Protocol Development for the NASA-JSC Lunar-Mars Life Support Test Project (LMLSTP) Phase III Project: A Report on Baseline Studies at KSC for Continuous Salad Production

G. D. Goins, N. C. Yorio, and H. R. Vivenzio; Dynamac Corp., Kennedy Space Center

NASA/TM-1998-207911 June 1998

ABSTRACT

During Fall 1997, the Phase III Lunar-Mars Life Support Test Project (LMLSTP) was conducted in a 20-foot chamber at Johnson Space Center. The overall objective of the Phase III project was to conduct a 90-day regenerative life support system test involving four human subjects to demonstrate an integrated biological and physiochemical life support system. A secondary objective of the Phase III LMLSTP was to demonstrate the ability to produce salad-type vegetable by integration of a small benchtop growth chamber located within the crew habitat area. This small chamber, commercially manufactured as the Controlled Environment Research Ecosystem (CERES 2010™), functioned primarily as a means to continuously provide fresh salad crops for crew members. The CERES 2010™ is a jointly assembled (Percival Scientific, Inc. and Quantum Devices, Inc.) growth chamber that utilizes hardware components developed for effective plant biomass production in spaceflight applications. These components include: (1) LED lighting (2) Astroculture™ Root Trays and (3) Zeoponic media. In planning for the LMLSTP Phase III, a request was put forward for KSC scientists to generate a protocol for successful continuous planting, culturing, and harvesting of the salad-crop, lettuce (*Lactuca sativa* L. cv. Waldmann's Green). By conducting baseline tests with components of the CERES 2010™, a protocol was developed and data were generated which characterizes the performance of lettuce.

Abbreviations used:

BF=Blue fluorescent; CWF=Cool-White Fluorescent; DAS=Days after seeding; JSC=Johnson Space Center ; KSC=Kennedy Space Center; LED=Light emitting diode; LMLSTP=Lunar-Mars Life Support Test Project; NFT=Nutrient Film Technique; PPF=Photosynthetic Photon Flux;

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ACKNOWLEDGMENTS

The authors thank John C. Sager, Raymond M. Wheeler, Jim D. Carr, Cheryl L. Mackowiak, Gary W. Stutte, Daniel Barta, and Keith Henderson for their advice and critical assistance with this study. LED arrays used in this study were supplied by NASA Ames Research Center. Quantum Devices, Inc. holds a patent (# 5012609) on light-emitting diodes as an illumination source for plant growth. This work was conducted under the NASA Life Sciences Suppport Contract NAS10-12180.

INTRODUCTION

The National Aeronautics and Space Administration (NASA) is conducting studies on plant growth for advanced life support for humans during long-term space missions. As part of the overall technology development effort in advanced life support, a 90-day Phase III Lunar-Mars Life Support Test Project (LMLSTP) is planned for Fall 1997 in a 20-foot diameter chamber at Johnson Space Center (JSC). The main objective of the LMLSTP Phase III is to demonstrate an integrated biological and physiochemical regenerative life support system. A secondary objective of the Phase III LMLSTP is to demonstrate the ability to produce salad-type vegetable by integration of a bench top growth chamber located within the crew habitat area.

A small (exterior 78.74 cm W x 66.0 cm D x 59.7 cm H) commercially manufactured growth chamber called the Controlled Environment Research Ecosystem (CERES 2010™, jointly assembled by Percival Scientific, Inc. and Quantum Devices, Inc.), will be located within the crew habitat area of the larger 20-foot diameter chamber. Although the basic design of the CERES 2010™ is for ground-based applications, the CERES 2010™ incorporates unique lighting and nutrient delivery hardware components that were specifically developed for plant cultivation in the spaceflight environment. These hardware components are: (1) light-emitting diode (LED) lighting (Quantum Devices, Inc. Barneveld, WI); (2) Astroculture™ water delivery system and root tray (Wisconsin Center for Space Automation and Robotics-WCSAR Madison, WI); and (3) Zeoponic growing media (NASA-JSC). In planning for the LMLSTP Phase III, Kennedy Space Center (KSC) scientists were tasked to conduct baseline tests which involved cultivation of lettuce (*Lactuca sativa* L. cv. Waldmann's Green) using these hardware components of the CERES 2010™. Lettuce is a versatile fresh salad crop which has an extensive information base for advanced life support applications (Wheeler *et al.*, 1994). Each of the CERES 2010™ technologies used in the present study are discussed more in detail below, and descriptions of the hardware configurations specific for KSC are described in MATERIALS AND METHODS.

LEDs

Light-emitting diodes (LEDs) are a promising electric light source for space-based plant growth chambers and bioregenerative advanced life support because of their small mass and volume, solid state construction, safety, and longevity (Barta *et al.*, 1992; Bula *et al.*, 1991). The CERES 2010™ is equipped with red and blue monolithic narrow spectrum LEDs light in a SNAP-LITE™ (Quantum Devices, Barneveld, WI) configuration. Although narrow spectrum LEDs have great potential for use as a light source to drive photosynthesis, plants are adapted to utilize a wide-spectrum of light to control photomorphogenic responses (Kendrick and Kronenberg, 1994). Both red light, via phytochrome, and blue light, via blue/UV photoreceptor(s), are effective in

inducing photomorphogenic responses (Barnes and Bugbee, 1991; Cosgrove, 1981; Mohr, 1987). Therefore, the growth, development, and seed production of different species of plants grown under specific wavelengths and narrow bandwidth must be characterized before LEDs can be universally accepted as an alternative light source for growing plants in space and in controlled environments.

Astroculture™ Root Trays

A reliable nutrient delivery system is essential for long-term cultivation of plants in space. The ultimate goal is to design a nutrient delivery system that is capable of sustaining plants for long periods under hypo-gravity, yet require minimal system maintenance and limited demands on crew time (Wright, 1984; Kliss *et al.*, 1994). The nutrient delivery system for the CERES 2010™ is based on the Astroculture™ root tray design similar in concept to that which has flown on-board the Space Shuttle (Morrow *et al.*, 1995; Brown *et al.*, 1996). In the Astroculture™ root tray, water under a slightly negative hydrostatic pressure is delivered to the root zone via stainless-steel porous tubes (Morrow *et al.*, 1995) that are fully covered by any suitable solid media. Therefore, plant roots can be in full contact with a high cation exchange capacity (CEC) solid medium such as arcillite (calcined montmorillonite clay) (Brown *et al.*, 1996) or manufactured zeolite (Morrow *et al.*, 1995). The sub-irrigated solid growing matrix provides root anchorage and a buffered source of nutrients. Just as important, the solid matrix also acts as a wick to transport nutrients and water to the roots (Cao and Tibbitts, 1996; Morrow *et al.*, 1994). By carefully controlling the pressure on the irrigation lines, the water potential in the medium can be managed at a constant level (Cao and Tibbitts, 1996; Morrow *et al.*, 1994).

Zeoponics

The external media proposed for the Astroculture™ root trays is a synthetic rooting media known as zeoponic substrate. Zeoponic substrate consists of two nutrient charged mineral phases: natural clinoptilolite or "zeolite" and either synthetic or natural apatite. Zeolites are crystalline, hydrated aluminosilicates that contain exchangeable cations within their infinite three-dimensional crystal structures (Ming, 1989). Zeoponic media is designed to release nutrients into solution via dissolution exchange reactions of the clinoptilolite and apatite (Allen et al., 1995a; Allen et al., 1995b; Ming *et al.*, 1993). Zeoponic media is being developed to supply all essential macro- and micronutrients (e.g. slow-release fertilization) to plants over several growth cycles. Hence, plant cultivation in nutrient delivery systems employing zeoponic material theoretically would require only the addition of water (Ming *et al.*, 1993).

OBJECTIVES

A protocol was needed for the LMLSTP Phase III test which described seeding, growing, and harvesting procedures for continuous lettuce production with the CERES 2010™ (See Appendix I). Continuous lettuce culture in a solid rooting media (such as zeoponics) presents a several challenges because of potential adverse plant responses to media and/or root disturbances from transplanting and harvesting. In addition, the canopy cover characteristics of lettuce requires special attention in order to most efficiently use available growing area (Wheeler *et al.*, 1994). Lettuce canopy cover changes over time are particularly significant in a continuous production system since adjacent plants may be at different growth stages at a given point in time. In a series of ground-based investigations at KSC, our primary focus was to develop a protocol which described continuous production of lettuce in zeoponic-containing Astroculture™ root trays under LED lighting. Along with this protocol development, specific comparisons were made between LED-grown lettuce and lettuce grown under cool-white fluorescent lighting. Media comparisons were made between zeoponic and peat-vermiculite media within the framework of Astroculture™ root trays. A nutrient thin-film technique (NFT) hydroponic system was located adjacent to the Astroculture™ root trays in each of these comparisons (See Appendix II).

Thus, the objectives of this study were:

1. Develop a general protocol for continuous production of lettuce in the Astroculture™ root tray

2. Evaluate LED lighting in terms of supporting lettuce production through comparisons with cool-white fluorescent lighting

3. Evaluate zeoponic media in terms of supporting lettuce production through comparisons with peat-vermiculite media

4. Evaluate zeoponic media in terms of supporting lettuce production through comparisons with nutrient thin-film technique (NFT) hydroponics (See Appendix II).

MATERIALS AND METHODS

Chamber Lighting and Environmental Conditions

In the Gravitational Biology Laboratory at KSC, three separate plant growth chambers were utilized in these tests. The first plant growth chamber housed Astroculture™ root trays (Figure 1) located under red LED (660 nm) arrays supplemented with blue fluorescent (BF) light (Figure 2). A vestibule of black, non-transparent plastic precluded outside light from entering the growth chamber. Discrete red LED circuit board arrays (four cards per array) were used for these tests at KSC. The arrays contained more than 2600 high-intensity discrete red LEDs (660 nm T1 3/4) at 100% packing density. The LED arrays used in the current study were contained in a black anodized aluminum shell structure mounted in a 0.17 m^2 ventilated enclosure over a 0.05 m^2 growing area (Figure 2). The blue fluorescent (BF) lamps (Philips 20-W F20T12/BB) were mounted around the LED arrays to supply approximately 10% (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of the total PPF as determined by quantum sensor (Model LI-189; LI-COR, Lincoln, NE) measurements at the top of the plant canopy. Spectral distribution scans (Figure 3) were taken from 300-1100 nm in 2 nm steps with a spectroradiometer (Model LI-1800; Li-Cor, Lincoln, NE).

A second plant growth chamber housed two Astroculture™ root trays, with both under cool-white fluorescent (CWF) lighting. One of these root trays contained zeoponic media, while the other contained peat-vermiculite media. A third chamber housed the Arasystem (Figure 4) under CWF lighting. The Arasystem served as a seedling nursery to prepare transplants. Lighting for all treatments used a 18 hour light/6 hour dark photoperiod) with approximately equal PPF at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All growth chambers had air temperature, relative humidity, and CO_2 concentrations maintained at 23°±2°C, 65±10%, and 1200±100ppm, respectively.

