general relationships between the nitrate-N : ammonium-N ratio and plant height, plant diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, plant fresh weight, and plant dry weight. All analyses were assessed for significance at the $P \leq 0.05$ level. Equations from quadratic regressions that were significant were used to calculate optimal nitrate-N : ammonium-N ratios for each dependent variable.

Results

Total N concentration experiment

There were no interaction effects for cultivar × total N concentration for both plant growth parameters and edible biomass parameters. Within the plant growth parameters, ‘Italian Large Leaf’ plants were taller than ‘Nufar’ plants, and they had a greater number of leaves $\geq$ 0.5 cm long (Table 1). There were no cultivar differences for the edible biomass parameters leaf fresh weight, plant fresh weight, and plant dry weight (Table 1). Over the total N concentration treatments, and within the plant growth parameters, plants that received 50 mg N/L were shorter and had fewer leaves than plants that received 100 to 250 mg N/L (Figure 1 A - C). For the edible biomass parameters, plants that received 50 mg N/L exhibited less leaf fresh weight, plant fresh weight, and plant dry weight, and plants that received 100 to 250 mg N/L were not different from each other (Figure 2 A - C).

Regression analyses of the means for plant growth parameters showed plant height $P = 0.055$ and $R^2 = 0.945$, plant diameter $P = 0.069$ and $R^2 = 0.931$, and number of leaves $\geq$ 0.5 cm long $P = 0.470$ and $R^2 = 0.530$. Regression analyses of the means for edible biomass parameters showed leaf fresh weight $P = 0.155$ and $R^2 = 0.845$, plant fresh weight $P = 0.145$ and $R^2 = 0.855$, and plant dry weight $P = 0.148$ and $R^2 = 0.852$.

Regression analyses of the raw data for plant growth parameters showed plant height $P = 0.001$ and $R^2 = 0.085$, plant diameter $P = 0.080$ and $R^2 = 0.034$, and number of leaves $\geq$ 0.5 cm long $P = 0.030$ and $R^2 = 0.047$. Regression analyses of the raw data for edible biomass parameters showed leaf fresh weight $P \leq 0.0001$ and $R^2 = 0.181$, plant fresh weight $P \leq 0.0001$ and $R^2 = 0.195$, and plant dry weight $P \leq 0.0001$ and $R^2 = 0.168$. Regression optima (optimum results calculated from regression equations) for plant height, plant diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, total plant fresh weight, and total dry weight were achieved at 225, 164, 200, 197, 195 and 170 mg N/L, respectively. Results centered around 200 mg N/L based on regression optima from raw data analyses of the six parameters. Therefore, a total N concentration of 200 mg N/L was used in this experiment because this treatment exhibited the greatest plant growth and edible biomass (Figure 1 A – C and Figure 2 A – C, respectively), but the 200 mg N/L treatment did not exhibit statistical differences among any treatment other than 50 mg N/L.

Nitrate-N: ammonium-N ratio experiment

There were no interaction effects of nitrate-N : ammonium-N ratio × cultivar, but there were main effects of cultivar and nitrate-N : ammonium-N ratio. Within the nitrate-N : ammonium-N ratio experiment, ‘Italian Large Leaf’ grew taller than ‘Nufar’, but all other growth and edible biomass parameters showed no cultivar effect (data not shown). Growth of basil varied among the five nitrate-N: ammonium-N ratio treatments. Main effects were present for all six parameters for nitrate-N: ammonium-N ratios (data not shown). All plants died by day 23 as a result the 0% nitrate : 100% ammonium treatment. The plant growth parameters of height and number of leaves ≥ 0.5 cm long were greatest at 50% nitrate-N : 50% ammonium-N and 75% nitrate-N : 25% ammonium-N, and diameter was greatest when the nutrient solution had ≥ 50% nitrate-N

(Fig. 3 A – C). Height and number of leaves ≥ 0.5 cm long declined at 100% nitrate-N : 0% ammonium-N (Fig. 3 A – C). Linear regression analysis produced differences in plant height ($P \leq 0.0001$), plant diameter ($P \leq 0.0001$), and number of leaves ≥ 0.5 cm long ($P \leq 0.0001$). Quadratic regression analysis produced differences in plant height ($P \leq 0.0001$), plant diameter ($P \leq 0.0001$), and number of leaves ≥ 0.5 cm long ($P \leq 0.0001$).

For the edible biomass parameters of leaf fresh weight, plant fresh weight, and plant dry weight, the 0% nitrate-N : 100% ammonium-N treatment caused all plants to die (Fig. 4 A – C). Linear and quadratic regression models that allowed analysis for optimum conditions suggest that the best ratio of nitrate-N to ammonium-N will be between 50:50 and 75:25 nitrate-N : ammonium-N (Fig. 3 A – C and Fig. 4 A – C). Linear regression analysis produced differences in leaf fresh weight ($P \leq 0.0001$), plant fresh weight ($P \leq 0.0001$), and plant dry weight ($P \leq 0.0001$). Quadratic regression analysis produced differences in leaf fresh weight ($P \leq 0.0001$), plant fresh weight ($P \leq 0.0001$), and plant dry weight ($P \leq 0.0001$).

Discussion

Total N concentrations greater than 100 mg N/L did not produce greater edible biomass in basil when compared with the 100 mg N/L treatment. This agrees with the study on field basil investigating growth responses to combinations of N and S (Zheljazkov et al., 2008). This also agrees with the greenhouse study on basil treated with four different all nitrate-N concentrations (Suh et al., 1999). Rocket (*Eruca sativa* L.) dry matter increased when less nitrate-N was used in fertilization; and, again, results from this basil study concur with more edible biomass production resulting from reduced total N concentration in the mineral nutrition regimen (Nicola et al., 2005).

