Sweet Basil Requires an Irradiance of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Greatest Edible Biomass Production

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Abstract. Energy conservation in controlled-environment agriculture is a major concern for both commercial and research facilities as well as extraterrestrial facilities for food production. Supplying optimal irradiance by using electrical lighting for the greatest edible biomass production potentially is the greatest draw on energy during earth-based or extraterrestrial food production in controlled environments. 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. Seedlings of the three cultivars were transplanted into soilless medium, one plant per pot, and grew for 17 days in reach-in growth chambers maintained at $25 \pm 4^\circ\text{C}$ with a 16-h photoperiod. Canopy-level irradiances of 300, 400, 500, and 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were provided by cool-white fluorescent and incandescent lamps. Shoot growth was measured as height, diameter, and number of leaves 0.5 cm long or greater; and edible biomass was measured as leaf fresh weight, shoot fresh weight, and shoot dry weight. There was no irradiance $\times$ cultivar interaction, but main effects of irradiance and cultivar were observed. Plant growth and edible biomass production were least at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and greatest at 500 or 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In several cases, 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ yielded intermediate 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 shoot fresh weight and dry weight. Under our environmental conditions that included ambient CO_2 concentration and ambient relative humidity, the rate of growth peaked at 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and no additional accumulation of edible biomass occurred at 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Based on our results, canopy-level irradiance of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provides maximum edible biomass production of basil in a controlled-environment production system.

Long-term, extraterrestrial missions require efficient use of area, volume, energy, and time for every task, particularly food production (Wheeler et al., 1996, 2008). Space-flight horticulturists will most likely depend on electrical lighting for crop production in near-term missions, in which energy generation and conservation are 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.

Salad crops 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 (Tibbits and Alford, 1982; 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 (1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), high temperature, elevated carbon dioxide concentrations, and vigorous air flow (Frantz et al., 2004). However, lettuce exposed to supraoptimal irradiance without additional environmental adjustments exhibited tip-burn damage (Seginer et al., 2006). In lettuce, biomass production is cultivar-dependent, and some lettuce cultivars exhibit decreased edible quality under constant illumination (Knight and Mitchell, 1983a, 1983b). In addition, supraoptimal irradiance and temperatures can 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 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for a longer period of time compared with transplants treated with irradiance greater than 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for shorter periods of time, indicating that daily light integral may be a key factor in edible biomass production of salad crops (Koontz and Prince, 1986; Oda et al., 1989).

Leaf photosynthesis in most C_3 plants saturates at $\approx 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, but whole plant photosynthesis of some species may not saturate until 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or greater (Taiz and Zeiger, 2006). 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 related to methemoglobinemia and carcinogenesis that may result from high nitrate consumption, issues that should be considered during the optimization of basil production (Alexander et al., 2008). Research indicates that essential oil content in basil increases with greater irradiance, whereas the concentration of the principal component of the essential oil, methyl eugenol, decreased with ultraviolet B treatment (Nitz and Schnitzler, 2004). Methyl eugenol is carcinogenic in large amounts and, therefore, a 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

Shoot growth. The basil cultivars Genovese, Italian Large Leaf, and Nufar (Johnny’s Selected Seeds, Inc., Winslow, ME) were used. Seeds were germinated in Oasis[®] LC-1 Horticubes[®] (Smithers-Oasis North America, Kent, OH) for 10 to 12 d in a growth chamber maintained at $25 \pm 4^\circ\text{C}$ with a 16-h photoperiod provided by cool-white fluorescent and incandescent lamps. During germination, irradiance at the canopy was 150 to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Seeds imbibed deionized water for 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 or greater. 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 iron/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 were grown in Conviron[®] reach-in growth chambers (Model E-15; Controlled Environment Limited, Winnipeg, Canada) maintained at $25 \pm 4^\circ\text{C}$. 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,

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four chambers were each set for a canopy irradiance of 300, 400, 500, or 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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 17 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, shoot canopy diameter, number of leaves 0.5 cm long or greater, leaf fresh weight, and shoot fresh weight were recorded on Day 29. Subsequently, individual shoots were placed into individual paper bags and placed in 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 analysis of variance 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.

Results

Our preliminary experiments showed that basil required a dark period for normal growth 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.

In our main experiment, no irradiance $\times$ cultivar interaction was detected, but main effects of irradiance and cultivar were observed. Plants that received an irradiance of 500 or 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were taller than those that received 300 or 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1A). Plants that received 500 or 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had a greater canopy diameter than plants receiving 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ resulting in an intermediate value (Fig. 1B). Plants that received 500 or 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had more leaves 0.5 cm long or greater than plants that received 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas plants treated with 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ developed an intermediate number of leaves (Fig. 1C). Regression analyses indicated linear and quadratic relationships between irradiance and plant height ($P \leq 0.0001$ and $P \leq 0.0001$, respectively), shoot canopy diameter ($P = 0.003$ and $P = 0.0074$, respectively), and number of leaves 0.5 cm long or greater ($P \leq 0.0001$ and $P \leq$

0.0001, respectively). Quadratic relationships between each of these parameters and irradiances of 300 to 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were especially strong (Fig. 1A–C). Quadratic regression peaks for plant height, plant diameter, and number of leaves that were 0.5 cm or greater correspond with irradiances of 600, 600, and 578 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively (Fig. 1A–C).