Seeding, Transplanting, and Harvesting

The Arasystem is a planting tray design with three components: Arabaskets, Araflats, and Aratrays. The Arasystem (Beta Developments, Gent, Belgium; supplier- Lehle Seeds, Round Rock, TX) is a commercial technology specifically developed for efficient germination and harvest of *Arabidopsis thaliana* L. seeds in laboratory growth chambers on shelves. Arabaskets are small 5 cm-tall netted crates that individually confine plants and their associated rooting structure and media. Arabaskets fit exactly into the pots of the Araflats which simplifies manual displacement of plants at all growth stages. Araflats (30 cm W X 50 cm L) contain 51 individual pots (Figure 3) which allows the user to conduct mass sowings of seed similar to a "plant nursery" design. For these studies, Arabaskets were filled with either 4th generation zeoponic or peat-vermiculite media (Metro-Mix 220; Scotts-Sierra

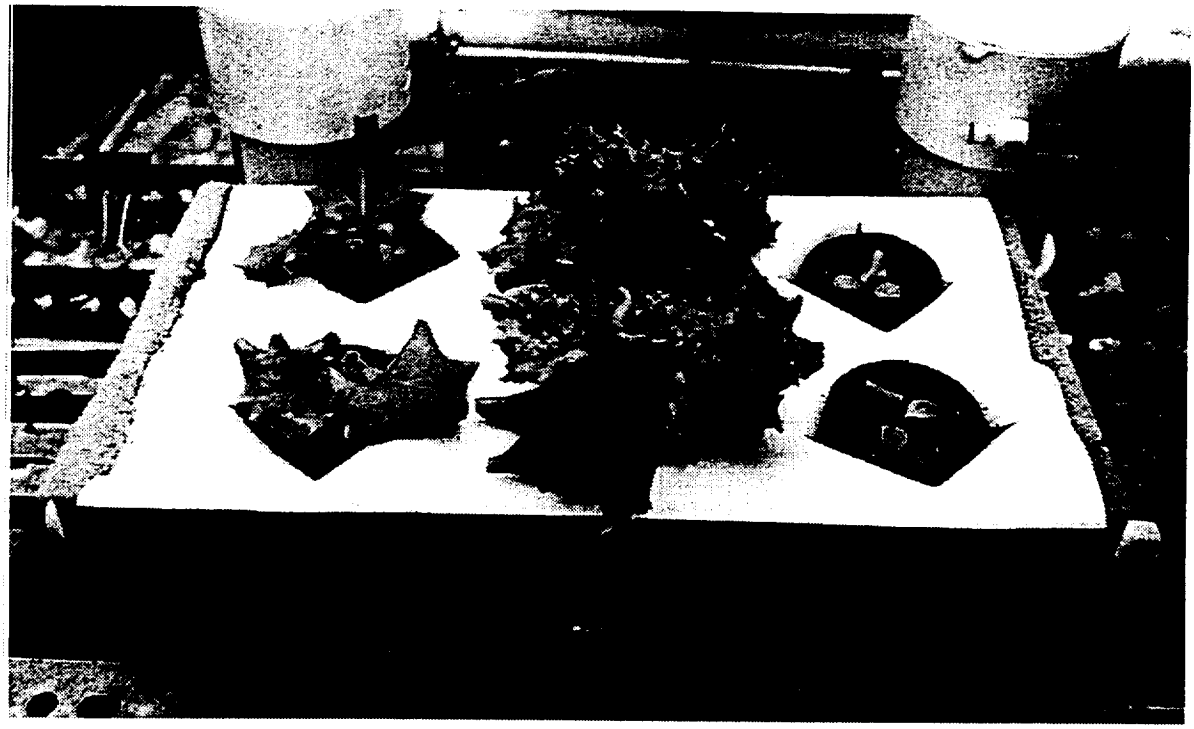

Figure 1. ASTROCULTURE™ root tray with lettuce plants at 8, 15, and 22 days after seeding in ARABASKETS.

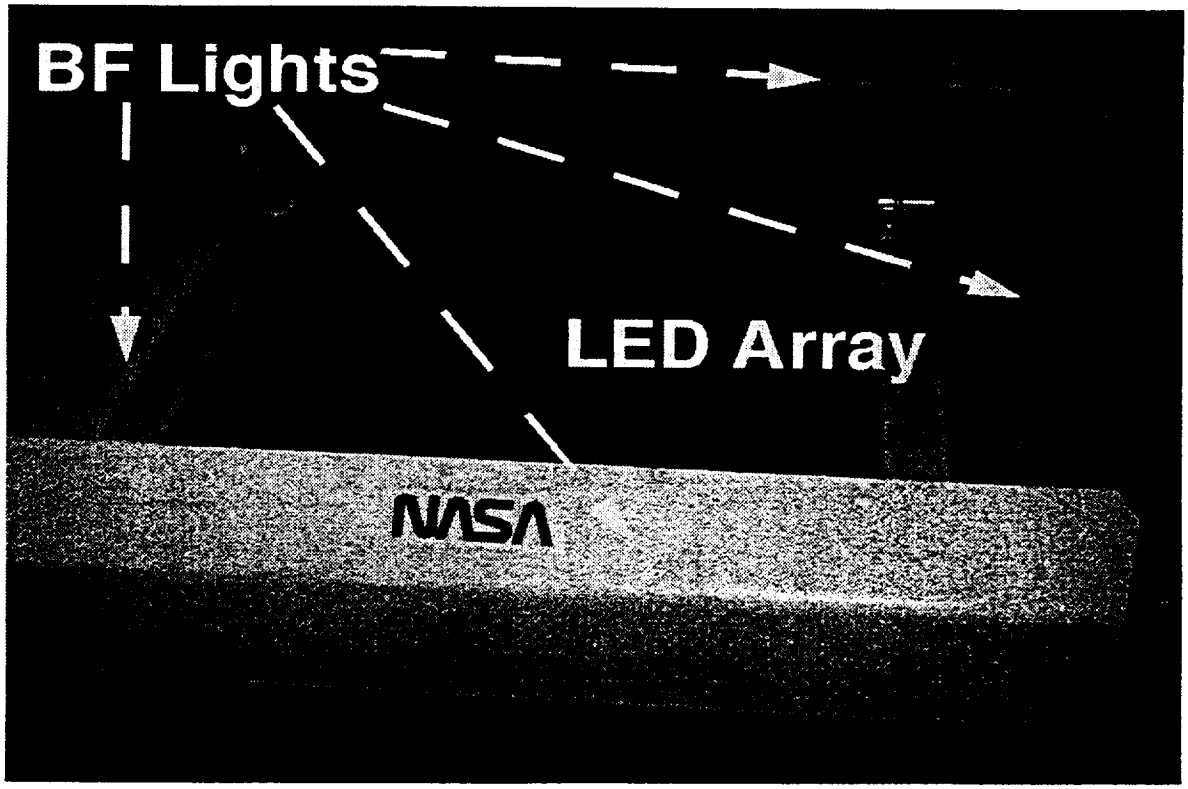

Figure 2. Top view of discrete LED arrays with supplemental blue fluorescent (BF) lamps.

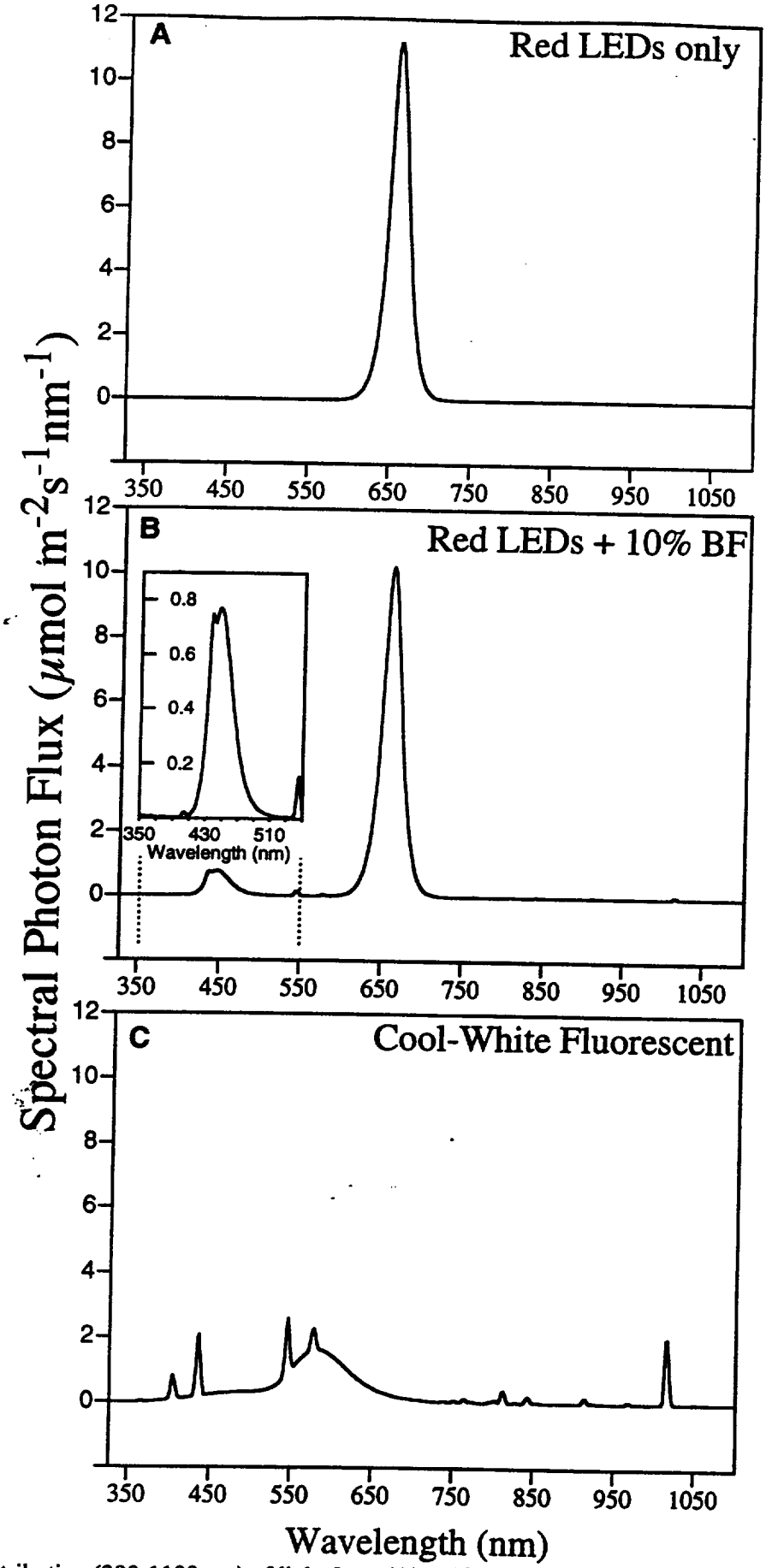

Figure 3. Spectral distribution (300-1100 nm) of light from (A) red LEDs only, (B) red LEDs + 10% blue fluorscent lamps, and (C) cool-white fluorscent lamps. Spectral scans were taken at the top of the plant canopy with a spectroradiometer.