Edible biomass production of basil would not be possible if all of the N were in the ammonium-N form because our results exhibited 100% plant death in this treatment. This is the first report of basil not surviving with all ammonium-N mineral nutrition. We suggest that basil is not a suitable crop to grow using all ammonium-N nutrition whether or not the basil was produced under high light and/or high temperature conditions or other conditions. Some food crops such as tomato (*Lycopersicon esculentum* Mill.) can survive with high ammonium-N mineral nutrition, but basil is not one of them (Tan et al., 2000). Rice (*Oryza sativa* L.) and maize (*Zea mays* L.) produce more edible biomass when ammonium-N serves as the major mineral nutrition regimen (Magalhaes and Huber, 1991; Anderson et al., 1991). Endive (*Chicorium endive* L.) grown in an NFT system with an all ammonium-N nutrition regimen did not exhibit ammonium-N toxicity, and did not contain any nitrates in the edible biomass (Bonasia et al., 2008). Endive produced comparable edible biomass when treated with either all ammonium-N or a nitrate-N nutrition regimen, but the all ammonium-N treated samples were nitrate-free whereas the nitrate-N treated samples had accumulation of nitrate in the edible tissues (Santamaria and Elia, 1997). Habañero pepper (*Capsicum chinense* Jacq.) increased flower and fruit formation along with plant height when treated with all urea-based N (Medina-Lara et al., 2008). Basil grown in peat-based substrate in a greenhouse did not exhibit any growth differences when treated with either all nitrate-N fertilizer or ammonium-N fertilizer, but there was a greater nitrate content in the edible biomass in the all nitrate-N treatment (Tesi et al., 1995). Basil seems to be similar to potato (*Solanum tuberosum* L.) and taro (*Colocasia esculenta* L.) in that greater edible biomass is produced with a combination of nitrate-N and ammonium-N mineral nutrition (Epstein and Bloom, 2005; Serio et al., 2004; Osorio et al., 2003). Also, basil is similar to greenhouse pepper (*Capsicum annuum* L.) in that edible biomass production is retarded when high ammonium-N nutrition is introduced to the regimen (Bar-Tal et al., 2001a). We did find that basil growth was more effective with at least some nitrate-N added to the nutrient solution. Lettuce (*Lactuca sativa* L.) fresh weight is limited when N is restricted in the nutrient solution supply along with high irradiance (Henriques and Marcelis, 2000). Because basil is considered a countercrop to lettuce, it may be prudent to investigate how basil responds to restricted total N content in the nutrient solution in combination with difference irradiance values. Perhaps basil may increase edible production with restricted nitrate-N and reduced irradiance. The accumulation of nitrate in edible biomass may be an issue to address for future research.

No cultivar differences were present in either the total N concentration experiment or the nitrate-N : ammonium-N ratio experiment, and therefore, we suggest that either Italian Large Leaf or Nufar could be used for edible biomass production in controlled-environment food production systems, based on our experimental conditions.

In the total N concentration experiment, the 50 mg N/L treatment produced the only difference in all six plant growth parameters and edible biomass parameters results compared with the 100, 150, 200, and 250 mg/L total N concentration (Fig. 1 A – C and Fig. 2 A – C, respectively). The lack of statistical differences in plant growth and edible biomass production parameters above a certain total N concentration is similar to other studies done with basil (Succop and Newman, 2004).

The optimal total N concentration and nitrate-N : ammonium-N ratio was 100 mg N/L and 50:50 and 75:25, respectively, based on our linear and quadratic regression models. Our regression models exhibited no differences in the plant growth parameters and edible biomass parameters that would enable predictability. The use of greater amounts of nitrate-N in the nutrient solution for basil production may lead to greater rates of accumulation of nitrate-N within the edible portions of the plant tissue. The preliminary investigation to the total nitrate content of edible biomass showed a potential for toxicity to humans. This is the first report to identify the potential problems with nitrate accumulation in edible tissues of basil. Further research is needed to determine the optimal nitrate-N : ammonium-N ratio for safe consumption of basil produced in controlled environments by astronauts.

In each of the experiments, seedlings received 210 mg N/L in the nitrate-N form for the first 10 to 12 days, as provided by the modified Hoagland’s nutrient solution, and this amount may have been absorbed and the plants may have retained the required N for optimal growth. In retrospect, the seedlings should have been treated with the respective total N concentration and nitrate-N : ammonium-N ratio after imbibition of deionized water. The first 40% of the growth cycle was provided with adequate N for optimal growth (Epstein and Bloom, 2005). This may have influenced the final results in regards to cultivar differences, and differences in treatments for all six measured parameters. In the 50 mg N/L treatment, the seedlings may have consumed the entire N reserve, and therefore exhibited the differences in growth parameters compared with the 100, 150, 200, and 250 mg N/L treatments. The 100, 150, 200, and 250 mg N/L treated seedlings may not have consumed the entire N reserve, and therefore, did not exhibit any differences in growth parameters.

In conclusion, our research determined that either ‘Italian Large Leaf’ or ‘Nufar’ would produce about the same edible biomass in a controlled-environment food production system. A total N concentration of 100 mg N/L using a 50% nitrate-N : 50% ammonium-N ratio exhibited the greatest edible biomass production for plant height, plant diameter, number of leaves ≥ 0.5 cm, leaf fresh weight, plant fresh weight, and plant dry weight for basil. Basil cannot grow with all ammonium-N nutrition. A 50% nitrate-N : 50% ammonium-N to 75% nitrate-N : 25% ammonium-N exhibited optimal plant growth parameters and edible biomass parameters for basil.

Edible biomass production of basil possibly may be done using limited space, limited irradiance, and with the recycling of astronaut waste products for nutrient solutions. Further studies are required to determine if basil would have a similar lower nitrate content as other food crops when treated with lower irradiance (Vos and Van der Putten, 1998; Seginer et al., 2006). Based on the fact that these experiments included 210 mg N/L total N for the first 40% of the growth cycle, we hypothesize that basil requires a small amount of total N during the beginning of the growth cycle, and may produce edible biomass with lower total N through to the end of the growth cycle. Further research is needed to quantify this hypothesis. NASA would benefit from the knowledge gained in this study in helping to keep their operating costs lower for edible biomass production in spacecraft as well as within outposts.

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Table 1

Cultivar differences for plant growth parameters plant height, plant diameter, and number of leaves ≥ 0.5 cm long, and edible biomass parameters leaf fresh weight, total plant fresh weight, and total dry weight of two cultivars of basil subjected to five total N concentrations at day 27. Plants grew in a growth chamber for 10 to 11 d with a 16-h photoperiod provided by cool-white fluorescent and incandescent lamps, and a constant temperature of 25 ± 3 °C and fertigated with modified full-strength Hoagland's Nutrient Solution No. 1. At day 10 or 12 plants were placed into a greenhouse until day 27, and then transplanted into silica sand and they were fertigated daily with 250 ml of total N concentrations of 50, 100, 150, 200, or 250 mg N/L. The greenhouse was maintained at 25 ± 5 °C with high-pressure sodium lamps providing supplementary lighting in addition to natural light. Data are averaged over all five total N treatments.

Cultivar	Plant height (cm)	Plant diameter (cm)	Number of leaves ≥ 0.5 cm long	Leaf fresh weight (g)	Plant fresh weight (g)	Plant dry weight (g)
Italian Large Leaf	17.8 a^{z}	13.9 a	35 a	10.1 a	13.1 a	1.1 a
Nufar	14.5 b	14.2 a	31 b	10.7 a	13.3 a	1.1 a

^{z}Means within each column followed by an identical letter are not different at $P \leq 0.05$ according to Fisher’s least significant difference test. N = 150.

Figure Captions

Fig. 1. Plant height (A), plant diameter (B), and number of leaves ≥ 0.5 cm long (C) of 'Italian Large Leaf' and 'Nufar' basil subjected to five total N concentrations at day 27. At day 10 or 12 after germination were transplanted into silica sand, fertigated daily with 250 ml of total N concentrations of 50, 100, 150, 200, or 250 mg N/L (50:50 ratio of NO_3^--N:NH_4^+-N), and placed into a greenhouse until day 27. The greenhouse was maintained at 25 ± 5 °C with high-pressure sodium lamps providing supplementary lighting in addition to natural light. Data are averaged over both cultivars.