Leaf fresh weight, shoot fresh weight, and shoot dry weight were greatest at 500 and 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, lowest at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and intermediate values were obtained at 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 2A–C). At our spacing of 1 plant/12.7 cm², the greatest yields were at 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and were as follows: 520.8 g·m⁻² for leaf fresh weight, 737.8 g·m⁻² for shoot fresh weight, and 55.8 g·m⁻² for shoot dry weight. Regression analyses showed linear and quadratic relationships between irradiance and leaf fresh weight ($P = 0.0004$ and $P = 0.0004$, respectively), shoot fresh weight ($P \leq 0.0001$ and $P \leq 0.0001$, respectively), and shoot dry weight ($P \leq 0.0001$ and $P \leq 0.0001$, respectively). Quadratic relationships between edible biomass (leaf fresh weight, shoot fresh weight, and shoot dry weight) and irradiances of 300 to 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were especially strong (Fig. 2A–C). Quadratic regression peaks for leaf fresh weight, shoot fresh weight, and shoot dry weight corresponded to irradiances of 600, 475, and 530 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively.

Cultivar main effects were present for plant height, plant diameter, leaf fresh weight, shoot fresh weight, and shoot 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 than 'Genovese', and 'Nufar' exhibited an intermediate amount of edible biomass (Table 1).

Discussion

Our results suggest that canopy photosynthetic capacity of basil saturated at an irradiance of $\approx 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in ambient CO₂ concentration and ambient relative humidity. Little, if any, additional growth occurred in plants that received 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The irradiance saturation point for leaf photosynthesis determined in this research is consistent with research on tropical conditions that inhibit lettuce growth (He et al., 2001) and C₃ plants in general (Taiz and Zeiger, 2006). However, we found the whole-plant growth response did not increase appreciably between 500 and 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, lower levels than those reported for many other species (Taiz and Zeiger, 2006). We recommend lighting that does not exceed 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to conserve energy while enabling the greatest edible biomass production of basil in a controlled-environment facility. Further research is needed to understand why these three cultivars of sweet basil did not benefit from greater irradiance in a controlled-environment system.

Our research investigated only instantaneous irradiance during a photoperiod of

Fig. 1. Plant height (A), plant diameter (B), and number of leaves 0.5 cm long or greater (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 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by a mixture of cool-white fluorescent and incandescent lamps on a 16-h photoperiod at 25 ± 4 °C day/night. No cultivar $\times$ irradiance interaction was found and, therefore, data for the three cultivars were combined. Values reflect means of three replicates with 60 observations per replicate.

16 h, not continuous irradiance, because preliminary results showed that basil suffered stem and foliar injury during a continuous photoperiod. This is the first report of basil requiring a dark period, and it is important to understand that basil requires at least some duration of dark period for successful controlled-environment production. The length of light and dark periods that maximize edible biomass production of basil, specifically, in a controlled-environment requires further investigation. Also, if environmental parameters such as temperature, relative humidity, and carbon dioxide concentration differ from our experimental conditions, the optimal irradiance for edible biomass production of basil may differ. This too requires further investigation.

The production of edible biomass in herbaceous plants such as basil requires maintenance of vegetative tissues while delaying 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

Fig. 2. Leaf fresh weight (A), shoot fresh weight (B), and shoot 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 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by a mixture of cool-white fluorescent and incandescent lamps on a 16-h photoperiod at 25 ± 4 °C day/night. No cultivar $\times$ irradiance interaction was found and, therefore, data for the three cultivars were combined. Values reflect means of three replicates with 60 observations per replicate.

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 may increase carotenoid concentration as well (Mou, 2005). Perhaps reduced irradiance and manipulation of photoperiod for basil may lower nitrate content and increase chlorophyll concentration, resulting in increased carotenoid concentration. The lower nitrate content and increased carotenoid concentration in the edible biomass of basil would make it a higher nutrient-containing food product.

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; Reuveni 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. Trends reflected in our quadratic regressions of growth in response to irradiance intensity suggest that edible biomass production in basil peaks $\approx 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas plant volume (height and diameter) peaked near $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Therefore, optimum production and efficiency in the growth chamber area and volume utilization occurred at an irradiance of $\approx 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These results would be important for long-term missions in which area and volume for food production are limited and must be used efficiently (Wheeler et al., 2008).

Production of edible biomass of basil was optimized at an irradiance of $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using our environmental conditions of 25 ± 4 °C uncontrolled 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. As it is with the production of any type of plant material, greater amounts of one or more environmental parameters may be used to increase productivity, but the efficiency of energy use may not be optimized appropriately for the particular species. Further research is needed to understand how production efficiency might be affected by overall daily light integral and by varying its components, instantaneous light intensity, and duration. Further research is also needed to develop controlled-environment practices that will optimize basil production, while limiting nitrate and methyl eugenol to concentrations acceptable for human consumption.

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Table 1. Plant height, plant diameter, number of leaves 0.5 cm long or greater, leaf fresh weight, shoot fresh weight, and shoot dry weight of three cultivars of basil grown at four irradiances.^z

Cultivar	Plant ht (cm)	Plant diam (cm)	Number of			
			leaves 0.5 cm long or greater	Leaf fresh wt (g)	Shoot fresh wt (g)	Shoot dry wt (g)
Genovese	19.7 a ^y	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
	13.9 c	16.8 a	37 a	7.4 a	9.9 ab	0.79 ab

^zPlants 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 overall four irradiances.

^yMeans 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.

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