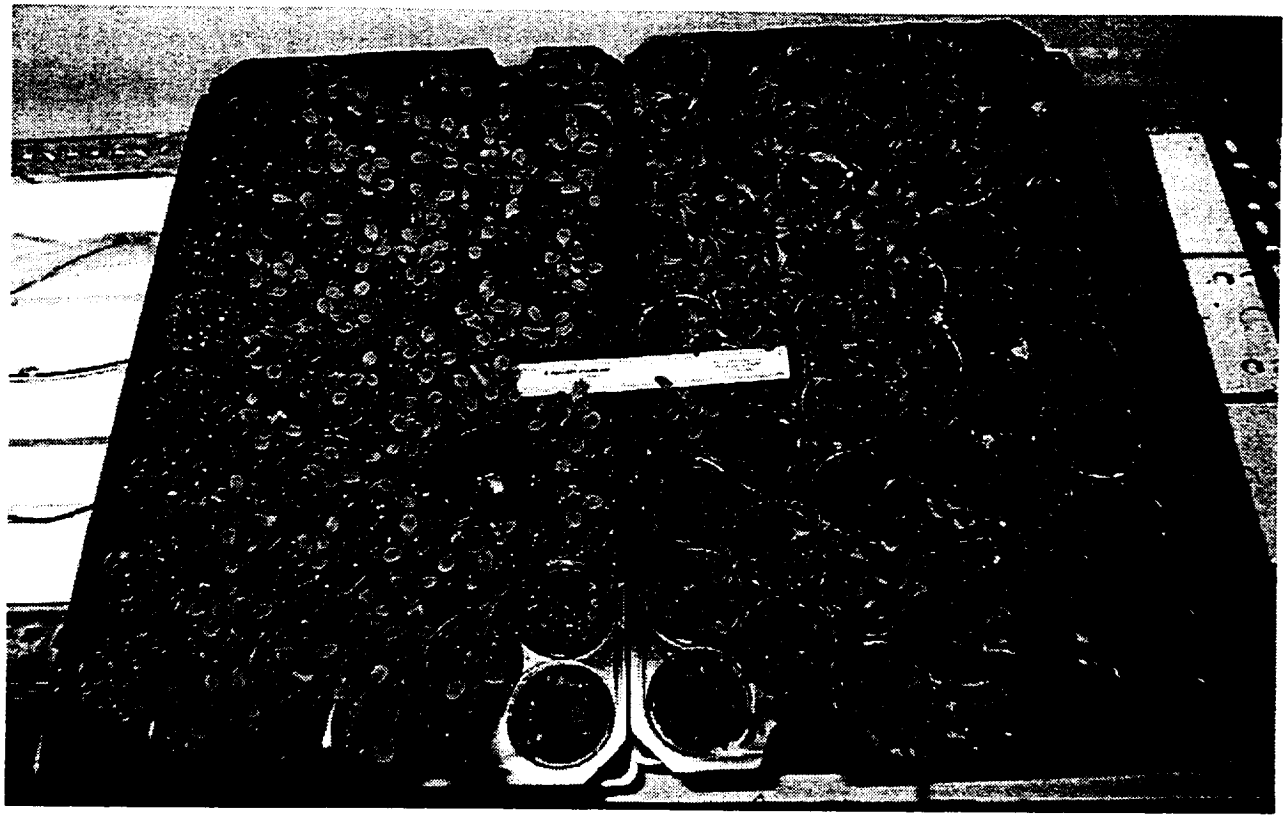

Figure 4. ARASYSTEM nursery system showing ARATRAYS and lettuce plants at 7 days after seeding in ARABASKETS filled with peat-vermiculite (left) or zeoponic media (right).

Horticultural Products Co., Marysville, OH). Once per week over a period of 2 months, three lettuce seed were sown per Arabasket (containing either zeolite or peat-vermiculite) under cool-white fluorescent lighting. At 4-7 days after seeding (DAS), plants were thinned to one plant per Arabasket.

In a continuous production process, five complete lettuce crop cycles (each lasting 24 days) were accomplished (Table 1). The 1st and 2nd crop cycles were transplanted when lettuce was at 7 DAS, while crop cycles 3, 4, and 5 were transplanted at 3 DAS. Transplanting was performed by manually removing two Arabaskets (each containing a plant at 3 or 7 DAS) from an Araflat (Figure 3) and manually inserting the Arabaskets into depressions located between the sub-irrigated porous tubes in the Astroculture™ root tray (Figure 5). Cycle 2 also tested the effects of re-transplanting plants that were already established in the Astroculture™ root trays. With the exception of crop cycle 2, plants remained at a fixed position in the Astroculture™ root tray until final harvest at 24 DAS. The surface of the Astroculture™ root trays were overlaid with an opaque polyethylene plastic cover sheet (white outer surface and black inner surface) to exclude light, prevent algae growth, and maintain high humidity in the root zone (Figure 1). Slits were made in the plastic to receive the transplant Arabaskets. The Astroculture™ root trays were prepared to hold a maximum of six Arabaskets in a 2X3 configuration (Figure 5). Final harvests (at 24 DAS) were completed by simply extracting the Arabaskets from the Astroculture™ root tray.

Astroculture™ Root Tray, Media, Nutrient Delivery

The baseline study at KSC used Astroculture™ root trays (17 cm W X 30 cm L X 5 cm H) configured for the proposed NASA Space Station Plant Research Unit (See Figure 5). In past nutrient delivery experiments conducted at KSC (Goins *et al.*, 1997a), and in the present discussion, of the entire Astroculture™ system, only the Astroculture™ root tray hardware component was employed. The Astroculture™ root trays housed an array of four parallel, slightly hydrophobic stainless-steel tubes (nominal pore size 30 μm, OD 0.953 cm, ID 0.635 cm, length 30 cm) positioned 1 cm above the tray bottom (Figure 5). The Astroculture™ stainless-steel porous tubes were evenly covered with a 4 cm layer of new unused zeoponic or peat-vermiculite substrate to give a final depth of 5 cm (total rooting media volume of 2.5 L). These substrates remained in the root trays without substitution for the duration of the study. In results presented in this paper, the zeoponic media was a fourth generation material with potassium- and ammonium-saturated Wyoming clinoptilolite, and generation 4 synthetic apatite (Doug Ming, NASA-JSC, personal communication). The total zeoponics active ingredients ratio was 30% zeolite:70% profile (porous ceramic inert material) with a particle size of approximately 0.5 - 1.0 mm.

From a separate reservoir, nutrient solution was provided to each Astroculture™ root tray and recirculated back to each reservoir using peristaltic pumps (Figure 6). In a parallel

Table 1. Crop Cycle Schedule and ASTROCULTURE™ Root Tray System Daily Water Use (L)

Crop cycle	I	II	III	IV	V	Peat-Verm.	Zeoponics	Zeoponics
	Days After Seeding (DAS)					CWF	CWF	LEDs+ 10%BF
9-Jun	0					0	0	0
10-Jun	1					0	0	0
11-Jun	2					0.8	0.06	0.06
12-Jun	3					0	0.04	0.02
13-Jun	4					0.08	0.02	0.04
14-Jun	5					0.08	0.14	0.02
15-Jun	6					0.05	0.1	0
16-Jun	7*	0				0.6	0.16	0.1
17-Jun	8	1				0	0.12	0.04
18-Jun	9	2				0.38	0.2	0.02
19-Jun	10	3				0.18	0.16	0.04
20-Jun	11	4				0.08	0.16	0.08
21-Jun	12	5				0.12	0.2	0.08
22-Jun	13	6				0.1	0.18	0.08
23-Jun	14	7*	0			0.1	0.16	0.6
24-Jun	15	8	1			0.1	0.2	0.6
25-Jun	16	9	2			0.16	0.2	0.06
26-Jun	17	10	3*			0.02	0.2	0.06
27-Jun	18	11	4			0.12	0.22	0.12
28-Jun	19	12	5			0.14	0.25	0.15
29-Jun	20	13	6			0.15	0.3	0.15
30-Jun	21	14	7	0		0.24	0.21	0.14
1-Jul	22	15	8	1		0.18	0.24	0.1
2-Jul	23	16	9	2		0.19	0.22	0.12
3-Jul	24	17	10	3*		0	0.24	0.14
4-Jul		18	11	4		0.33	0.25	0.1
5-Jul		19	12	5		0	0.23	0.1
6-Jul		20	13	6		0	0.2	0.13
7-Jul		21	14	7	0	0	0.16	0
8-Jul		22	15	8	1	0.05	0.2	0.08
9-Jul		23	16	9	2	0	0.35	0.1
10-Jul		24	17	10	3*	0.75	0.24	0.14
11-Jul			18	11	4	0	0.12	0.8
12-Jul			19	12	5	0	0.26	0.31
13-Jul			20	13	6	0.04	0.19	0.14
14-Jul			21	14	7	0.15	0.16	0.13
15-Jul			22	15	8	0.27	0.17	0.2
16-Jul			23	16	9	0.18	0.22	0.18
17-Jul			24	17	10	0.2	0.24	0.2
18-Jul				18	11	0.02	0.16	0.08
19-Jul				19	12	0.14	0.17	0.1
20-Jul				20	13	0.09	0.15	0.14

*Indicates day of seeding within each crop cycle

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Table 1. Crop Cycle Schedule and ASTROCULTURE™ Root Tray System Daily Water Use (L)

Crop cycle	I	II	III	IV	V			
Days After Seeding (DAS)								
21-Jul				21	14	0.2	0.2	0.16
22-Jul				22	15	0.14	0.18	0.2
23-Jul				23	16	0.18	0.2	0.18
24-Jul				24	17	0.26	0.1	0
25-Jul					18	0.16	0.1	0.08
26-Jul					19	0.71	0.3	0
27-Jul					20	0.16	0.2	0.12
28-Jul					21	0.08	0.2	0.16
29-Jul					22	0.08	0.2	0.16
30-Jul					23	0.08	0.2	0.16
31-Jul					24	0.5	0.6	0.21
Totals						**17.64**	**9.93**	**7.18**

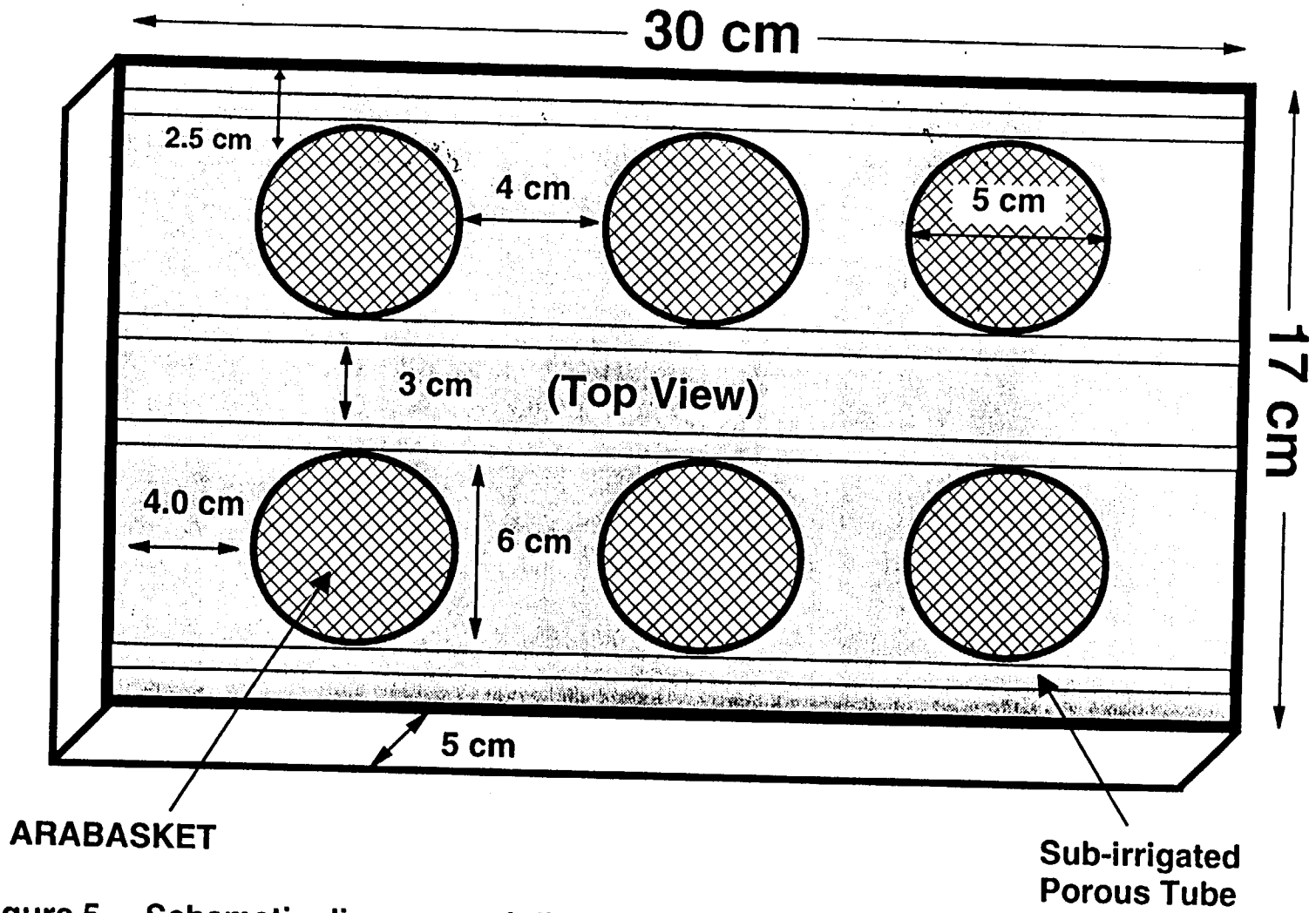

Figure 5. Schematic diagram and dimensions of Astroculture™, root trays used at the Kenndy Space Center Gravitational Biology Laboratory