Fig 2. Leaf fresh weight (A), plant fresh weight (B), and plant dry weight (C) of 'Italian Large Leaf' and 'Nufar' basil subjected to five total N concentrations at day 27. At day 10 or 12 after germination plants were transplanted into silica sand, fertigated daily with 250 ml of total N concentrations of 50, 100, 150, 200, or 250 mg N/L (50:50 ratio of NO_3^--N:NH_4^+-N), and placed into a greenhouse until day 27. The greenhouse was maintained at 25 ± 5 °C with high-pressure sodium lamps providing supplementary lighting in addition to natural light. Data are averaged over both cultivars.

Figure Captions

Fig. 3. Plant height (A), plant diameter (B), and number of leaves ≥ 0.5 cm long (C) of ‘Italian Large Leaf’ and ‘Nufar’ basil subjected to five nitrate-N: ammonium-N ratios at day 27. At day 10 or 12 after germination plants were transplanted into silica sand, fertigated daily with 250 ml of nitrate-N: ammonium-N ratios of 0:100, 25:75, 50:50, 75:25, or 100:0 (using a total N concentration of 200 mg N/L) and placed into a greenhouse until day 27. The greenhouse was maintained at 25 ± 5 °C with high-pressure sodium lamps providing supplementary lighting in addition to natural light. Data are averaged over both cultivars.

Fig. 4. Leaf fresh weight (A), plant fresh weight (B), and plant dry weight of ‘Italian Large Leaf’ and ‘Nufar’ basil subjected to five nitrate-N: ammonium-N ratios at day 27. At day 10 or 12 after germination plants were transplanted into silica sand, fertigated daily with 250 ml of nitrate-N: ammonium-N ratios of 0:100, 25:75, 50:50, 75:25, or 100:0 (using a total N concentration of 200 mg N/L) and placed into a greenhouse until day 27. The greenhouse was maintained at 25 ± 5 °C with high-pressure sodium lamps providing supplementary lighting in addition to natural light. Data are averaged over both cultivars.

Figure 1

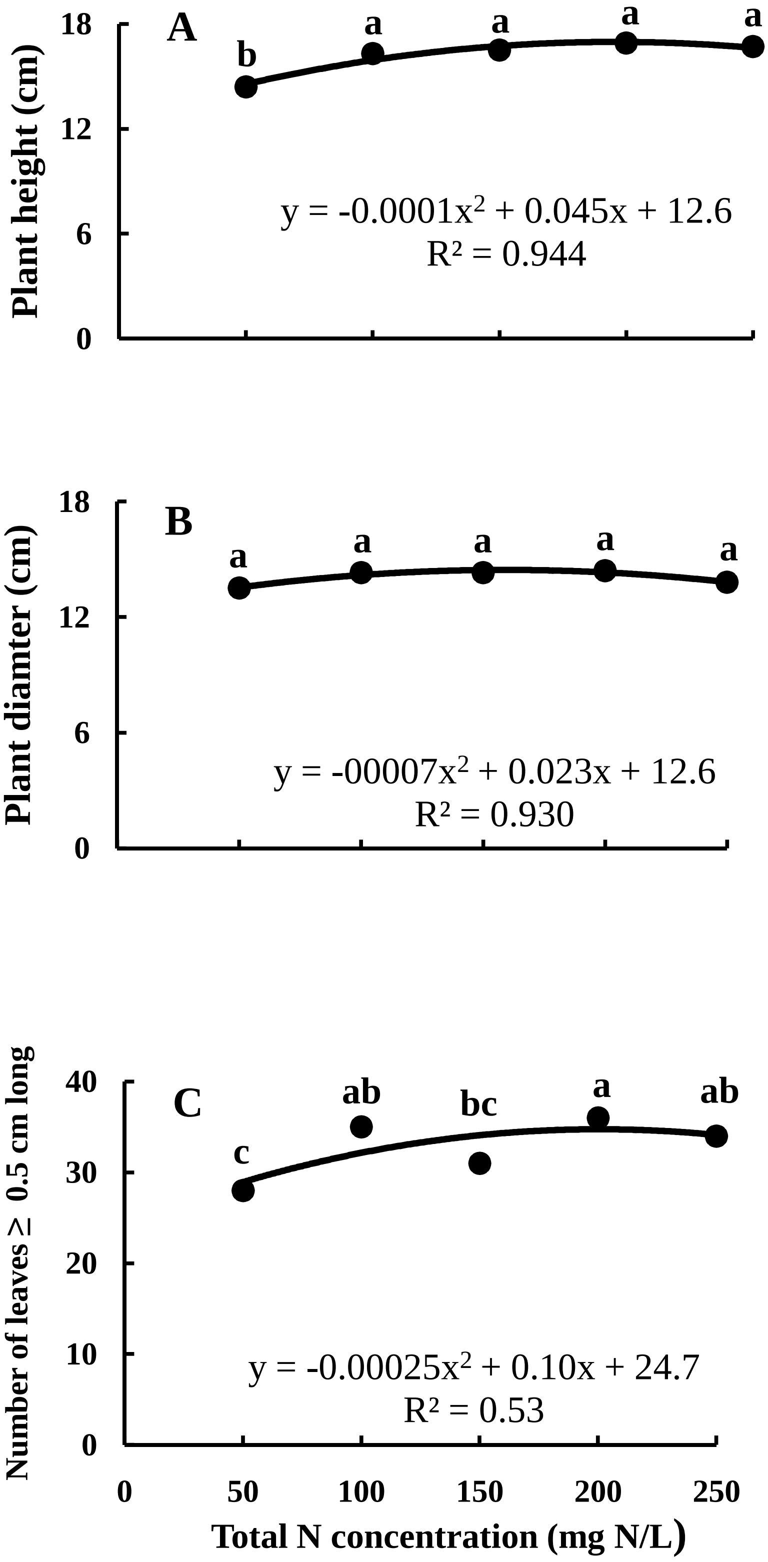

A
Plant height (cm)
18
12
6
0
b
a
a
a
a
y = -0.0001x² + 0.045x + 12.6
R² = 0.944
B
Plant diamter (cm)
18
12
6
0
a
a
a
a
a
y = -00007x² + 0.023x + 12.6
R² = 0.930
C
Number of leaves ≥ 0.5 cm long
40
30
20
10
0
c
ab
bc
a
ab
y = -0.00025x² + 0.10x + 24.7
R² = 0.53
0
50
100
150
200
250
Total N concentration (mg N/L)

Figure 2

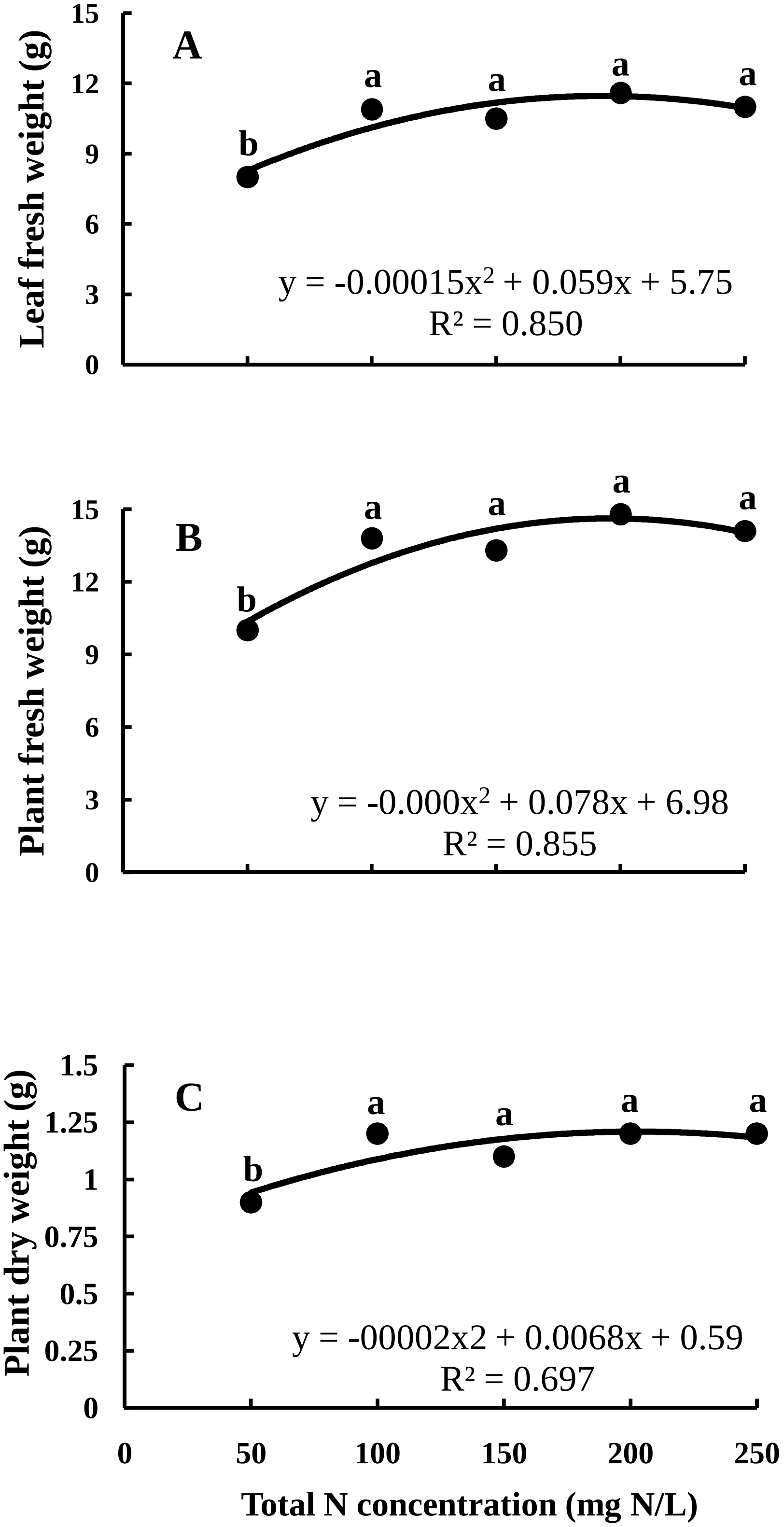

A
Leaf fresh weight (g)
15
12
9
6
3
0
b
a
a
a
a
y = -0.00015x² + 0.059x + 5.75
R² = 0.850
B
Plant fresh weight (g)
15
12
9
6
3
0
b
a
a
a
a
y = -0.000x² + 0.078x + 6.98
R² = 0.855
C
Plant dry weight (g)
1.5
1.25
1
0.75
0.5
0.25
0
b
a
a
a
a
y = -00002x2 + 0.0068x + 0.59
R² = 0.697
0
50
100
150
200
250
Total N concentration (mg N/L)