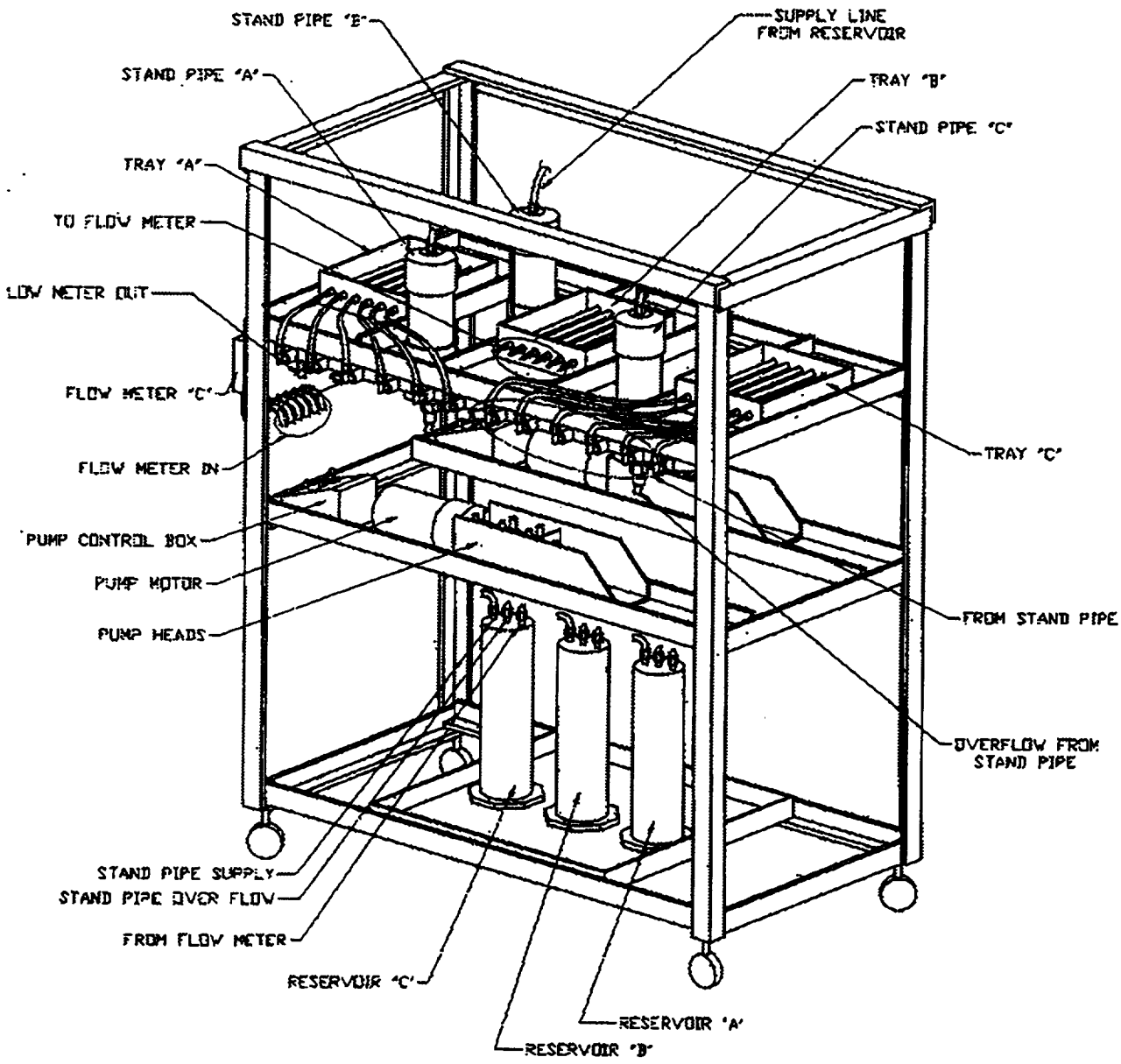

Figure 6. Astroculture™ root trays and associated nutrient delivery system configuration at the Kennedy Space Center Gravitational Biology Laboratory.

configuration, the tubes in each tray of the Astroculture™ system were supplied a constant flow (approximately 4 - 5 $mL \cdot min^{-1}$) of nutrient solution via an adjustable stand-pipe siphon connected to a peristaltic pump plumbed to the reservoir. The suction (-0.23 kPa) on the porous tubes was induced by adjusting the standpipe siphon such that a constant hydrostatic head was maintained approximately 2.3 cm below the center line height of the porous tubes. The flow rate through each porous tube was controlled by adjustable 65mm flowmeters (Cole-Parmer 03219-55) in a parallel inlet/common return configuration.

Nutrient solution pH and electrical conductivity (EC) were measured daily from each reservoir with portable hand-held meters. For the Astroculture™ root tray containing peat-vermiculite media, nutrient solution electrical conductivity (EC) was maintained at approximately 1200 $\mu S \cdot cm^{-1}$ by adding a modified (NO_3-N only) concentrated Hoagland's stock solution (Hoagland and Arnon, 1950; Mackowiak *et al.*, 1989) to the reservoirs, and nutrient solution pH was maintained at 6.5-7.0 by manual additions of dilute HNO_3. Zeoponic media containing Astroculture™ root trays received no nutrient replenishment. De-ionized water was added daily to all reservoirs to replenish water transpired by the plants.

Plant and Nutrient Solution Measurements

On a weekly basis, canopy cover area was determined from digitized images of the plants *in situ* using a public domain image program (National Institutes of Health, Springfield, VA). From these images, change in canopy cover over time was determined for each plant in the Astroculture™ root trays. Plants were harvested at 24 DAS and shoot fresh mass was measured. Shoots were oven dried at 70°C for 48 h and shoot dry mass was measured. Data were subjected to analysis of variance, and mean separation was by least significant difference (LSD). Nutrient solution from each Astroculture root tray was sampled weekly and individual nutrient concentrations were determined using inductively coupled plasma (ICP) spectrometry.

RESULTS AND DISCUSSION

Seeding, Transplanting, and Harvesting

At final harvest (24 DAS) within a given rooting media and lighting regime, shoot fresh mass (Figure 7A), shoot dry mass (Figure 7B), and canopy cover (Figures 8 A-C) were consistent across lettuce crop cycles (except cycle 2, see discussion below). When comparing transplants at 3 DAS as opposed to 7 DAS within each media or lighting regime, there were no significant differences in final harvest shoot fresh mass (Figure 7A), shoot dry mass (Figure 7B), or canopy cover (Figures 8A-C). This indicated that during the first week after seeding in the Arabaskets, there is at least a 4-day option in transplant time without significantly affecting final harvest yields. The Arabaskets appeared to be a simple mechanism to seed and transport lettuce seedlings from the "nursery" Araflat to the Astroculture™ root tray, without adversely affecting older plants already in the Astroculture™ root tray. Overall, these data suggest that Arabaskets were an effective transplant mechanism for consistently producing lettuce biomass.

Crop cycle 2 was a test to determine if plants already transplanted in the Astroculture™ root tray could be removed (at 10 or 17 DAS) and transferred to a different location within the root tray without affecting final harvest yield. Significant reductions in lettuce shoot fresh mass (Figure 7A), shoot dry mass (Figure 7B), canopy cover (Figure 8A-C) resulted from transferring plants at 10 or 17 DAS. Unlike plants at 3 and 7 DAS, where roots were encompassed by the Arabaskets, plants at 10 and 17 DAS had a substantial number of roots emerged beyond the perimeter of the Arabaskets. Such roots were already established in the bulk media of the Astroculture™ root tray, and these roots were most likely injured by the removal and re-transplanting process.

At a planting density of 120 plants•m^{-2} (6 plants per 0.05 m^2 Astroculture™ root tray), leaves from the oldest plants (24 DAS) began to overlap, and thus, compete with adjacent younger plants (17 DAS). Therefore, a determination was made to harvest plants in each crop cycle at 24 DAS to avoid shading of younger plants by older ones. Prior research has indicated that net photosynthesis per unit lettuce canopy cover may decrease due to mutual shading of leaves within and between plants (Wheeler *et al.*, 1994).

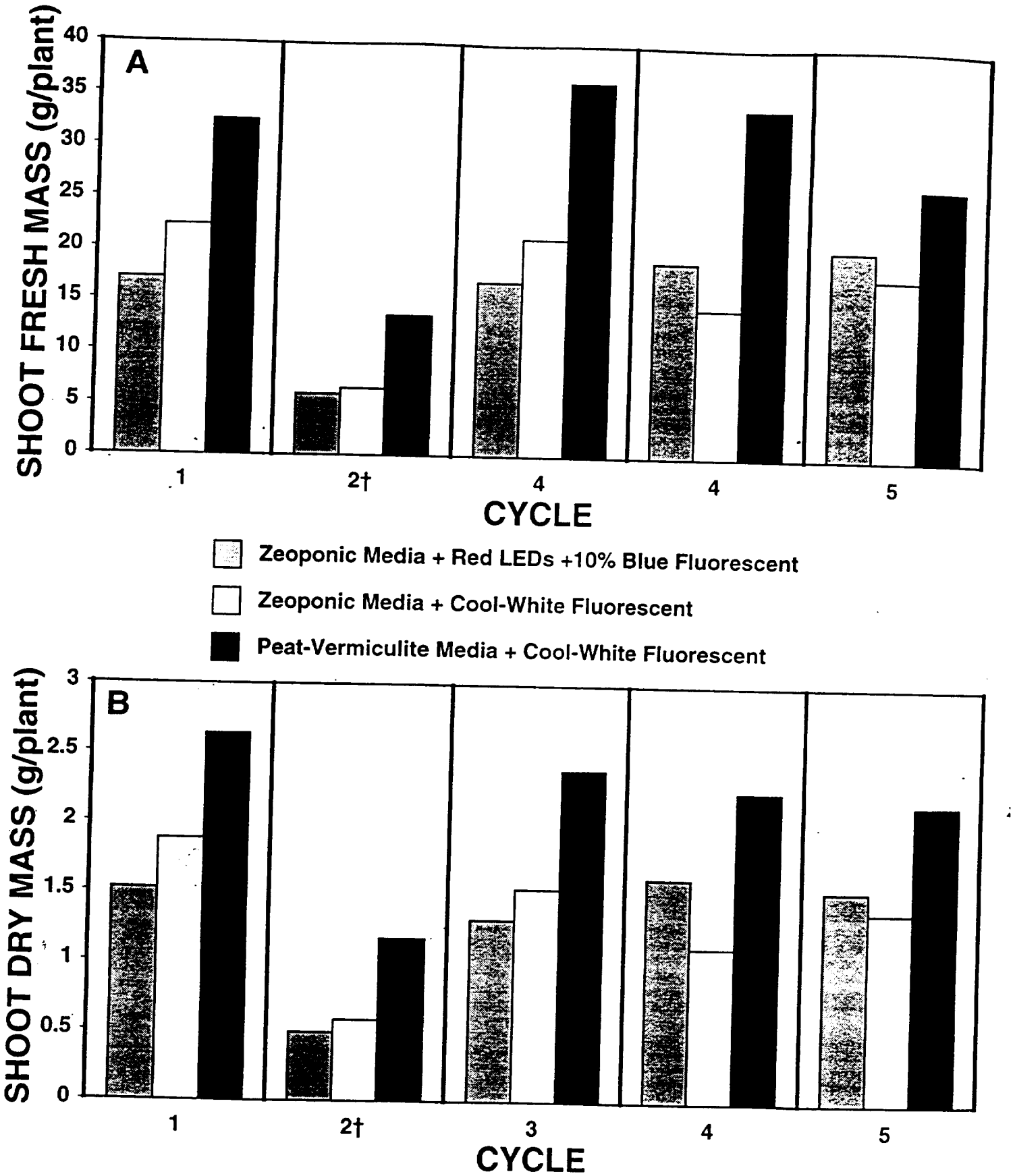

Figure 7. Final harvest (24 days after seeding) shoot fresh (A) and dry mass (B) for 5 crop cycles of lettuce through the Astroculture™ root trays.

†Cycle 2 tested effects of re-transplanting at 10 and 17 DAS.

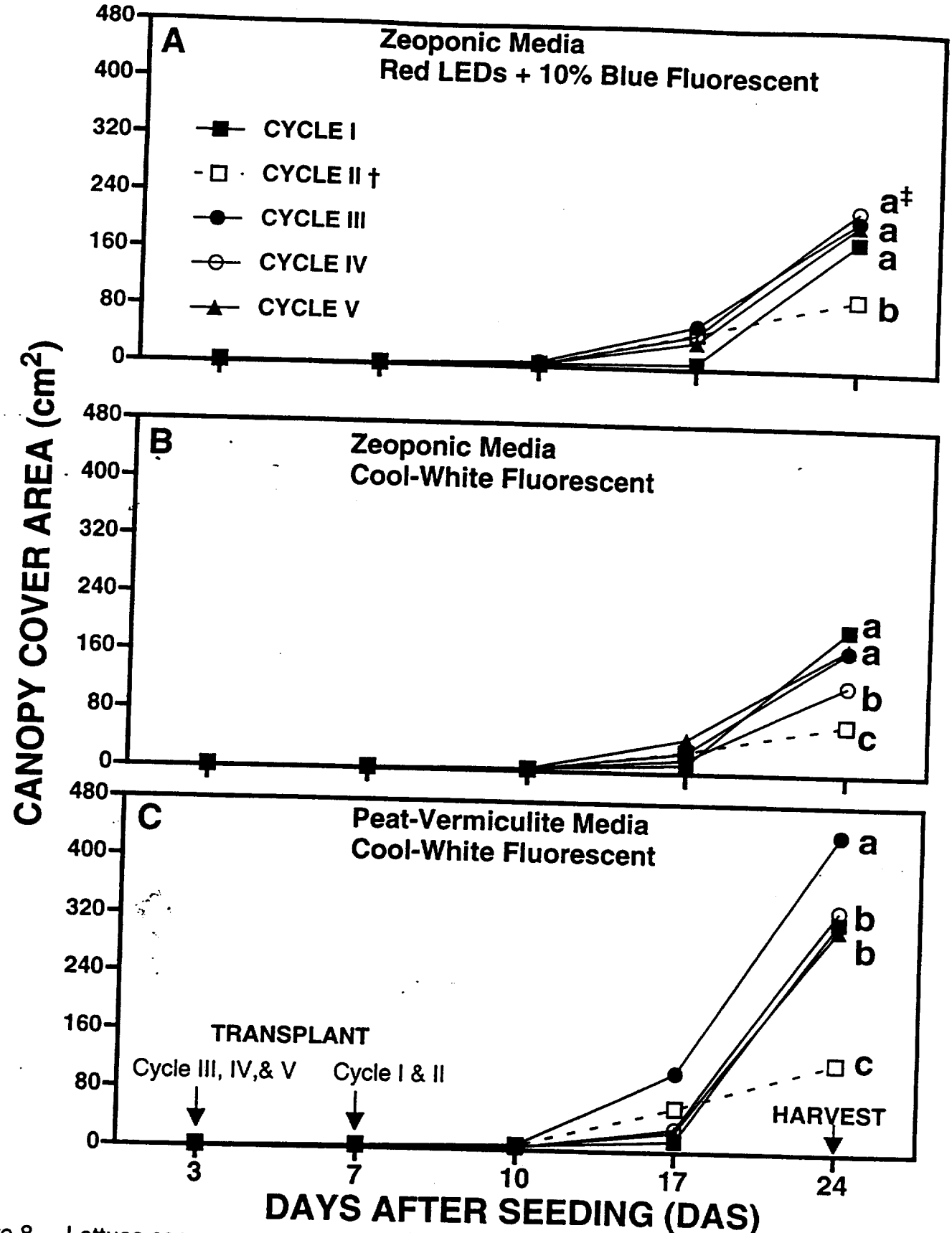

Figure 8. Lettuce canopy area cover over time for 5 crop cycles through the Astroculture™ root trays using zeolite or peat-vermiculite media under different lighting regimes.

†Cycle 2 (dashed line) tested effects of re-transplanting at 10 and 17 DAS. ‡At 24 DAS within each chart, symbols followed by different letters indicate significant difference at the 5% probability level.

LEDs versus Cool-White Fluorescent Lamps

Averaging over crop cycles, there were no significant differences between plants grown in zeoponic media under red LEDs + 10% blue fluorescent (BF) light as opposed to plants grown in zeoponic media under cool-white fluorescent light in terms of shoot fresh mass (Figure 9A), shoot dry mass (Figure 9B), or canopy cover (Figure 10) at final harvest. However, similar studies conducted in the KSC laboratory have shown that when grown under red LEDs without supplemental blue light, lettuce (See Appendix II), wheat (Goins *et al.*, 1997b), and *Arabidopsis* (Goins *et al.*, 1996) all had significantly lower shoot fresh and dry weight at harvest compared to plants grown in the presence of white light or red LEDs with supplemental blue light. Typical responses of plants from plants grown under red LEDs without supplemental blue light are increased length of hypocotyls, cotyledons, stems, and leaves, and well as a decreased overall biomass yield (Goins *et al.*, 1997b; Hoenecke *et al.*, 1992).

Zeoponic Media versus Peat-Vermiculite

When compared to plants grown in peat-vermiculite media, lettuce grown in zeoponic media had a significantly lower shoot fresh mass (Figure 9A), shoot dry mass (Figure 9B), and canopy cover (Figure 10) at final harvest within each crop cycle, irrespective of lighting source. Reductions in shoot fresh mass, shoot dry mass, and canopy cover at final harvest were 43%, 37%, and 47%, respectively for zeoponic-grown lettuce as compared to lettuce grown in peat-vermiculite. Previous work in our laboratory has also demonstrated that wheat seed yield was significantly lower in plants grown in zeoponic media when compared to peat vermiculite (Goins *et al.*, 1997a). Interestingly, lettuce grown in zeoponic media, irrespective of lighting regime, displayed leaves with a deeper green color as compared to plants grown in peat-vermiculite. We have speculated that differences in biomass assimilation in comparisons of plants grown in zeoponic media versus peat-vermiculite may be a consequence of nitrogen (N) source. In zeoponics, the predominant N source is NH_4 (Allen *et al.*, 1995a; Allen *et al.*, 1995b) as opposed to NO_3 in the modified Hoagland's solution (Mackowiak *et al.*, 1989) used with peat-vermiculite. The form of nitrogen supply (NH_4 versus NO_3) has a major role in the cation-anion balance in plants (Marschner, 1995). The pH of the nutrient solution recirculated through zeoponic media decreased over time (Table 2; Goins *et al.*, 1997a). Poor growth of NH_4-fed plants has been associated with a low pH of the external rooting media (Marschner, 1995). Net proton excretion from roots is depressed in low pH media, and thus, regulation of root cytoplasm pH is diminished (Marschner, 1995).

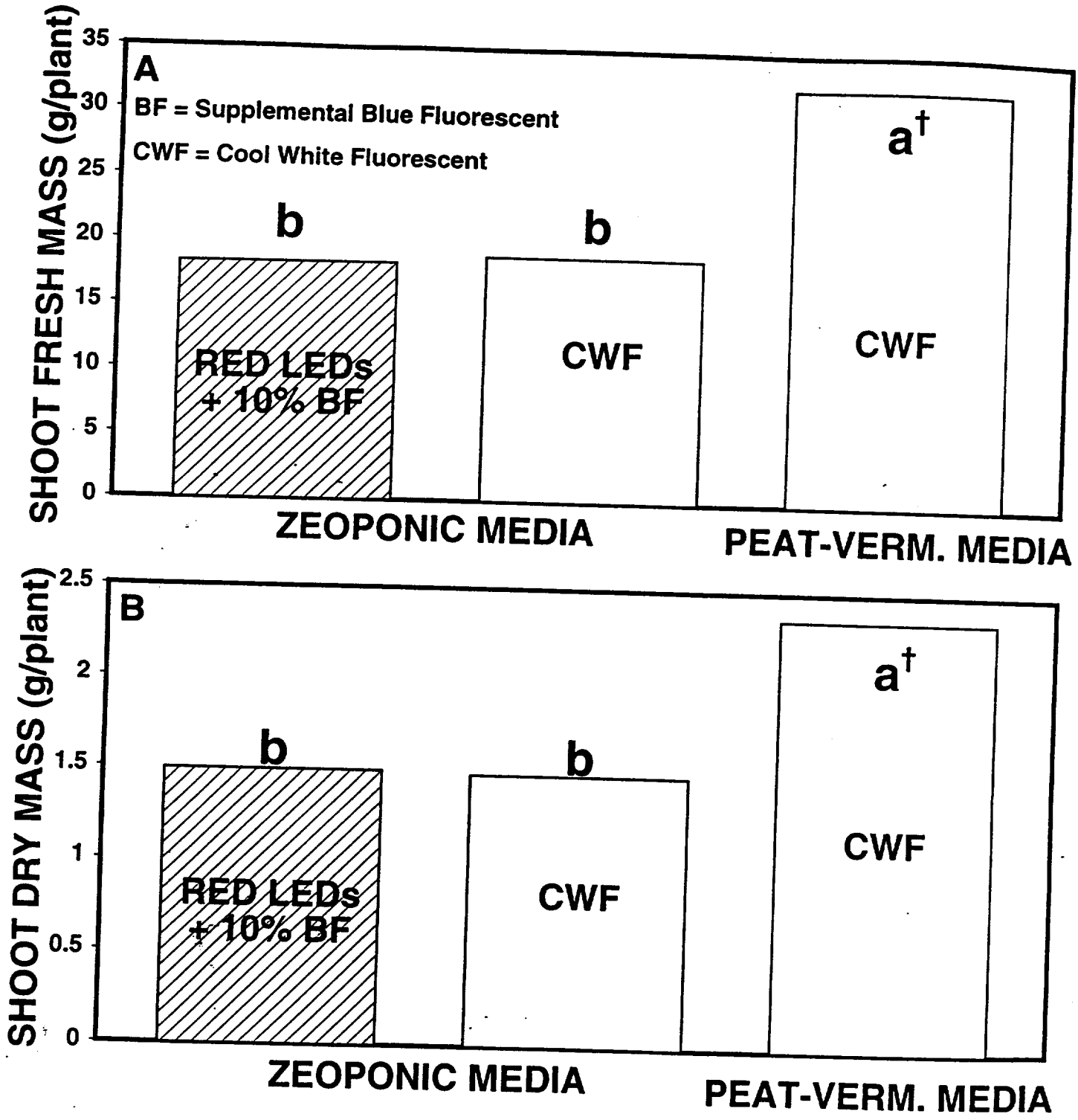

Figure 9. Final harvest (24 days after seeding) shoot fresh (A) and dry mass (B) measurements averaged over 4 crop cycles of lettuce through in Astroculture root trays™. Note: cycle 2 (not shown) which tested effects of re-tranplanting at 10 and 17 DAS, was not included in the data shown above.

†Within each chart, bars with different letters are significantly different at the 5% probability level.