Figure 3

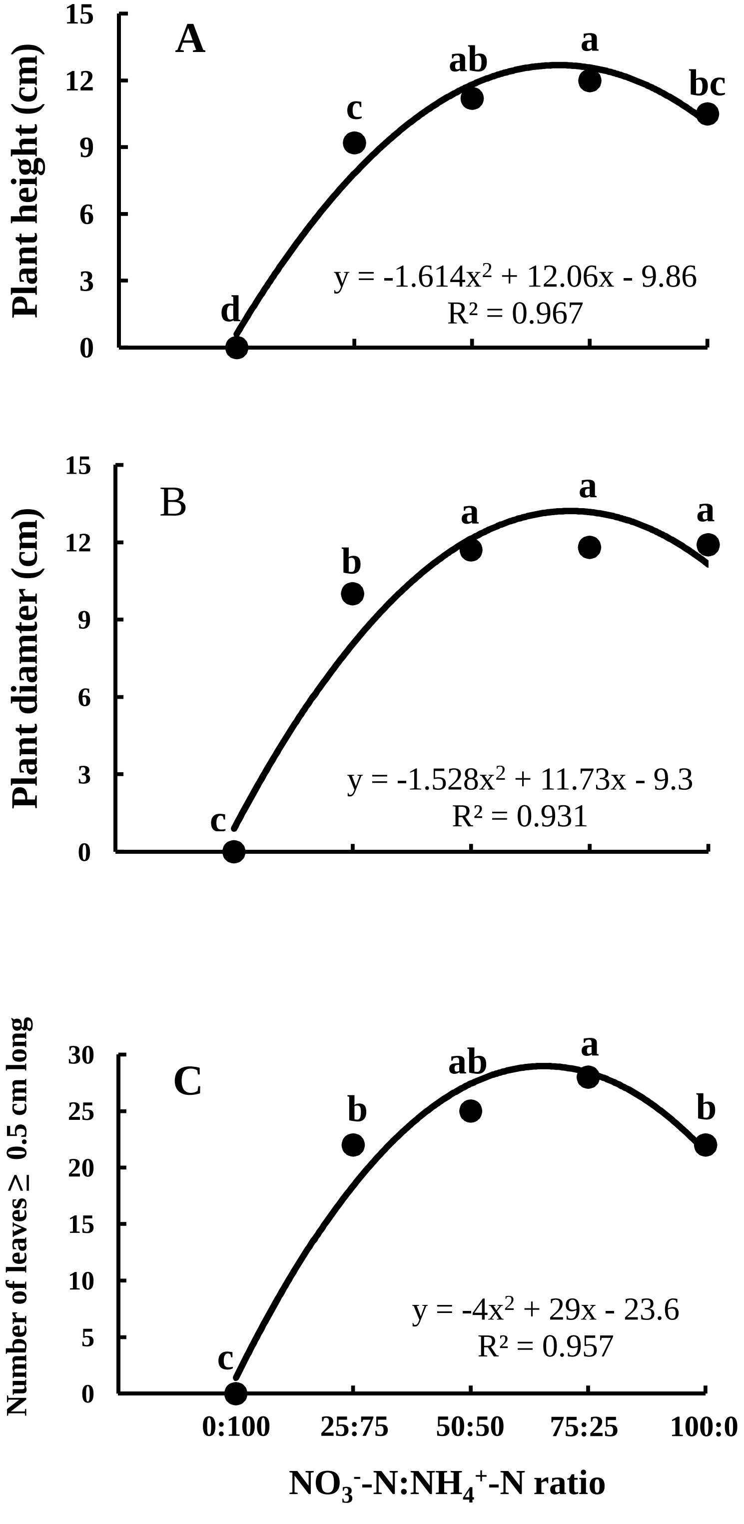
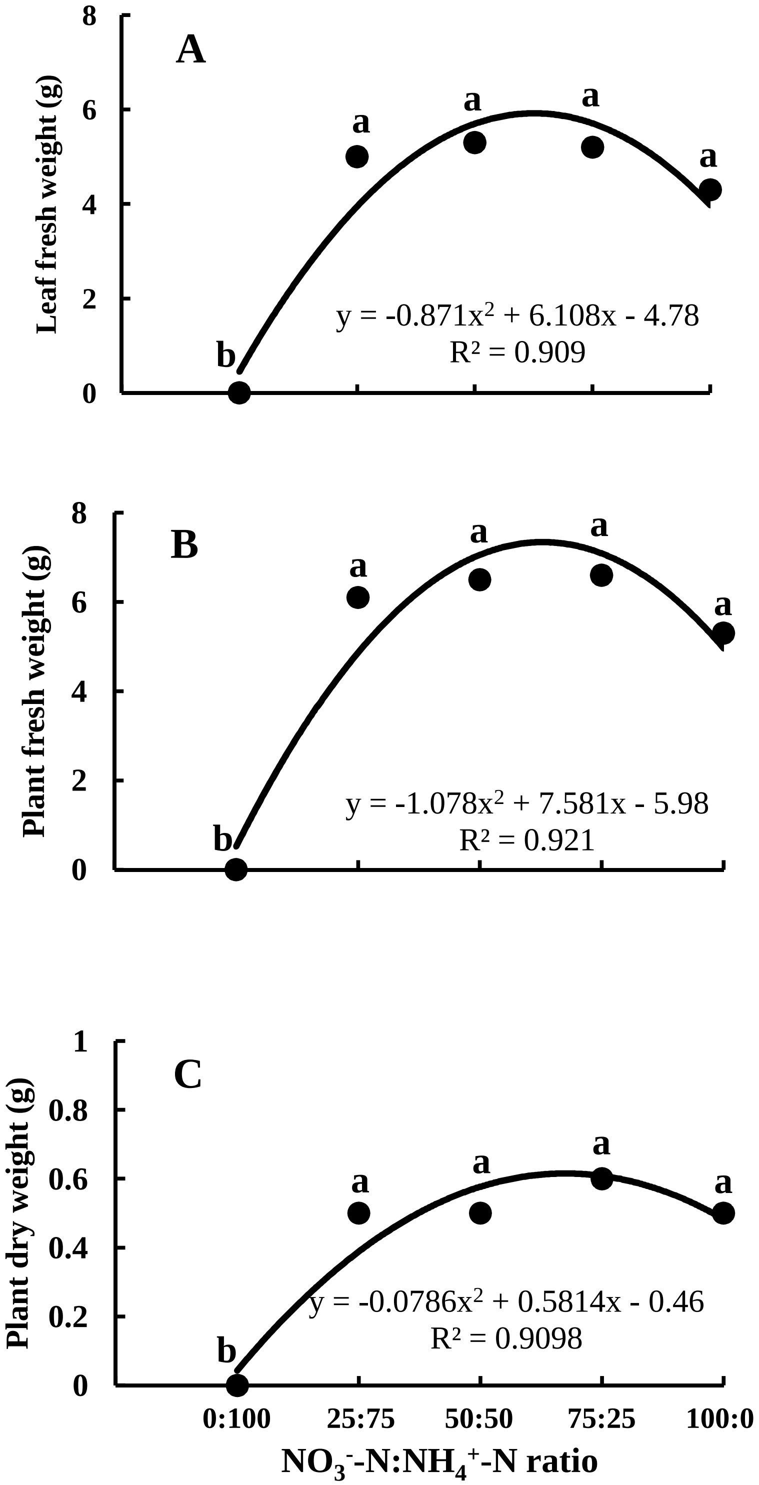

A
15
12
9
6
3
0
Plant height (cm)
d
c
ab
a
bc
y = -1.614x² + 12.06x - 9.86
R² = 0.967
B
15
12
9
6
3
0
Plant diamter (cm)
c
b
a
a
a
y = -1.528x² + 11.73x - 9.3
R² = 0.931
C
30
25
20
15
10
5
0
Number of leaves ≥ 0.5 cm long
c
b
ab
a
b
y = -4x² + 29x - 23.6
R² = 0.957
0:100
25:75
50:50
75:25
100:0
NO3⁻-N:NH4⁺-N ratio

Figure 4


A
Leaf fresh weight (g)
8
6
4
2
0
a
a
a
a
b
y = -0.871x² + 6.108x - 4.78
R² = 0.909
B
Plant fresh weight (g)
8
6
4
2
0
a
a
a
a
b
y = -1.078x² + 7.581x - 5.98
R² = 0.921
C
Plant dry weight (g)
1
0.8
0.6
0.4
0.2
0
a
a
a
a
b
y = -0.0786x² + 0.5814x - 0.46
R² = 0.9098
0:100
25:75
50:50
75:25
100:0
NO3-N:NH4+-N ratio

General Conclusions

Edible biomass production systems require a practical understanding of the irradiance saturation point and mineral nutrition requirements for efficient use of energy inputs such as irradiance and temperature and the output such as fresh, edible, plant material (Frantz et al., 2004). Extraterrestrial food production systems used on the International Space Station attempt to replicate ideal Earth-based conditions such as irradiance, photoperiod, and temperature, with the added challenges of conserving energy, area, volume, and other costs (Monje et al., 2002; Salisbury, 1991). In this research, I attempted to determine the optimal irradiance, total N concentration, and nitrate-N : ammonium-N ratio requirements for basil grown in a controlled environment.

The irradiance experiments were completed in growth chambers maintained at 25 ± 4 °C, with 16-h photoperiod lighting provided by a mixture of cool-white fluorescent and incandescent lamps for 29 d. The basil cultivars used in the irradiance experiment were 'Genovese', 'Italian Large Leaf', and 'Nufar'. The total N experiments and the nitrate-N : ammonium-N experiments were completed in a greenhouse maintained at 30 ± 5 °C under natural irradiation, with 16-h photoperiod lighting provided by high-pressure sodium lamps at 400 to 500 μmol/m^2/s for 27 d. The basil cultivars used in the total nitrogen and nitrate-N : ammonium-N ratio experiments were 'Italian Large Leaf' and 'Nufar'. In early experiments, plants were harvested at day 27 to 29, but plants exhibited stem lignification at day 27 to 29. Plants that exhibited this stem lignification were considered inedible, and therefore, subsequent experiments were deemed complete when plants were at day 27.