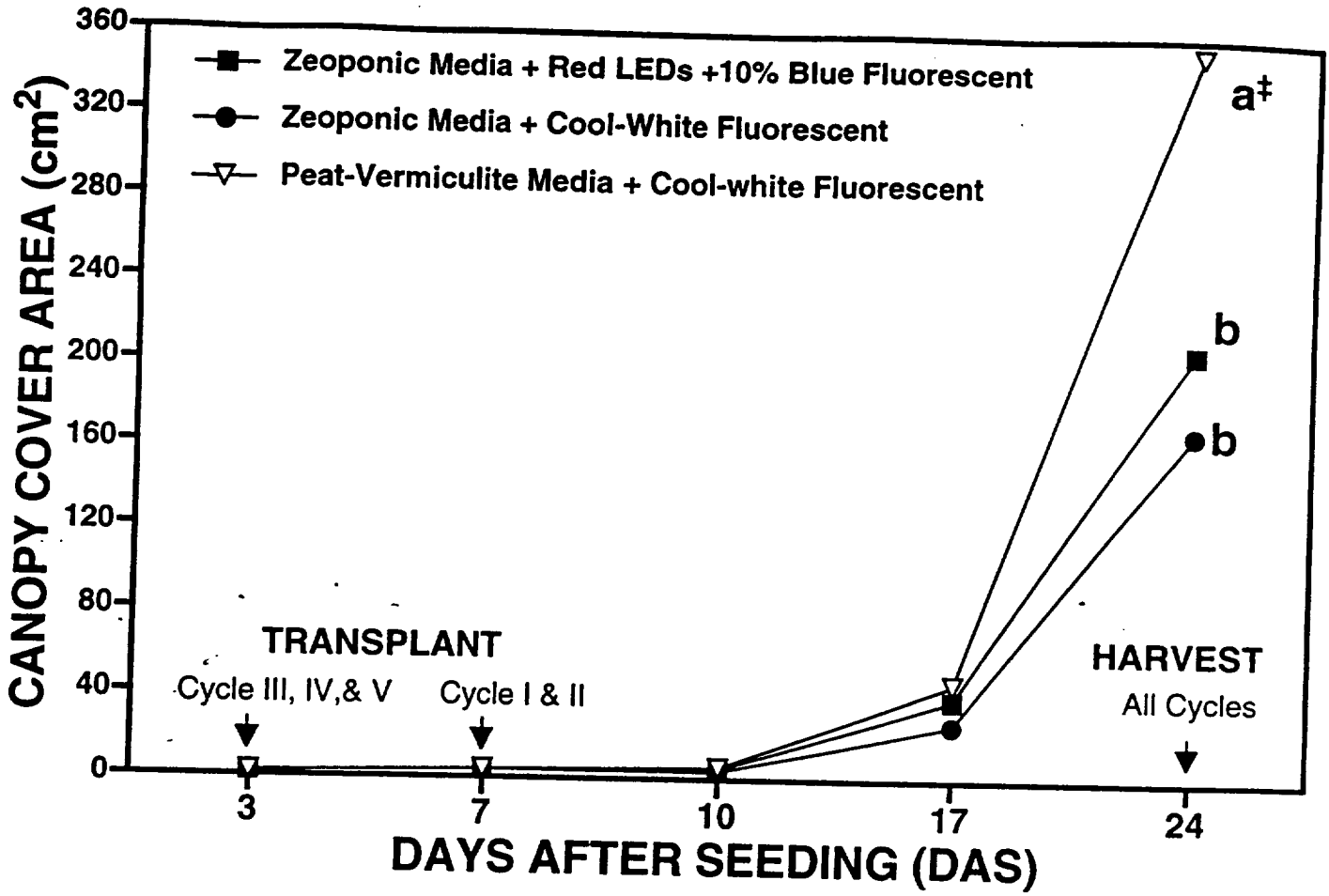

Figure 10. Lettuce canopy area cover over time measurements averaged over 4 crop cycles through the Astroculture™ root trays. Note: cycle 2 (not shown) which tested effects of re-transplanting at 10 and 17 DAS, was not included in the data shown above.

‡At 24 DAS, symbols followed by different letters indicate significant difference at the 5% probability level.

Table 2. ASTROCULTRE™ Root Tray Recirculated Nutrient Solution Analysis (mg/L)

	zeolite red LEDs+10%BF	zeolite CWF	peat-verm. CWF
EC			
6/16/97	795	1417	1417
6/23/97	769	1083	1223
6/30/97	795	977	1045
7/7/97	1182	1091	1159
7/14/97	1534	1091	1045
7/28/97	2718	1276	1229
pH			
6/16/97	5.3	6.09	6.55
6/23/97	5.04	5.19	6.71
6/30/97	5.98	5.69	6.7
7/7/97	5.93	4.53	7.02
7/14/97	4.85	4.85	7.04
7/28/97	4.84	4.86	6.77
NO3			
6/16/97	2.85	2.75	60
6/23/97	0	5.1	62.35
6/30/97	11.55	7.95	30
7/7/97	34.35	10.5	23.65
7/14/97	106.8	9	18.75
7/28/97	318.05	57.6	40.2
Mg			
6/16/97	21.82	47.43	109.4
6/23/97	19.99	35.13	88.01
6/30/97	26.04	42.44	77.2
7/7/97	47.83	47.11	82.55
7/14/97	71.55	48.97	75.16
7/28/97	132.1	61.54	69.92
Na			
6/16/97	1.456	1.63	12.91
6/23/97	0.6762	0.9076	10.17
6/30/97	1.19	0.9576	10.55
7/7/97	1.794	1.196	10.75
7/14/97	1.082	0	7.552
7/28/97	3.743	0.8094	5.376
Cu			
6/16/97	0.02	0.0073	0.0506
6/23/97	0.0151	0.0147	0.0322
6/30/97	0.0136	0.0093	0.0367
7/7/97	0.0178	0.0138	0.0353
7/14/97	0.0132	0.0113	0.0352
7/28/97	0.015	0.0222	0.0424

	zeolite Red LEDs+10%BF	zeolite CWF	peat-verm. CWF
NH4			
6/16/97	31.35	61.1	0
6/23/97	34.95	49.15	0
6/30/97	31.85	42.3	0
7/7/97	54.9	47.25	0
7/14/97	62.4	44.2	0
7/28/97	0	23.05	0
PO4			
6/16/97	0	0	0
6/23/97	0	0	0
6/30/97	0	0	5.02
7/7/97	1.445	2.345	8.165
7/14/97	12.56	4.575	10.985
7/28/97	45.82	3.205	12.73
K			
6/16/97	53.29	92.22	75.66
6/23/97	54.58	66.27	72.87
6/30/97	60.52	64.44	96.19
7/7/97	80.29	68.61	94.56
7/14/97	89.17	66.88	95.96
7/28/97	111.5	63.01	133.2
Ca			
6/16/97	32.68	59.63	146.7
6/23/97	27.01	42.34	115.3
6/30/97	26.19	39.19	92.27
7/7/97	46.4	43.55	102.3
7/14/97	86.93	48.76	99.01
7/28/97	202.3	67.13	98.51
Fe			
6/16/97	0.0964	0.014	0.3057
6/23/97	0.086	0.0415	0.0928
6/30/97	0.0372	0.0111	0.1314
7/7/97	0.036	0.0487	0.0625
7/14/97	0.0559	0.0387	0.082
7/28/97	0.0479	0.0529	0.1272
B			
6/16/97	0.2512	0.3813	0.0518
6/23/97	0.2741	0.293	0.0472
6/30/97	0.2874	0.314	0.0517
7/7/97	0.4747	0.4026	0.0551
7/14/97	0.5569	0.434	0.0548
7/28/97	0.7469	0.4516	0.0977

continued on next page

Table 2. ASTROCULTRE™ Root Tray Recirculated Nutrient Solution Analysis (mg/L)

	zeolite Red LEDs+10%BF	zeolite CWF	peat-verm. CWF
Zn			
6/16/97	0.9722	0.5016	0.8688
6/23/97	0.6821	0.6261	0.7347
6/30/97	0.7035	0.4857	0.7205
7/7/97	0.5336	0.7154	0.8617
7/14/97	0.5012	0.3159	0.761
7/28/97	0.2552	0.3937	0.8359
Mn			
6/16/97	0.0315	0.016	0.0151
6/23/97	0.0243	0.014	0.0088
6/30/97	0.0177	0.0128	0.07
7/7/97	0.017	0.0145	0.0146
7/14/97	0.0327	0.0185	0.032
7/28/97	0.0772	0.0275	0.0376
Mo			
6/16/97	0.0242	0.0426	0
6/23/97	0.0262	0.01	0
6/30/97	0	0	0
7/7/97	0	0	0
7/14/97	0	0	0
7/28/97	0	0	0

Interestingly, nutrient solution analysis of the zeoponic media over the five crop cycles showed that NH_4 concentrations steady declined, while NO_3 concentrations increased substantially. This may suggest a rise in nitrification activity as more lettuce crops were completed in the zeoponic media. Previous studies have shown that growth of different crops supplied a mixed N source (NH_4 and NO_3) exceeds that of crops supplied a single N-form alone (Camberato and Bock, 1990; Knight and Mitchell, 1983; Marschner, 1995). Therefore, lettuce yields may have increased over that reported in the present study if additional crop cycles were continued in this zeoponic substrate. It is important to note that since control of pH and EC was not obligatory with zeoponic media, labor requirements were much less for plants grown in zeoponic media as opposed to peat-vermiculite. Current research efforts at JSC include further refinement of zeoponic media (Personal Communication, Doug Ming, JSC).

Appendix I.
A Protocol for Continuous Lettuce Crop Production in the CERES 2010™

Materials Needed
Lettuce seed
Zeoponic media
Astroculture™ root tray
De-ionized water
Arasystem- Arabaskets, Aratrays, Araflats
Clear plastic sheets
Water bottle with fine spray mist nozzle
Growth chamber conditions:
Air Temperature 23°C; 65-75% RH; PAR 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$; CO_2 1200 $\mu mol \cdot mol^{-1}$

1. **Prepare zeoponic media**
   Saturate zeoponic media with de-ionized water. Thoroughly mix zeoponic media to achieve uniform wetness, and drained off excess water.

2. **Prepare Arabaskets for seeding**
   Fill each Arabasket with moist zeoponic media, leaving approximately 0.5 cm of unfilled space at the top of the basket. Dry zeoponic media will not stay contained within the Arabasket. Do not compress or pack moist media in Arabasket. Packing moist media will create hard pans, which causes root penetration problems.

3. **Prepare Aratrays**
   Place zeoponic media-containing Arabaskets in the Aratrays, and then place the Arabasket-containing Aratray on top of an Araflat. Place Aratrays under a light source (preferably PAR 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Flood Araflat to capacity with de-ionized water so that zeoponic media in Arabaskets can wick up water through bottom of the Aratrays. Once per day, check the water level in the tray to be sure media does not desiccate.

4. **Seed Arabaskets**
   Place 3 dry lettuce seeds on surface of zeoponic media within each basket. Thoroughly moisten seeds and zeoponic media surface by applying de-ionized water from a spray bottle. It is recommended to prepare about twice the minimum required number of Arabaskets per Aratrays for each specific seeding date. This will give the experimenter extra plants in situations where there are less than optimal seed germination or unsuccessful transplant establishment in the ASTROCULTURE™ root tray. Cover seed-containing baskets with a clear plastic sheet to maintain a high humidity zone near seeds.

5. **Irrigate Arabaskets**
   Lift clear plastic sheet cover and observe seeds daily for germination progress. Irrigate Arabaskets at least once a day by lightly re-spraying zeoponic media surface with water for the first 3 days after seeding (DAS). Also add water to Araflat if water level has gone below wicking point to Aratrays. Re-spray of seedlings is crucial for environments with low relative humidity.

6. **Uncover Arabaskets and Thin Plants**
Leave clear plastic sheet over baskets until first cotyledon leaf of germinating seedlings is observed (usually about 3 DAS). At this point, seedlings should be able to withstand being uncovered without risk of desiccation. Leave plants uncovered to harden, but make sure seedlings do not desiccate if chamber humidity is low (e.g. below 65% RH). Re-spray seedlings with water if necessary. Use small scissors to thin plants to 1 plant per Arabasket.

7. **Prepare Astroculture™ root trays**
In empty Astroculture™ root trays (containing only porous tubes), place non-seeded media-filled Arabaskets in desired transplant locations between the sub-irrigated porous tubes in the root tray (see Figures 1 and 5 in attached report). Fill in remaining root tray void space with moist media. As each of these Arabaskets are removed, a hollow depression will be left behind. As long as the zeoponic media remains moist, the hollow depression will hold its original form when an Arabasket is removed. A negative pressure of -0.23 kPa has been found to be sufficient for maintaining adequate rooting media moisture and plant growth.

8. **Transplant into Astroculture™ root trays**
At 3-7 DAS, lettuce seedlings are ready to be transplanted into Astroculture™ root trays in the CERES 2010™ by removing Arabaskets from an Araflat and manually inserting the Arabaskets into pre-prepared depressions (see step 7) located in the Astroculture™ root tray. Firmly seat the Arabasket in the Astroculture™ root tray by gently rotating the Arabasket 360°. Arabaskets may be transplanted more than once in this manner without detrimental plant yield loss if plants are less than 7 DAS (see attached report). If transplanted after 10 DAS, plants have shown significant yield reduction.

9. **Plant Maintenance and Growth**
Plant growth data over continuous cycles is presented in the attached report. In addition, trends in Astroculture™ root tray water use, pH, and nutrient ion concentrations over continuous cycle are outlined in the attached report.

10. **Harvest Plants in Arabaskets**
At desired harvest date, remove the Arabaskets from the Astroculture™ root tray by grasping Arabasket lip and gently turning Arabasket and applying steady upward pressure. With enough pressure, the Arabasket will begin to loosen from the bulk media. Arabasket will lift out of the Astroculture™ root tray with plant and media contents largely intact. Roots that have emerged outside the perimeter of the Arabaskets are severed and left behind in the Astroculture™ root tray. Minimum rooting media disturbance should be observed with neighboring plants in the Astroculture™ root tray. Empty depression in Astroculture™ root tray can immediately be filled with next transplant.

APPENDIX II.

Ceres 2010™ Specifications Data Sheet.

THERMAL MANAGEMENT:

Temperature Range: +15 to + 40 deg C.
Tolerance +/- 0.5 deg C.

Heating and cooling on the CERES 2010™ is accomplished by using solid state Thermoelectric Coolers (TEC). The TEC's have an excellent response time when coupled with the computer interface. TEC's offer a quiet alternative to the noisy mechanical refrigeration compressors of the past and there is no CFC refrigerant to leak into the atmosphere thereby making the CERES 2010™ very environmentally friendly.

HUMIDITY:

Humidity Range 20 to 80% relative
Tolerance +/- 2.0 %

The CERES 2010™ HygroThermoElectric (HTE) humidity control system was developed for NASA to maintain precise humidity control for the research plant chambers on board the Space Shuttle. The HTE system has been designed to be used in the humidifier or dehumidifier mode. The solid state construction of the HTE provides long term reliability and easy interface with CERES 2010™ computer interface.

AIR MANAGEMENT:

The CERES 2010™ has been designed to have a laminar counter flow controlled internal air velocity of 0.5m/sec. The low velocity has been designed to enhance the overall temperature and humidity control and to minimize mechanical stress on the experiment.

CARBON DIOXIDE:

CO^2 range 500 to 3,000 ppm
Tolerance +/- 25 ppm

The CERES 2010™ is equipped with computer assisted CO^2 monitoring and control system that can be used to maintain a predetermined level of CO^2 during the illuminated cycle of the experiment. In low level applications the CO^2 control opens a port to the room ambient. For higher level requirements a reagent tank of CO^2 can be connected to the port.

ETHYLENE MANAGEMENT:

The CERES 2010™ has been designed to accept an optional non-consumable ethylene removal unit when required. The Ethylene Management Unit (EMU) is a non-consumable photocatalytic ethylene oxidation unit and is offered as an option. The EMU has been expressly developed for the CERES 2010™ product line and is easy to install.

ASTROCULTURE™ ROOT TRAY:

The optional Astroculture™ Root Tray has been designed to provide a favorable root environment while providing the nutrient and water requirements for proper plant response. This root tray is similar to the hardware flown on several successful missions on board the U.S. Space Shuttle and has been designed to interface with the CERES 2010™ hardware and computer interface.

GENERAL SPECIFICATIONS

ELECTRICAL

115/60 - 10 amps.

GROWING HEIGHT

23" (58 cm) without root tray
20" (50.8 cm) with root tray

GROWTH AREA

2.76 Sq Ft. (.223 m^2)

INTERIOR

(W) 26.5" x (D) 15" x (H) 23.5"
(W) 58.6 cm x (D) 38.1 cm x (H) 59.7 cm

EXTERIOR

(W) 31" x (D) 26" x (H) 46"
(W) 78.74 cm x (D) 66.0 cm x (H) 116.8 cm

CONSTRUCTION

Interior and exterior constructed of 22-gauge electro-zinc plated steel. All seams and joints on the outer and inner shells are welded. Inner shell is solidly supported by a non-compressing and non-thermal conducting material to lock the inner liner in place without a metal-to-metal bond to the outer case. The chamber is completely self-contained, suitable for stacking one above the other.

INSULATION

Insulated with rigid urethane. Overall wall thickness is 2" (5.1 cm) providing ample insulation for maintenance of stated temperature range and vapor isolation in a 35 degree C room ambient.

DOOR

One door opening 26 13/16" W x 24" H provides full access to chamber interior. A magnetic gasket and latch provide a tight seal to door frame. (Window is for demonstration purposes only)

SHELVING

One tier of zinc and acrylic coated steel wire shelving adjustable vertically on 1/2" (1.27 cm) centers. Shelf dimensions are 12" x 26" (30.48 cm x 66 cm).

FINISH

Interior/exterior painted with highly reflective white baked enamel.

APPENDIX III.
Comparison of lettuce performance in the Astroculture Root Tray versus NFT Hydroponics

Nutrient Thin-Film Technique (NFT) Design and Cultural Practices

Lettuce was grown using a hydroponic nutrient film technique (NFT) located adjacent to each Astroculture™ root tray (Figure 11) under each lighting regime in each growth chamber as described in MATERIALS AND METHODS. An NFT system was also located in an additional plant growth chamber that was equipped with an array of red LEDs without supplemental blue fluorescent light. There were no Astroculture™ root trays placed in this additional chamber. Each NFT system contained 3 separate sloping rectangular plastic troughs (Figure 11) spaced 2 cm apart. Each trough (10 cm W X 35 cm L) contained 3 plants spaced 12 cm apart to give an effective planting density of 73 plants•m^{-2} (actual 9 plants•0.123 m^2). Seeding was done by placing a dry seed between two nylon (Nitex) fabric wicks supported above the bottom of the troughs as described by Wheeler *et al.* (1994).

Recirculating solution flowed through the troughs at a rate of approximately 1 L•min^{-1} to give a steady stream or film of nutrient solution to the lettuce roots. Nutrient solution was continuously pumped and recirculated from separate reservoirs under the different light regimes. Nutrient solution electrical conductivity (EC) was maintained at approximately 1200 µS•cm^{-1} by adding a modified (NO_3-N only) concentrated Hoagland's stock solution (Hoagland and Arnon, 1950; Mackowiak *et al.*, 1989) to the reservoirs. On a daily basis, water replenishment and nutrient solution EC levels were monitored and controlled manually. An automatic pH controller added 0.1M HNO_3 as needed to maintain solution pH at 5.5 - 6.0. Plants were harvested at 21 days after seeding (DAS) and shoot fresh and dry mass measurement were recorded. Two crops were completed in a batch process, and the results are presented below.

NFT and Astroculture™ Root Tray Comparisons

Lettuce grown in the NFT system appeared to have a faster rate of shoot expansion (Figure 12) and greater shoot fresh mass (Figure 13A) and dry mass (Figure 13B) at 21 DAS than plants in the Astroculture™ root trays under the same lighting regime, which were harvested 3 days later. Interestingly, the fresh to dry mass ratio of the NFT-grown plants (at 21 DAS) were much greater than the plants grown in the Astroculture™ root trays (at 24 DAS). This may suggest that NFT-grown seedlings become established more rapidly and/or plants were more hydrated than the Astroculture™ root tray-grown plants. Especially under cool-white fluorescent lamps, the NFT systems (Table 3) consumed considerably more water than the Astroculture™ root

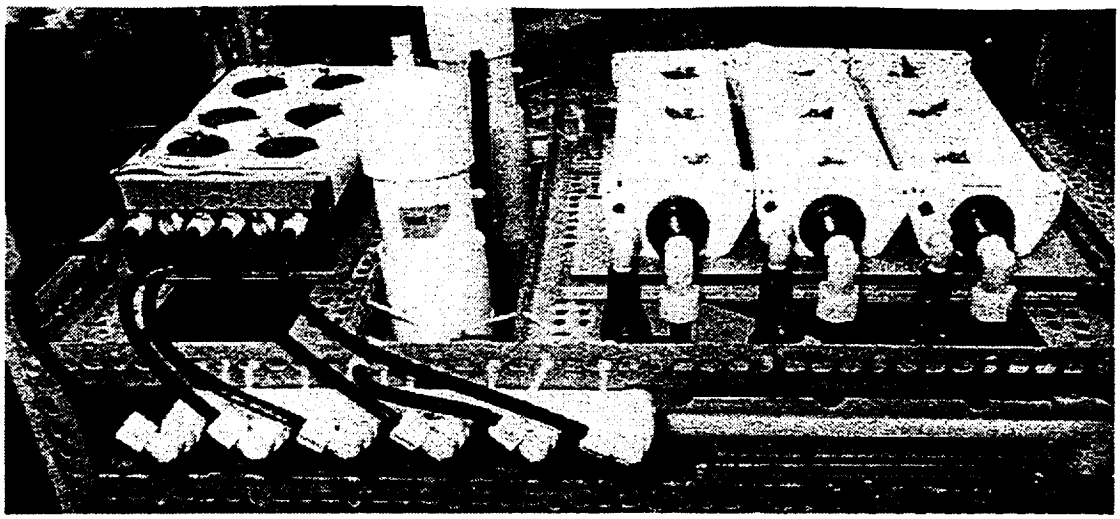

Figure 11. Astroculture root tray (left) and NFT hydroponic system (right) in side-by-side comparison at the Kennedy Space Center Gravitational Biology Laboratory.

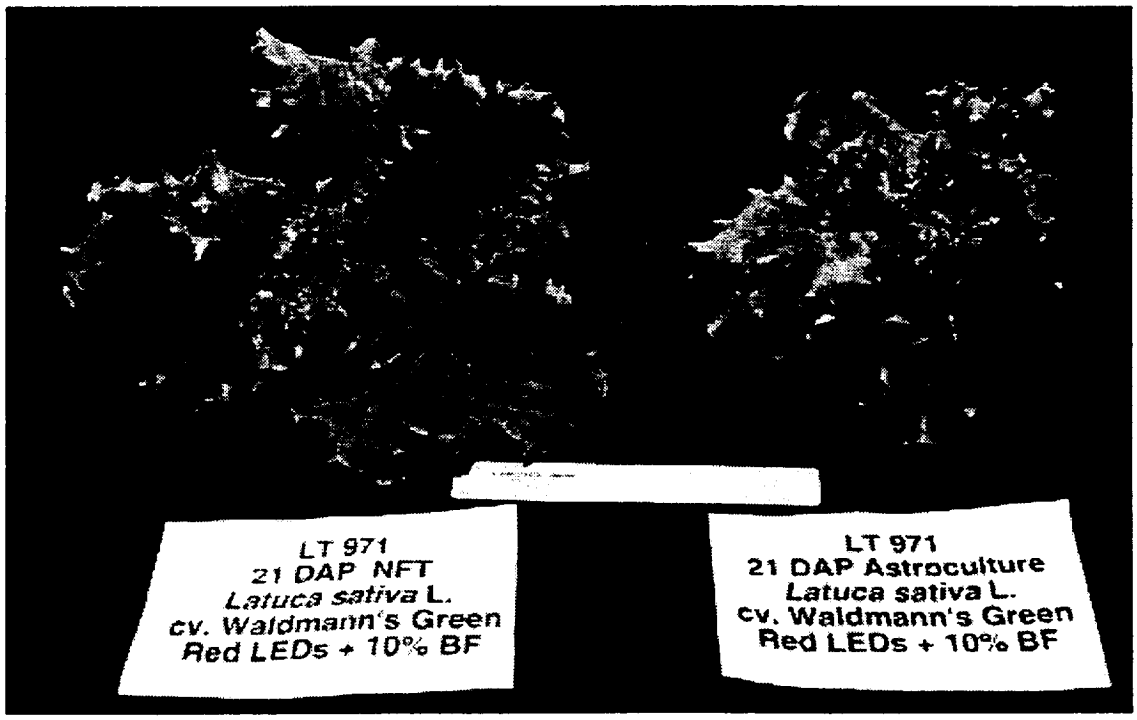

Figure 12. Comparison of Waldmann's Green lettuce grown in the NFT hydroponic system (left) or the Astroculture root tray (right) after 21 days (15-cm ruler shown in foreground).