In the irradiance, total nitrogen, and nitrate-N : ammonium-N experiments, we measured plant growth parameters of plant height, plant diameter, and number of leaves $\geq$ 0.5 cm long, along with the edible biomass production parameters of leaf fresh weight, plant fresh weight, and plant dry weight. All three experiments were replicated over time. Only one replication of the nitrate-N : ammonium-N experiment was tested for nitrate content of the dry plant tissues due to incorrect protocols used in the first two replications.

Basil growth did not increase as the irradiance increased between 500 and 600 μmol/m^2/s, and little, if any, additional growth occurred at 600 μmol/m^2/s in all three basil cultivars. We recommend irradiance no greater than 500 μmol/m^2/s in basil production in controlled environments.

Basil plants grew poorly at 50 mg N/L, but they grew equally well at 100, 150, 200, and 250 mg N/L. Modeling analyses suggested that the best growth occurred at about 200 mg N/L.

No plants survived in the 0% nitrate-N : 100% ammonium-N treatment. There were varied responses to the nitrate-N : ammonium-N ratios that contained nitrate-N, but plants in all ratios grew equally well, and modeling analyses showed that either 50% nitrate-N : 50% ammonium-N or 75% nitrate-N : 25% ammonium-N yielded the greatest edible biomass.

Future research is needed on specific irradiance sources to provide the light needed for basil production in controlled environments. Light-emitting diodes (LEDs) are being used more often for nonscientific activities such as stoplight lamps, automobile lamps, etc., and may be a suitable, low-cost, low-heat output, long-lasting irradiance source for fresh basil production in controlled environments such as on the International Space Station (Yorio et al., 2001; Kim et al., 2004). Future research also needs to address the problem of nitrate-N accumulation in basil and other plant tissues grown in extraterrestrial outposts. Very preliminary data from this work showed nitrate-N may accumulate to amounts toxic to humans (WHO, 2007; European Communities, 2008).

Recycling astronaut body waste products probably will be essential for continuous inhabitation of the International Space Station and long-term missions, and within future outposts such as lunar and Mars habitats. Use of recycled astronaut waste products, after cleaning and reprocessing, may prove to be a good source for the base of nutrient solutions for food crop production in controlled-environment systems such as the International Space Station. However, further studies are needed on how well plants will respond to cleaned and reprocessed astronaut waste products in a hydroponic system. Urea or another form of ammoniacal N may be a starting point for plant nutrient solutions in such extreme environments because basil responded so well to 25 to 50% ammonium-N in this study.

More research is required to quantify and understand the optimal controlled-environment conditions for edible biomass production of basil in systems such as the International Space Station. This research has provided a beginning toward better understanding of efficient energy inputs for basil production in controlled-environment systems. As is usual in science, more questions presented themselves than answers were concluded.

References for General Conclusions

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APPENDIX: PRELIMINARY EXPERIMENTS

Germination Experiment

Five basil cultivars were evaluated for germination percentage and germination uniformity. The five cultivars were ‘Aroma 2’, ‘Genovese’, ‘Genovese Compact Improved’, ‘Italian Large Leaf’, and ‘Nufar’ (Johnny’s Selected Seeds, Inc., Winslow, ME). The five basil cultivars were selected based on preliminary research done in spring 2005. Four replications of 100 seeds of each cultivar were germinated in reach-in growth chambers. The growth chambers were maintained at 25 ± 3 °C, and irradiance was provided by a mixture of cool-white fluorescent and incandescent lamps set to a 16-h photoperiod. Irradiance was 150 to 200 μmol/m^2/s. Relative humidity was not measured. Oasis LC-1 Horticubes® (Smithers-Oasis North America, Kent, OH) served as the substrate, with a planting density of one seed per cell. Seeds imbibed deionized water for 24-h, and were then fertigated with modified full-strength Hoagland’s Nutrient Solution No. 1 every 12-h for 10 days. Germination counts were done every 12-h until increases in germination stopped. Germination was counted as complete when the radicle emerged from the seed coat. The greatest germination percentage occurred in ‘Aroma 2’ and ‘Nufar’, followed by ‘Genovese Compact Improved’ and ‘Italian Large Leaf’. The lowest germination percentage occurred in ‘Genovese’ (Table 1). In all four replications, ‘Genovese Compact Improved’ was the least uniform in germination, and the least uniform in the development of the first two true leaves, whereas ‘Nufar’ was the most uniform in germination and the development of the first two true leaves (data not shown). ‘Aroma 2’ was similar to ‘Nufar’ in germination uniformity and the development of the first two true leaves. ‘Italian Large Leaf’ and ‘Genovese’ were moderate in germination uniformity and the development of the first two true leaves when compared with ‘Genovese Compact Improved’ and ‘Nufar’. This experiment was completed in the fall of 2006.

Table 1

Table 1. Means of four replications of 100 seeds each for germination percentage of five basil cultivars (Aroma 2, Genovese, Genovese Compact Improved, Italian Large Leaf, and Nufar) grown in growth chambers maintained at 25 ± 3 °C, with a 16-h photoperiod, and fertigated every 12-h with modified full-strength Hoagland’s Nutrient Solution No. 1. Germination was counted when the radicle emerged from the seed coat.

Cultivar	Germination %
Aroma 2	94.2 a[z]
Genovese	80.8 c
Genovese Compact Improved	87.3 b
Italian Large Leaf	87.3 b
Nufar	92.5 ab

[z] Means within each column followed by an identical letter are not different at $P \leq 0.05$ according to Fisher’s least significant difference test.

Five Basil Cultivars in Nutrient Film Technique Experiment

Five basil cultivars (Aroma 2, Genovese, Genovese Compact Improved, Italian Large Leaf, and Nufar) (Johnny's Selected Seeds, Inc., Winslow, ME) were evaluated for greatest biomass production in a recirculating nutrient film technique (NFT) system. Seeds germinated in growth chambers maintained at 25 ± 3 °C, and irradiance was provided by a mixture of cool-white fluorescent and incandescent lamps set to a 16-h photoperiod. Irradiance was 150 to 200 µmol/m^2/s. Relative humidity was not measured. Oasis LC-1 Horticubes® (Smithers-Oasis North America, Kent, OH) served as the substrate, with a planting density of two seeds per cell. Seeds imbibed deionized water for 24-h, and were then fertigated with modified full-strength Hoagland's Nutrient Solution No. 1 every 12-h for 10 to 11 days, until the first two true leaves measured ≥ 0.5 cm long. Plants were thinned to one plant per Oasis LC-1 Horticube® cell when the first two true leaves measured ≥ 0.5 cm long, and then they were transplanted into the NFT system (SureGro™, Crop King, Seville, OH), set on 10 cm centers. Plants grew for 15 days in the NFT system and were harvested at day 26. The greenhouse was maintained at 26 ± 5 °C D/N. Relative humidity was not measured. Supplemental lighting was provided by high-pressure sodium lamps set on a 16-h photoperiod (on at 0600, off at 2200), and the supplemental irradiance was 220 to 500 µmol/m^2/s. The recirculating nutrient solution was maintained at 25 ± 5 °C by a heating mat. The nutrient solution pH and soluble salts (EC) were measured and recorded twice daily. The NFT troughs were set atop a 4.0 mm sheet of aluminum that sat approximately 3 to 5 mm above soil warming cables (Heavy-duty Gro-Quick™; Wrap-On Company, Inc., Beford

Park, IL) that were stapled to plywood that maintained bottom heat at 25 ± 5 °C. The aluminum sheet was used to better distribute the bottom heat over the entire NFT trough footprint. The plant growth parameters measured were plant height, plant diameter, and number of leaves ≥ 0.5 cm long. The edible biomass parameters measured were leaf fresh weight, and plant fresh weight. Plant dry weight was not measured. This experiment had three replications done over time.

Italian Large Leaf and Nufar produced the greatest edible biomass of the five basil cultivars in this experiment. ‘Aroma 2’ and ‘Genovese’ exhibited the tallest plants, but did not exhibit the greatest edible biomass parameters of leaf fresh weight and plant fresh weight. Genovese Compact Improved exhibited the least edible biomass parameters of the five cultivars (Table 2). Space-flight growth chambers are indeed small in area available for edible biomass production, and dwarf cultivars of edible plants would seem to be ideal. However, the dwarf basil cultivar Genovese Compact Improved did not produce as much edible biomass compared with Italian Large Leaf and Nufar. We thought that harvesting nondwarf, younger plants with more edible biomass potential would be a smarter and more practical cultivar selection rather than relying on industry-lead quantified dwarf status. At around day 30, all cultivars exhibited lignified stems that appeared inedible. We decided to terminate future experiments at around day 26 to 29 to retain edible qualities of the entire shoot tissue including the stem, petiole, and leaves. The two greatest edible biomass producers, ‘Italian Large Leaf’ and ‘Nufar’, were selected for future experiments based on the results of this experiment. This experiment was started in the fall of 2006 and completed in the fall of 2007.

Table 2

Means of four replications of plant height, plant diameter, number of leaves ≥ 0.5 cm, leaf fresh weight, and plant fresh weight of five basil cultivars grown for 15 d in a recirculating NFT system fertigated with modified full-strength Hoagland's Nutrient Solution No. 1, under high-pressure sodium lamps set for 16-h photoperiod for supplementation of natural photoperiods. The nutrient solution and bottom heat under the troughs was maintained at 25 ± 5 °C. N = 28.

Cultivar	Plant height (cm)	Plant diameter (cm)	Number of leaves ≥ 0.5 cm long	Leaf fresh weight (g)	Plant fresh weight (g)
Aroma 2	14.1 a[z]	9.1 c	31 a	4.5 c	6.4 cd
Genovese	12.7 ab	9.6 bc	31 a	5.2 bc	7.2 bc
Genovese Compact Improved	6.0 c	7.2 d	16 b	3.4 c	4.2 d
Italian Large Leaf	11.2 b	10.8 ab	30 a	6.8 ab	9.2 ab
Nufar	10.6 b	11.7 a	33 a	7.9 a	10.8 a

[z] Means within each column followed by an identical letter are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

Plants Per Unit Area Experiment

Two basil cultivars (Italian Large Leaf and Nufar) (Johnny's Selected Seeds, Inc., Winslow, ME) were evaluated for greatest biomass production in a planting density experiment. The planting density was one plant, two plants, three plants, and four plants per 12.5 cm standard pot. Seeds germinated in growth chambers maintained at 25 ± 3° C, with irradiance provided by a mixture of cool-white fluorescent and incandescent lamps set to a 16-h photoperiod. Irradiance was 150 to 200 μmol/m^2/s. Relative humidity was not measured. Oasis LC-1 Horticubes® (Smithers-Oasis North America, Kent, OH) served as the substrate, with a planting density of five seeds per cell. Seeds imbibed deionized water for 24-h, and then were fertigated with modified full-strength Hoagland's Nutrient Solution No. 1 every 12-h for 10 to 11 days, until the first two true leaves measured $\geq$ 0.5 cm long. Plants were thinned to one, two, three, or four plants per Oasis LC-1 Horticube® cell when the first two true leaves measured $\geq$ 0.5 cm long, and then they were transplanted into silica sand (Unimin® #2040, Unimin® Corporation, New Canaan, CT) in 12.5 cm standard pots set pot-to-pot on a greenhouse bench. The greenhouse was maintained at 26 ± 5 °C D/N. Relative humidity was not measured. Plants grew for 15 days in the silica sand culture and were harvested at day 27. Supplemental lighting was provided by high-pressure sodium lamps set on a 16-h photoperiod. The supplemental irradiance was 450 to 500 μmol/m^2/s. There were no cultivar differences, or cultivar × seeds per cell treatment interaction for all six measured parameters. The one plant per pot treatment exhibited the greatest in all of the six measured parameters. The two plants per pot, three plants per pot, and four plants per pot treatments were different from the one plant per pot treatment (Table 3), but were not different from each other. More research is needed to gain a better understanding of the optimal planting density for edible biomass production of basil in a controlled environment. This experiment started October 2007 and was completed in February 2008.

Table 3

Means of three replicates for plant height, plant diameter, number of leaves ≥ 0.5 cm long, leaf fresh weight, plant fresh weight, and plant dry weight^z of two cultivars of basil (Italian Large Leaf and Nufar) subjected to four planting densities. Plants were grown in a greenhouse in silica sand, under high-pressure sodium set for 16-h photoperiod for supplementation of natural photoperiod for 15 days. Values represent the means for individual plants within each planting density treatment. N = 30.

Planting density (Plants / pot)	Plant height (cm)	Plant diameter (cm)	Number of leaves ≥ 0.5 cm	Leaf fresh weight (g)	Plant fresh weight (g)	Plant dry weight (g)^z
1	10.5 a^y	14.2 a	12 a	3.4 a	4.3 a	0.6 a
2	4.6 b	6.5 b	4 b	1.2 b	1.5 b	0.2 b
3	2.9 b	3.9 c	2 c	0.6 c	0.8 c	0.1 b
4	2.2 b	2.8 c	2 c	0.4 d	0.5 d	0.1 ab

^{z}Plant dry weight was recorded on one replication only.
^{y}Means within each column followed by an identical letter are not different at $P \leq 0.05$ according to Fisher's least significant difference test.

Vegetative Propagation Experiment

Three preliminary experiments evaluated the effectiveness of vegetative propagation techniques for two cultivars of basil, Italian Large Leaf and Nufar, and these experiments are detailed below. Stem-tip cuttings (8.0 cm long) were used in all experiments. No statistical analyses were conducted on these three experiments as they were preliminary, observational experiments only. We thought to conduct these types of experiments to decrease the time investment for greater and more rapid edible biomass production in controlled environments compared with conventional seed propagation techniques. These experiments began fall 2007 and were completed in spring 2008.

Subirrigation with three substrates

Three substrates, silica sand (Unimin® #2040, Unimin® Corporation, New Canaan, CT), Oasis LC-1 Horticubes® (Smithers-Oasis North America, Kent, OH), and Rockwool®, were placed into standard flats and submerged into deionized water to 1.5 cm deep for seven days in a greenhouse maintained at 25 ± 5 °C D/N. Supplemental lighting was provided by HPS lamps at a 16-h photoperiod (450 to 500 μmol/m^2/s). Relative humidity was not measured. One stem-tip cutting was inserted into each cell of the Oasis and Rockwool. One stem-tip cutting was placed every 1.5 cm in the silica sand. A total of ten stem-tip cuttings per cultivar, per treatment were evaluated per replication. A total of three replications were conducted. The greatest rooting response occurred in silica sand and the least rooting response occurred in the Rockwool, with an intermediate rooting response occurring in the Oasis substrates. We were greatly encouraged with the rapid root development in this experiment. This experiment was done in winter to early spring 2008.

Adventitious rooting in high-humidity environments

Stock plants of Italian Large Leaf and Nufar basil cultivars were maintained in 15-cm standard pots filled with silica sand and fertigated daily with modified full-strength Hoagland's Nutrient Solution No. 1, in the same greenhouse used for the five cultivars in NFT system experiment, total N concentration experiment, nitrate-N : ammonium-N ratio experiment, and subirrigation experiments. Careful scouting for pests and pathogens resulted in noting adventitious root development on some of the lignified stem tissues. The plants were set pot-to-pot until the leaves touched and then were set on about 30.5 cm centers. We think that the humidity levels were high enough within the plant canopy to warrant adventitious root development on the stems. Therefore, we cut the plant at the soil line and submerged the entire shoot into moist perlite for seven days, under supplemental lighting provided by HPS lamps (450 to 500 $\mu mol/m^2/s$) set on a 16-h photoperiod. One plant per 2.5 liter nursery pot filled with moist perlite was used, and this was conducted three times per cultivar. The perlite was kept moist with twice daily irrigation with deionized water. Both basil cultivars exhibited adventitious rooting starting at the cut end of the stem to several centimeters below the first set of leaves. Root number and length was not measured or recorded. We think basil may have preformed root initials that may be useful for vegetative propagation in controlled-environments such as the growth chambers in the International Space Station and extraterrestrial outposts such as on the moon and on Mars. Further research is needed to investigate the possibilities of propagating basil from stem tissue that exhibits adventitious root tissue. This experiment was conducted in spring 2008.

Five relative humidity chambers

This preliminary experiment was done out of the observations made in the adventitious rooting in high humidity environments experiment. We wondered if adventitious rooting on basil plants cut at the soil line would occur in different relative humidity controlled-environments. Stock plants of Italian Large Leaf and Nufar basil cultivars were maintained in 15-cm standard pots filled with silica sand and fertigated daily with modified full-strength Hoagland's Nutrient Solution No. 1, in the same greenhouse used for the five cultivars in the NFT system experiment, total N concentration experiment, nitrate : ammonium ratio experiment, and subirrigation experiments. We cut the plant at the soil line and submerged the bottom 4.0 cm of main stem tissue into a 50-ml glass flask filled with deionized water. Parafilm® was wrapped around the top of the glass flask onto and around 1.0 cm of the main stem to prevent water evaporation and plant tissue desiccation. The humidity chambers were two desiccator chambers set on top of one another, creating a sealed controlled environment to which a system of capillary tubing attached to cylinders of air administered 0%, 25%, 50%, 75%, or 100% relative humidity to individual chambers. There were a total of five chambers used in this experiment. This experiment was conducted for seven days, three times, and each chamber contained six samples, three of 'Italian Large Leaf' and three of 'Nufar'. The chambers were set in a laboratory maintained at 21 °C, and irradiance was provided by fluorescent lamps as is typical in a laboratory. The lights were on a 24-h photoperiod; therefore, no dark period was introduced in this experiment. No bottom heat was provided in this experiment. We expected to see vigorous adventitious rooting in the 100% relative humidity chamber, but no plants exhibited rooting in this chamber. The only plants that exhibited notable adventitious root development were in the 75% relative humidity chamber. There was very little adventitious root development in the 0%, 25%, and 50% relative humidity chambers. Root number and length was not measured or recorded. Further research is needed to investigate the possibilities of propagating basil in different controlled-environments that have different relative humidity levels. This experiment was conducted in spring 2008.

Additional questions

Throughout this series of experiments and research work, we kept asking more and more questions. Knowing what makes basil flower could prove to be useful in keeping basil in the vegetative state. Knowing how long of a dark period basil requires for optimal edible biomass production may be useful in calculating energy input costs. Knowing if basil has preformed root initials may be useful in rapid vegetative propagation protocols that could be used to maintain uniformity in controlled environments. We also wondered if basil flowering and/or rooting could be controlled with ethylene and/or 1-MCP. Each of these questions could develop into their own research project for future researchers and students.

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I would like to take this opportunity to express my thanks to those who helped me with various aspects of conducting research and the writing of this thesis. First and foremost is my immense gratitude for my mom who believed in me and my abilities especially when I felt I had taken on something increasingly overwhelming and totally out of my league. Thanks go out to Dr. Richard J. Gladon for having patience, insight, and advice throughout my graduate studies and research. I would also like to thank my committee members for their efforts and contributions to this work: Dr. Gail R. Nonnecke, Dr. Loren C. Stephens, and Dr. Lester A. Wilson. Additional thanks are due to Dr. Nonnecke for her humanistic and inspirational teaching style. She helped me see the big picture well enough to not give up on myself. Dr. James Schrader's wisdom and perspectives were instrumental in synthesizing the data into something worth reading. His sense of humor was a great help in staying on track through to completion of this thesis. The Iowa State University Department of Horticulture faculty, Dr. Jeffery Iles, Barb Osborn, Arlen Patrick, and Rose Rollenhagen were instrumental in keeping a sense of humor and practicality throughout my graduate studies and work. Rose Rollenhagen, in particular, helped me the most in regards to following my dream of teaching and helping students do their best. The gentlemen at NASA's Kennedy Space Center, Dr. Raymond Wheeler, Dr. John Sager, Dr. Gary Stutte, and Dr. Neil Yorio, provided me a healthy dose of reality and humor and taught me how to not take myself too seriously and still accomplish great things. Last of all, great thanks go out to Ty Alchin, who inspired me to hold onto an unusual dream in an unwelcoming arena. This has been a difficult path, but well worth the knowledge and skills gained.