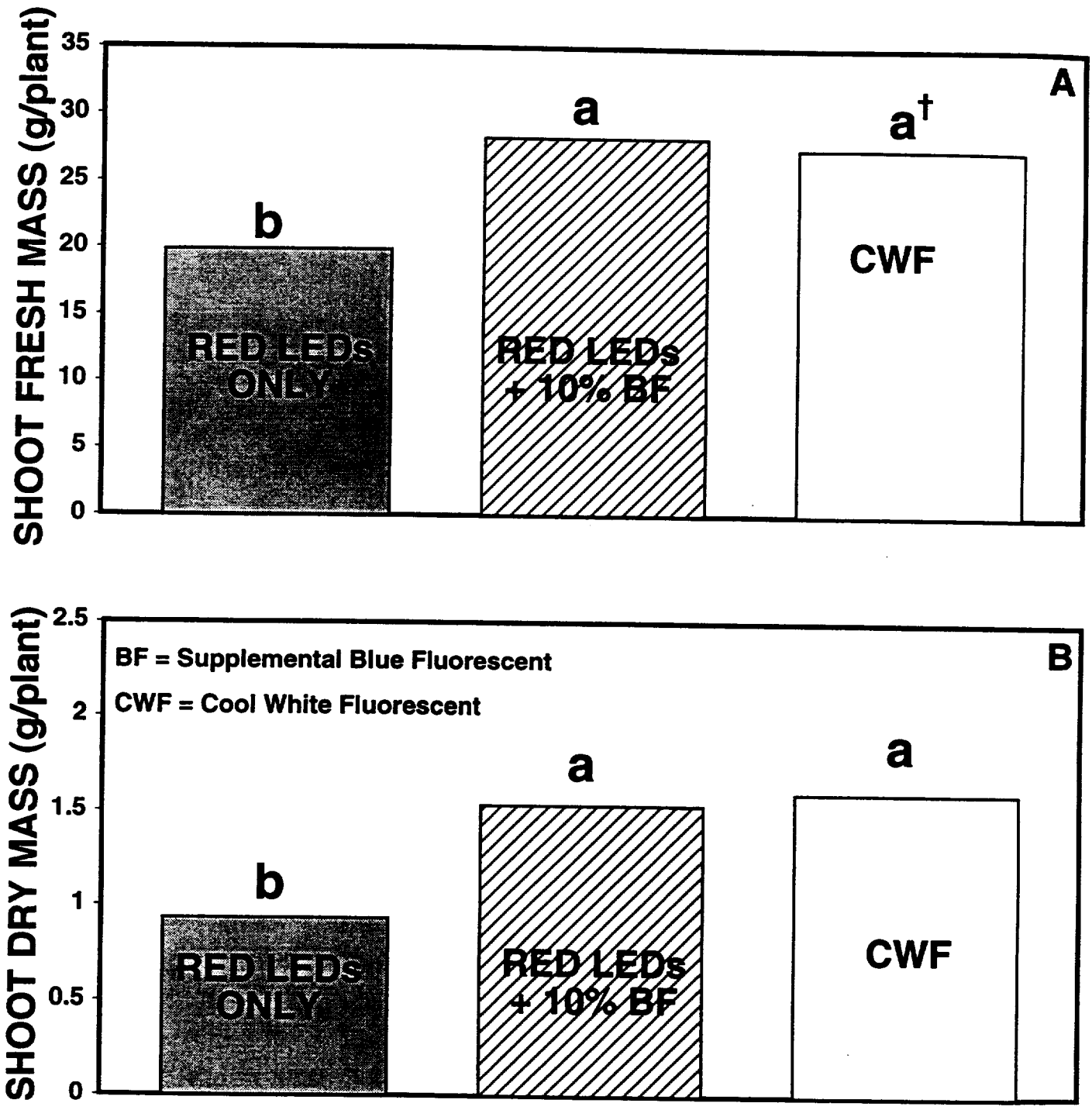

Figure 13. Final harvest (21 days after seeding) shoot fresh (A) and dry mass (B) measurements averaged over 2 crop cycles of lettuce through the NFT sytems under different lighting regimes.

†Within each chart, bars with different letters are significantly different at the 5% probability level.

Table 3. NFT System Daily Water Use (L)

Crop 1	CWF	Red LEDs only	Red LEDs+ 10%BF
9-Jun	0	0	0
10-Jun	0.62	0	0
11-Jun	0.4	0	0
12-Jun	0.5	0	0
13-Jun	0.26	0	0
14-Jun	0.5	0	0
15-Jun	0.35	0	0
16-Jun	0.92	0	0
17-Jun	0.25	0	0
18-Jun	0.66	0	0
19-Jun	0.66	0	0
20-Jun	0.64	0.55	0.44
21-Jun	0.55	0.32	0.36
22-Jun	0.6	0.66	0
23-Jun	0	0.35	0.64
24-Jun	1	0	0.15
25-Jun	0.9	0.67	0.67
26-Jun	0.56	0.5	0.86
27-Jun	0.5	0.48	0.44
28-Jun	0.9	0.4	0.5
29-Jun	1.2	0.7	0.6
30-Jun	1	0.62	0.37
Totals	**12.97**	**5.25**	**5.03**

Crop 2	CWF	Red LEDs only	Red LEDs+ 10%BF
7-Jul	0	0	0
8-Jul	0.65	0.13	0.1
9-Jul	0.4	0.6	0.4
10-Jul	0.56	0.24	0.63
11-Jul	0.28	0.51	0.3
12-Jul	0.77	0.41	0
13-Jul	0.42	0	0.46
14-Jul	0.42	0.36	0
15-Jul	0.5	0.36	0.3
16-Jul	0.76	0.3	0.34
17-Jul	0.45	0.32	0.38
18-Jul	0.45	0.25	0.25
19-Jul	0.62	0.58	0.27
20-Jul	0.66	0.45	0.32
21-Jul	0.59	0.25	0.27
22-Jul	0.5	0.47	0.37
23-Jul	1	0.29	0.29
24-Jul	0.35	0.7	0.42
25-Jul	1.05	0.32	0.68
26-Jul	1	0.51	0.44
27-Jul	1	0.71	0.47
28-Jul	1.4	0.5	0.5
Totals	**13.83**	**8.26**	**7.19**

tray system (Table 2). The capacity of porous tube systems to conserve water has been observed previously (Berry et al., 1992; Dreschel and Sager, 1989). Plants are generally assumed to have all water requirements met by an NFT system, whereas plants in the Astroculture™ root tray system receive water chiefly via capillary forces (Morrow *et al.*, 1994; Morrow *et al.*, 1995). Lettuce in the Astroculture™ root trays may have experienced water stress, although the zeoponic media appeared to receive more than adequate moisture to support lettuce growth. Several growth trials by other researchers have indicated that the amount of negative pressure and pore size of porous tubes can have significant effects on plant growth (Berry et al., 1992; Dreschel and Sager, 1989).

NFT-Grown Lettuce Lighting Comparisons

In comparison to NFT lettuce grown under red LEDs alone, NFT lettuce grown under red LEDs + 10% BF light or cool-white fluorescent light had significantly greater amounts of shoot fresh and dry mass at final harvest (Figure 13). Supplementing red LEDs with 10% BF light produced NFT lettuce similar to cool-white light-grown NFT lettuce with respect to amounts of shoot fresh and shoot dry mass at final harvest. This agreed with the findings from lettuce grown in Astroculture™ root trays (Figure 9). Decreased biomass yield is a typical response of plants grown under red LEDs without supplemental blue light (Goins *et al.*, 1997b). Moreover, we have observed that net leaf photosynthesis rates were significantly lower in wheat grown under red LEDs alone as compared to wheat grown under daylight fluorescent. Thus, the low biomass at harvest for plants grown under red LEDs alone may be related to a lower CO_2 assimilation rate relative to plants grown under a white light or blue light-supplemented red LED source.

SUMMARY

1. A reliable protocol was developed for simple seeding, transplanting and harvesting of lettuce in Astroculture™ root trays. (See Protocol, pg. 23)

2. Compared to lettuce grown under cool-white fluorescent light, lettuce grown under red LEDs + 10% blue fluorescent light showed no significant differences in shoot fresh mass, shoot dry mass, or canopy cover area at final harvest.

3. Lettuce grown in zeoponic media had significantly lower shoot fresh mass, shoot dry mass, and canopy cover area at final harvest than lettuce grown in peat-vermiculite, irrespective of lighting source.

4. Lettuce grown in zeoponic media had a slower rate of shoot expansion, and a lower shoot fresh mass and dry mass than NFT-grown lettuce, irrespective of lighting source.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1998	Technical Memorandum

4. TITLE AND SUBTITLE
Protocol development for the NASA-JSC Lunar-Mars Life Support Test Project (LMLSTP) Phase III Project: A Report on Baseline Studies at KSC for Continuous Salad Production

5. FUNDING NUMBERS

6. AUTHOR(S)
G.D. Goins, N.C. Yorio, and H.R. Vivenzio

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dynamac Corporation, Mail Code: DYN-3 and
NASA Biological Research and Life Support Office: JJ-G
J. F. Kennedy Space Center, FL 32899

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Biomedical Operations and Research
Mail Code: JJ-G
Kennedy Space Center, FL 32899

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
NASA/TM-1998-207911

11. SUPPLEMENTARY NOTES
Category 51

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Unclassified/Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

During Fall 1997, the Phase III Lunar-Mars Life Support Test Project (LMLSTP) was conducted in a 20-foot chamber at Johnson Space Center. The overall objective of the Phase III project was to conduct a 90-day regenerative life support system test involving 4 human subjects to demonstrate an integrated biological and physicochemical life support system. A secondary objective of the Phase III LMLSTP was to demonstrate the ability to produce salad-type vegetable by integration of a small benchtop growth chamber located within the crew habitat area. This small chamber, commercially manufactured as the Controlled Environment Research Ecosystem (CERES 2010™), functioned as a means to continuously provide fresh lettuce crops for crew members. The CERES 2010™ growth chamber utilized hardware components developed for effective plant biomass production in spaceflight applications. These components included: (1) LED lighting (2) Astroculture™ Root Trays, and (3) Zeoponic media. In planning for the LMLSTP Phase III, a request was put forward for KSC scientists to generate a protocol for successful continuous planting, culturing, and harvesting of the salad-crop, lettuce (*Lactuca sativa* L. cv. Waldmann's Green). By conducting baseline tests with components of the CERES 2010™, a protocol was developed.

14. SUBJECT TERMS
LEDs, Zeoponics, plant growth, vegetable salad crops

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited