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**Crop Production for Advanced Life Support Systems –  
Observations From the Kennedy Space Center  
Breadboard Project**

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***February 2003***

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## ABSTRACT

The use of plants for bioregenerative life support for space missions was first studied by the US Air Force in the 1950s and 1960s. Extensive testing was also conducted from the 1960s through the 1980s by Russian researchers located at the Institute of Biophysics in Krasnoyarsk, Siberia, and the Institute for Biomedical Problems in Moscow. NASA initiated bioregenerative research in the 1960s (e.g., *Hydrogenomonas*) but this research did not include testing with plants until about 1980, with the start of the Controlled Ecological Life Support System (CELSS) Program. The NASA CELSS research was carried out at universities, private corporations, and NASA field centers, including Kennedy Space Center (KSC). The project at KSC began in 1985 and was called the CELSS Breadboard Project to indicate the capability for "plugging in" and testing various life support technologies; this name has since been dropped but bioregenerative testing at KSC has continued to the present under the NASA's Advanced Life Support (ALS) Program. A primary objective of the KSC testing was to conduct pre-integration tests with plants (crops) in a large, atmospherically closed test chamber called the Biomass Production Chamber (BPC). Test protocols for the BPC were based on observations and growing procedures developed by university investigators, as well as procedures developed in plant growth chamber studies at KSC. Growth chamber studies to support BPC testing focused on plant responses to different carbon dioxide (CO<sub>2</sub>) concentrations, different spectral qualities from various electric lamps, and nutrient film hydroponic culture techniques.

This Technical Memorandum (TM) gives an overview of activities leading up to the KSC Breadboard Project, a description of the BPC, and summaries of crop test data, including the following:

- Environmental and horticultural approaches.
- Observations on growth and development.
- Crop biomass and oxygen yields.
- Proximate composition of biomass.
- Whole stand photosynthesis and respiration rates and responses.
- Nutrient solution measurements, including water use, nutrient uptake, and acid additions for pH control.
- Ethylene and other volatile organic compound production by plants.

Because of the extent and diversity of crop testing at KSC during the 1985 to 2002 time frame, the results and discussion are limited primarily to BPC studies with wheat, potatoes, soybeans, lettuce, and tomato. An expanded bibliography of plant-related CELSS/ALS research is included at the end of the document.

The intent of this TM is to provide both a general summary of the crop studies at KSC as well as a template for compiling a user's guide or handbook for growing crops for Advanced Life Support systems in space. Because knowledge on growing plants for life support is continually expanding, the specific set points and outputs from ALS cropping systems will continue to be refined and improved. A crop handbook detailing these outputs and horticultural approaches may one day provide an invaluable reference for sustaining life support "farms" and the success of future space missions.

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## ABBREVIATIONS AND ACRONYMS

A	ampere
AIBS	American Institute of Biological Sciences
ALS	Advanced Life Support
AOAC	Association of Official Analytical Chemists
ARC	Ames Research Center
BLSS	Bioregenerative Life Support Systems
BLT	BPC Lettuce (Experiment Series)
BPC	Biomass Production Chamber
BSAB	Breadboard Scale Aerobic Bioreactor
BSB	BPC Soybean (Experiment Series)
BTM	BPC Tomato (Experiment Series)
BWP	BPC Potato (Experiment Series)
BWT	BPC Wheat (Experiment Series)
°C	degree Celsius
ca	circa
CE	controlled environment
CEA	controlled environment agriculture
CEEF	closed ecology experiment facility
CELSS	Controlled Ecological Life Support System; Closed Ecology Life Support System
CO <sub>2</sub>	carbon dioxide
CPEF	closed plant experiment facility
CSTR	continuous stirred tank reactor
dc	direct current
DI	deionized water
EC	electrical conductivity (units typically as dS m <sup>-1</sup> )
FID	flame-ionization detector
GC	gas chromatography
h	hour
HEPA	high-efficiency particulate air
HPS	high-pressure sodium
ICP	inductively coupled plasma
IES	Institute of Environmental Science
incan	incandescent
JSC	Johnson Space Center
KSC	Kennedy Space Center
kW	kilowatt
LED	light-emitting diode
m	meter
MELISSA	Micro-Ecological Life Support System Alternative
MH	metal halide
mol	mole
NFT	Nutrient Film Technique (form of hydroponic culture)

## ABBREVIATIONS AND ACRONYMS (cont)

PAR	photosynthetically active radiation (units typically as $\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\text{W m}^{-2}$ )
PID	photo-ionization detector
ppb	parts per billion
PPF	photosynthetic photon flux (units as $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
ppm	parts per million
TIF	tuber induction factor
TM	Technical Memorandum
VHO	very high output
VOC	volatile organic compound
VPD	vapor pressure deficit
$\mu\text{M}$	micromole per liter

# CROP PRODUCTION FOR ADVANCED LIFE SUPPORT SYSTEMS – OBSERVATIONS FROM THE KENNEDY SPACE CENTER BREADBOARD PROJECT

## 1. INTRODUCTION

The concept of using biological systems for life support in space has been studied since the 1950s and was considered even before this (Myers, 1954; Krall and Kok, 1960; Golueke and Oswald, 1964). Early studies were sponsored by the US Air Force and focused on algae (e.g., *Chlorella*) for atmospheric regeneration (Krall and Kok, 1960, Golueke and Oswald, 1964; Eley and Myers, 1964; Miller and Ward, 1966), while related studies sponsored by NASA included tests with chemoautotrophic organisms, such as *Hydrogenomonas*, in combination with physico-chemical methods (electrolysis) for atmospheric regeneration and biomass production (Bongers and Medici, 1968). Testing was expanded in the late 1960s and 1970s by Russian researchers to include higher plants, and studies using crops to support human crews (the BIOS Projects) were carried out in closed systems (Gitelson et al., 1989; Salisbury et al., 1997). In the late 1970s, NASA initiated the Controlled Ecological Life Support System (CELSS) program to continue research on bioregenerative life support, with much of the effort focused on controlled environment production of higher plants (MacElroy and Bredt, 1985; Galston, 1992). CELSS studies were conducted at several universities, corporations, and NASA field centers throughout the 1980s and early 1990s, after which the program merged with NASA's physico-chemical life support research efforts under the Advanced Life Support Program (Averner, 1993). Studies of bioregenerative life support concepts have continued under the NASA ALS Program, as well as at the Japanese Institute for Environmental Sciences (Tako et al., 2001) and the European Space Agency's Micro-Ecological Life Support System Alternative or MELISSA Project (Lasseur and Savage, 2001).

As interest in the CELSS program expanded to include testing with higher plants (crops), questions arose as to which species should be considered for space life support. The topic was first addressed at a "Space Biologistics Symposium" held at Wright-Patterson Air Force Base in 1962, which produced a list of suggested leafy vegetables and root crops (Boeing, 1962). Additional crop recommendation panels were commissioned by CELSS and ALS programs and findings from these panels and other groups have been published in several documents, including Tibbitts and Alford, 1982; Hoff et al., 1982a; Salisbury 1991; Salisbury and Clark, 1996a, b; and Mitchell et al., 1996 (Table 1). Criteria considered for selecting these crops included crop yield, nutritional value, harvest index (edible dry mass/total dry mass), horticultural requirements, and processing requirements (Tibbitts and Alford, 1982; Hoff et al., 1982a). In addition, all of the crops were C<sub>3</sub> photosynthesis types, which are slightly more efficient than C<sub>4</sub> types at enriched CO<sub>2</sub> concentrations. Generally, the lists focused on "staple" crops to address bulk dietary requirements (carbohydrate, protein, and fat) but also included supplemental vegetables/fruits to provide some vitamins, minerals, and dietary variety. It is important to note that none of these lists provided a complete diet, and for some missions meeting all the nutritional demands for space crews might be achieved more efficiently through a combination of in situ grown plant foods along with some imported supplements, e.g., highly processed foods, flavorings, vitamins, minerals, etc. (Hoff et al., 1982a; Tibbitts and Alford, 1982).

Table 1. Possible crops for Advanced Life Support (ALS) systems.

Boeing Company (1962)	Tibbitts and Alford (1982)	Hoff, et al. (1982a)	Salisbury and Clark (1996)	Gitelson and Okladnikov (1994) BIOS-3 Tests
Sweetpotato	Wheat	Wheat	Wheat	Wheat
Tampala	Soybean	Soybean	Rice	Potato
Lettuce	Potato	Potato	Sweetpotato	Carrot
Chinese Cabbage	Lettuce	Rice	Broccoli	Radish
Cabbage	Sweetpotato	Peanut	Kale	Beet
Cauliflower	Peanut	Dry Bean	Lettuce	Nut Sedge
Kale	Rice	Tomato	Carrot	Onion
Collards	Sugar Beet	Carrot	Canola	Cabbage
Turnip	Taro	Chard	Soybean	Tomato
Swiss Chard	Winged Bean	Cabbage	Peanut	Pea
Endive	Broccoli		Chickpea	Dill
Dandelion	Onion		Lentil	Cucumber
Radish	Strawberry		Tomato	Salad Species
New Zealand Spinach			Onion	
			Chili Pepper	

## 2. CROP TESTING AT KENNEDY SPACE CENTER

Interest in bioregenerative life support at NASA's Kennedy Space Center began in the late 1970s when a proposal was submitted to NASA Headquarters to develop a Closed Ecology Life Support System (CELSS) testbed (Buchanan et al., 1978). This project proposed to retrofit the two large (Apollo) vacuum chambers in the Operations and Checkout Building at KSC and develop a plant production system in one chamber and then link this to a human habitat and waste processing system in the second chamber (Buchanan et al., 1978) (Figure 1).

This proposal was not funded; subsequently, a smaller project was proposed to develop a testbed for crop production and biological waste processing only (i.e., no attached human habitat) for the CELSS program (Prince and Knott, 1986, 1989; Knott, 1992). Dr. Arnauld Nicagossian, NASA Life Sciences Director, approved the latter project, named the CELSS Breadboard Project, and facilities construction activities began in 1985. These Breadboard Project objectives initially encompassed biological approaches for food and atmospheric regeneration (through crop production), biological waste treatment and recycling approaches, and food processing tests using controlled-environment-grown crops (Prince and Knott, 1989). External advisory panels were established through the American Institute of Biological Sciences (AIBS) to provide oversight and annual reviews in each focus area. The food processing research program was abandoned due to funding and staff limitations, and the project continued with crop testing and waste treatment/recycling approaches.



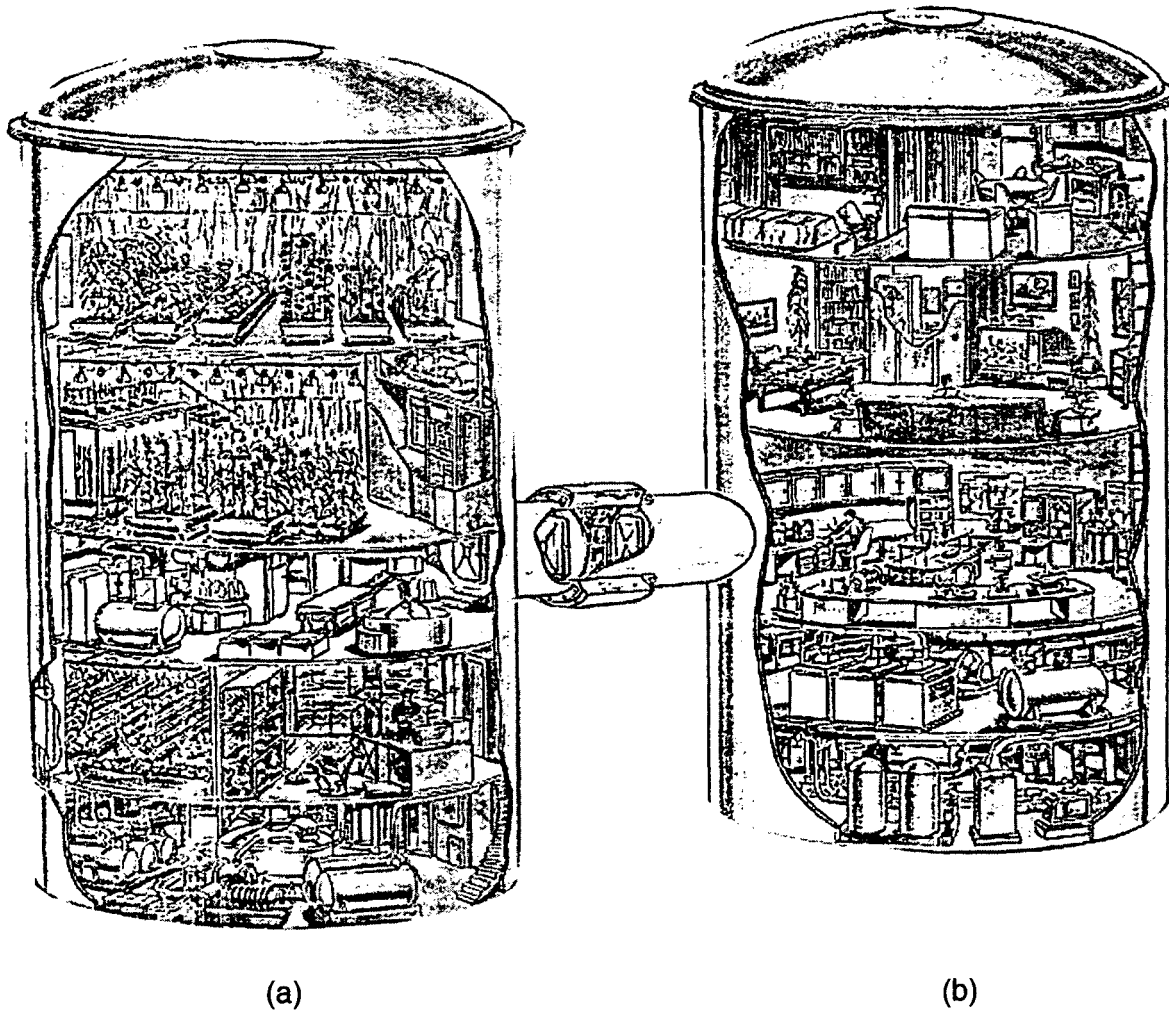


Figure 1. Proposed CELSS testbed at KSC.  
(Two large vacuum chambers used during the Apollo Program would have served as (a) a plant (crop) production module to supply food, oxygen, and clean water to the crew (Buchanan et al., 1978) and (b) human living quarters with control center and waste processing systems.)

The following provides a general summary of crop test results from the KSC Breadboard Project. Results and discussion from waste treatment/recycling tests are reported only where they were part of crop studies and hence are generally not included in this document.

## 2.1 Plant growth facilities at KSC

Crop research for the KSC Breadboard Project involved both conventional growth chambers (Tibbitts and Kozlowski, 1979), as well as a large, atmospherically closed chamber that formerly served as a hypobaric test chamber for the Mercury Program (Prince and Knott, 1986, 1989). This large chamber became known as the Biomass Production Chamber or BPC (Figures 2 and 3). The BPC's design and sizing were chosen to provide the food, water, and atmospheric regeneration needs for approximately 1 person using a bioregenerative approach (Prince and Knott, 1986, 1989).

The BPC was an upright cylindrical chamber (7.5 meters (m) high, 3.7 m in diameter) that provided two-stories with direct access (Figures 2 and 3). The chamber enclosed four annular crop growing shelves stacked vertical (two per story), with each shelf providing approximately 5 m<sup>2</sup> of crop growing area (20 m<sup>2</sup> total). Internal volume including air ducting was 113 m<sup>3</sup>, with air circulation of about 400 m<sup>3</sup> min<sup>-1</sup> provided by two 30-kilowatt (kW) fans. Cooling and dehumidification were provided by two copper heat-exchange coils (one in the upper and one in the lower air handling system) using chilled water from two 15-ton (53 kW) chilling units (these were later replaced by a single 40-ton (140 kW) unit; Appendix A). Following each cold coil was a reheat coil supplied by hot water from resistance heating elements (up to 150-kW capacity). Lighting was provided by 96 400-W high-pressure sodium (HPS) lamps operated on dimming ballasts, thereby providing about 2 kW of input electrical power per m<sup>2</sup> of growing area. Lamps were separated from the plants by clear glass or acrylic barriers. Plants were grown in 64 plastic (PVC or ABS) hydroponic growing trays (~0.3 m<sup>2</sup> each). Nutrient solution circulated continuously through the trays and back to four tanks (one for each shelf) located outside the chamber (Figure 4). Tank headspaces were vented back to the chamber to maintain atmospheric closure. Although the chamber was used for hypobaric testing during the Mercury Program, the addition of air ducting and plumbing penetrations increased leakage and prevented large atmospheric pressure differentials from developing. Nonetheless, atmospheric leakage could be kept as low as 5 to 10% of the chamber volume per day when the chamber doors were closed (Wheeler et al., 1991a). This allowed close tracking of CO<sub>2</sub> exchange rates (net photosynthesis and respiration), evapotranspiration, and the quantification of some volatile organic compounds using closed or semi-closed gas exchange calculations (Coombs et al., 1985; Wheeler, 1992). Further engineering details and operational specifications can be found in Prince et al., 1987, 1992; Sager et al., 1988a; Wheeler et al., 1990, 1996; and Wheeler 1992. A development time-line for systems additions/improvements to the Biomass Production Chamber is show in Appendix A.

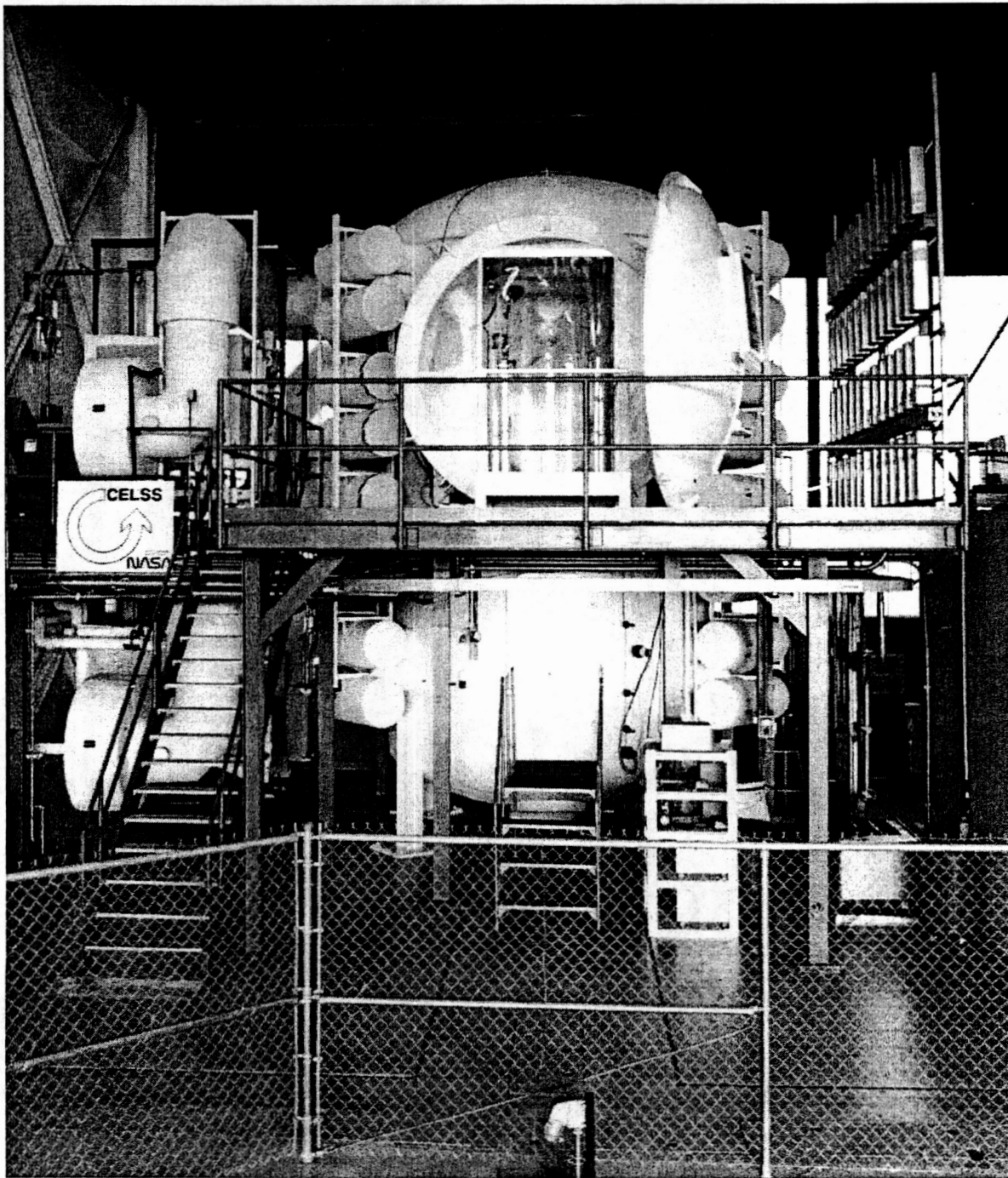


Figure 2. BPC located at Hangar L at KSC, FL (front view, 1986). (The chamber provided a closed atmospheric volume of about 113 cubic meters (including air ducting) with 20 square meters of crop growing area. External nutrient solution tanks were not in place at the time of this photo.)



Figure 3. BPC located at Hangar L at KSC, FL (rear view, 1986). (Banks of ballasts for the 96 400-W HPS lamps are visible at the left. Construction began on the chamber in 1985, with operational capabilities completed for most subsystems by 1988.)

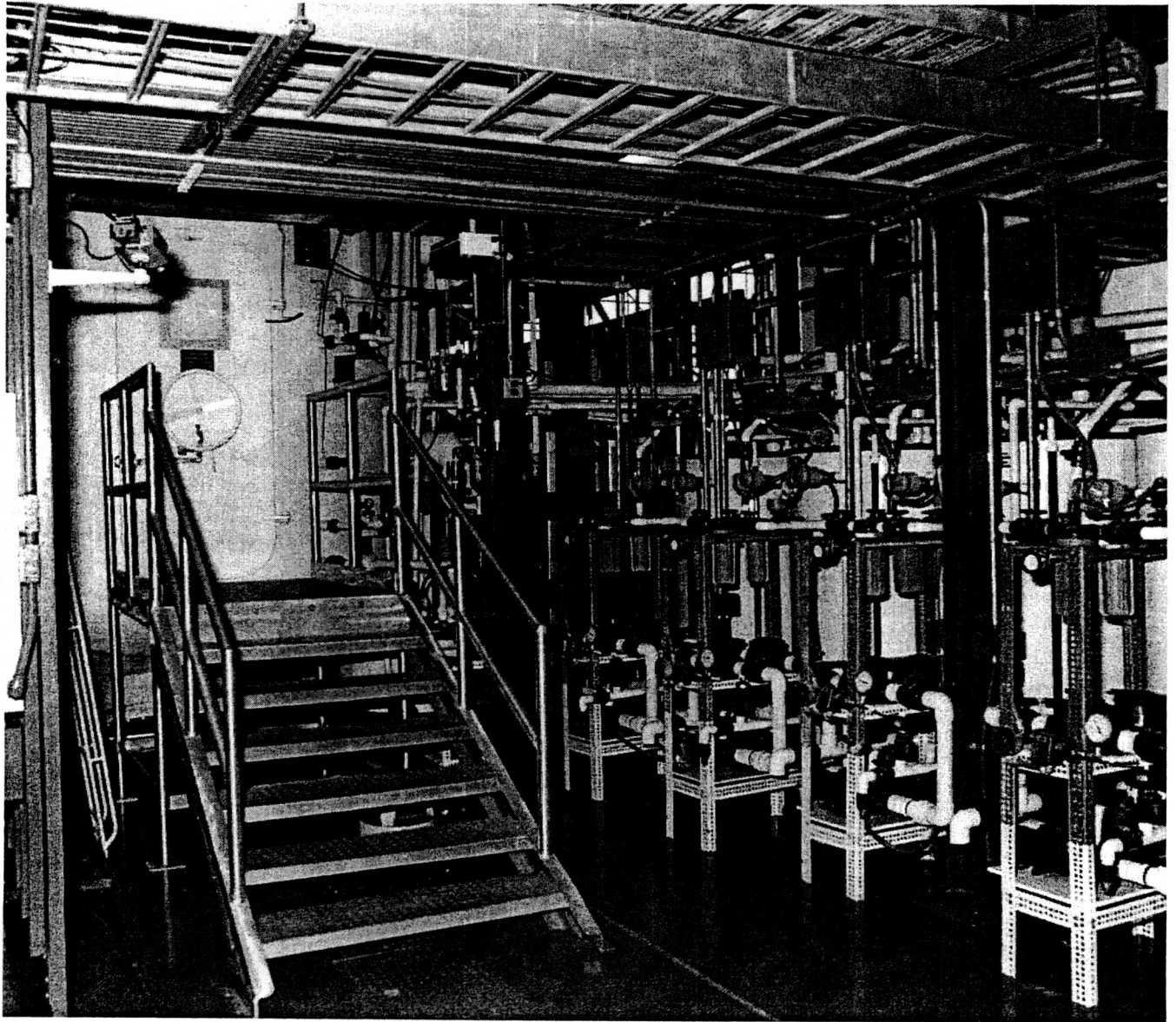


Figure 4. Nutrient solution delivery tanks and plumbing system used to support crop growth in the BPC (1988). (Tanks typically ran with a volume of 225 liters of half strength, modified Hoagland/Arnon (1950) solution that circulated continuously to trays inside the chamber (Wheeler et al., 1998). Trays were inclined slightly to maintain a thin film of nutrient solution.)



Smaller plant growth chambers for KSC studies included both reach-in and walk-in commercial chambers that controlled lighting, temperature, humidity, and CO<sub>2</sub> concentrations (Sager et al., 1988a). Lighting was provided by fluorescent (48 or 96 inch, VHO—1.5 A), high-pressure sodium (400-W), or metal halide (400-W) lamps. For most studies in the BPC and growth chambers, temperature, humidity, and CO<sub>2</sub> were monitored continuously using a computer with custom-developed monitoring and control software (Sager et al., 1988a; Bledsoe et al., 1993). The sequences of smaller growth chamber and lab-scale tests with waste processing/resource recovery and how they both supported the larger, Breadboard Scale tests are shown in Appendix B.

## 2.2 Environmental management and horticultural approach

Most crops differ in their environmental requirements for producing acceptable morphological traits and high yields (Langhans and Tibbitts, 1997). For example, rice, sweetpotato, and peanut prefer warm temperatures (Bonsi et al., 1994; Mackowiak et al., 1998; Frantz and Bugbee, 2002), whereas potato and wheat prefer cool temperatures (Wheeler et al., 1986c; Bugbee, 1995a); soybean, potato, rice, and strawberry are short-day crops, whereas wheat grows well under long days (Salisbury, 1981). The entire issue is complicated by the fact that environmental factors can interact with regard to their effects on crop growth (Thomas and Raper, 1983a, b; Wheeler et al, 1986c, 1991b). Due to time and staffing constraints, exhaustive testing of environmental responses for each crop was not conducted; consequently, environmental settings for KSC crop tests were based largely on findings by university researchers (e.g., Bugbee, 1995a; Bugbee and Salisbury, 1988; Knight and Mitchell, 1983a, 1988a; Thomas and Raper, 1983a, Wheeler et al, 1986c, 1991b). Environmental tests conducted in growth chambers focused mainly on features that were either unique to the BPC or conditions that were not commonly addressed in university studies. These included studies on the effects of HPS lighting (Wheeler et al., 1999b, Yorio et al, 1995), the effects different CO<sub>2</sub> concentrations (Wheeler et al., 1993b, 1999b; Mackowiak and Wheeler, 1996), and crop growth and development using nutrient film technique (Mackowiak et al., 1989, 1998; Wheeler et al., 1990, 1999). For most crop tests, maximum growth chamber lighting was used, with HPS lighting studies ranging from ~500 to 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and fluorescent lighting studies from ~250 to 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 2). For some studies, HPS lighting was deliberately dimmed to produce desired growth traits, e.g., to reduce tipburn injury in lettuce (Collier and Tibbitts, 1982). Dimming was also used in several growth chamber studies with HPS lamps. A summary of environmental set points, modes of propagation, planting densities, and developmental characteristics for a range of crops tested in the KSC Breadboard Project are presented in Tables 2, 3, and 5.

For each crop species, several cultivars were usually compared in early testing, which provided an initial selection of genotypes for controlled environment performance (Table 3). But as with environmental response testing, cultivar testing at KSC was limited and selections relied heavily on recommendations by university researchers. The best performing cultivars from KSC tests are shown in Table 3, but these results are somewhat dated by continuing tests of new cultivars by other ALS researchers. For example, recent studies with rice by Utah State University investigators (Frantz and Bugbee, 2002) indicate the 'Super Dwarf' rice is more appropriate for ALS applications than 'Ai-Nan Tsao,' and continued improvements in genotype (cultivar) selections for other species are inevitable.

After considering a range of horticultural approaches, a recirculating nutrient film technique (Graves, 1983) was selected as the method for water and nutrient delivery (Prince and Knott, 1989). Nutrient film technique was amenable to a range of species, including root zone crops, reduced the need for large volumes of standing water, eliminated the need for solid media, and permitted close tracking of water and nutrient uptake. On the other hand, because of the limited water volume and buffering capacity, NFT systems are susceptible to crop stress or possible loss if system malfunctions are not dealt with promptly (e.g., loss of circulating pumps). A typical NFT system at KSC continuously pumped nutrient solution from a reservoir to a slightly elevated end of a tray, after which the solution flowed through the tray and back to the reservoir (Graves, 1983; Mackowiak et al., 1989). Clearly, such hydroponic approaches are gravity-dependent and would not function in the microgravity of spaceflight, yet the findings should still be pertinent for planetary settings (e.g., Moon or Mars) where gravity can be used for return flow. Although not reviewed in this document, testing has been and continues to be carried out at KSC to develop watering techniques for microgravity (e.g., low-Earth orbit and/or planetary transit), and these are reported in Wright et al., 1988; Dreschel and Sager, 1989; Koontz et al., 1990; Brown et al., 1992; Goins et al., 1997b; Levine, 1999; and Levine et al., 2002.

Use of recirculating hydroponics requires monitoring and control of solution pH, electrical conductivity (EC), and water volume (Graves, 1983; Bugbee, 1989). For KSC studies, water levels and nutrient concentrations of hydroponic solutions were maintained by first adding either deionized water or condensate passed through ion-exchange columns (BPC studies) to the reservoirs to maintain a constant volume. Following this, a concentrated nutrient stock solution was added to maintain an EC set point of  $0.12 \text{ S m}^{-1}$  (Table 4) (Mackowiak et al., 1989; Wheeler et al., 1999a). The starting solutions and make-up stock solutions provided all the essential elements for plant growth and were based on formulations developed by Hoagland and Arnon, 1950. For most studies,  $\text{NO}_3$  salts provided the sole source of nitrogen; consequently solution pH levels would usually rise over time as a result of nutrient uptake (Marschner, 1995). To offset this, dilute nitric acid ( $0.2$  or  $0.4 \text{ mol L}^{-1}$ ) was added to maintain a pH set point of  $5.8 \pm 0.2$ . For most KSC studies, including early BPC tests, nutrient solution temperatures were not controlled. For BPC studies after 1992 and certain growth chamber studies, water temperatures were controlled using stainless-steel coils submerged in the nutrient solution reservoirs. Cooling was then provided by chilled water circulated through the coils.

Most of the species were seed-propagated, where either dry or imbibed seeds were placed against nylon (Nitex) wicks supported between tensioned strips of white-on-black polyethylene film (Prince and Knott, 1989; Mackowiak et al., 1989; 1996a) (Table 3; Figure 5). The ends of nylon wicks were kept in contact with the flowing nutrient solution in the trays. Potatoes, sweetpotatoes, and strawberry were propagated vegetatively using *in vitro* grown plantlets (potato), stem cuttings (sweetpotato), or runner plantlets (strawberry). Plantlets or cuttings were wrapped with pliable foam (sponge) collars and placed in white-on-black polyethylene tray covers with the roots kept in contact with the flowing nutrient solution (Wheeler et al., 1985, 1990). Following planting, trays were typically covered for 2 to 4 days with white translucent covers to maintain high humidity to promote germination and establishment. Seeds, seedlings, and plantlets under these covers were sprayed manually each day with deionized water to reduce water stress. Planting densities were highest for the grass species wheat and rice. A summary of planting densities, mode of propagation, seedling establishment periods, and times of plant thinning (to final spacing) for a range of crops

Table 2. Environmental set points used for ALS candidate crops in KSC testing.

Crop ( <i>Genus species</i> )	Nutrient Solution Temperature	Air Temperature (Light/Dark)	Photosynthetic Photon Flux (PPF)	Photoperiod (Light/Dark)
Staple Crops	(°C)	(°C)	( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	(h)
Wheat ( <i>Triticum aestivum</i> ) <sup>1</sup>	~18	20/16	750 – 800	20/4 or 24/0
Soybean ( <i>Glycine max</i> ) <sup>2</sup>	~24	26/22	500 – 800	12/12
Potato ( <i>Solanum tuberosum</i> ) <sup>3</sup>	~18	20/16	500 – 800	12/12
Sweetpotato ( <i>Ipomoea batatas</i> ) <sup>4</sup>	~24	26/22	500 – 800	12/12
Peanut ( <i>Arachis hypogaea</i> )	~24	26/22	500 – 750	12/12
Rice ( <i>Oryza sativa</i> ) <sup>5</sup>	~24	28/24	750 – 800	12/12
Bean ( <i>Phaseolus vulgaris</i> )	~26	28/24	350 – 400	18/6
<b>Supplemental Crops</b>				
Lettuce ( <i>Lactuca sativa</i> ) <sup>6</sup>	~ 23	23	300	16/8
Spinach ( <i>Spinacia oleracea</i> ) <sup>7</sup>	~ 23	23	300	16/8
Tomato ( <i>Lycopersicon esculentum</i> ) <sup>8</sup>	~ 24	24	500 – 750	12/12
Chard ( <i>Beta vulgaris</i> )	~ 23	23	300	16/8
Radish ( <i>Raphanus sativus</i> )	~ 23	23	300	16/8
Red Beet ( <i>Beta vulgaris</i> )	~ 23	23	300	16/8
Strawberry ( <i>Fragaria x ananassa</i> ) <sup>9</sup>	~ 18	20/16	400 – 600	12/12

<sup>1</sup> Higher PPFs and long photoperiods (up to 24 h) can be used for wheat, with yields being a strong function of total light provided ( $\text{mol m}^{-2} \text{d}^{-1}$ ).

<sup>2</sup> Soybean leaves can become chlorotic under high PPF from HPS lamps, but photosynthetic rates and productivity seem to remain high.

<sup>3</sup> Warm temperatures (e.g.,  $>24^{\circ}\text{C}$ ) should be avoided for potato roots and shoots as these tend to suppress tuber initiation.

<sup>4</sup> High PPF ( $\sim 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) from HPS lamps can cause chlorosis and bleaching of young sweetpotato leaves, but effects are usually transient.

<sup>5</sup> Recent evidence from Frantz and Bugbee, 2002 suggests rice does well at warmer temperatures (e.g.,  $\sim 30^{\circ}\text{C}$ ).

<sup>6</sup> Lettuce is susceptible to leaf tipburn when plants are grown rapidly to a heading stage. Mild tipburn was observed in BPC studies with 'Waldmann's Green' lettuce grown 1000 ppm  $\text{CO}_2$  and  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPF with a 16-h photoperiod ( $\sim 17 \text{mol m}^{-2} \text{d}^{-1}$ ).

<sup>7</sup> Spinach is a long-day plant for flowering but can be grown successfully under long days if harvested prior to bolting.

<sup>8</sup> Long photoperiods ( $> 18 \text{h}$ ) can be injurious for tomatoes and some potato cultivars.

<sup>9</sup> Many strawberry cvs. require short photoperiods and cool temperatures for flowering and fruit set (Durner and Poling, 1988). Manual pollination of enhances both fruit quality and total yield. Different environmental set points (e.g., longer photoperiods) may be needed to force "runner" for vegetatively propagating strawberry.



Table 3. Crop propagation/establishment considerations.

Crop ( <i>Genus species</i> )	Best Performing Cultivar (from KSC tests)	Propagation and Germination	Plant Density (initial/final)	Seedling Establishment	Thinning Time
Staple Crops			(plants m <sup>-2</sup> )	(days)	(days)
Wheat ( <i>Triticum aestivum</i> )	Apogee	Seed <sup>1</sup>	~1200	4	NA
Soybean ( <i>Glycine max</i> )	Hoyt	Seed <sup>2</sup>	32/12-16	5	10
Potato ( <i>Solanum tuberosum</i> )	Norland	Plantlets <sup>3</sup>	12/6-8	NA	10
Sweetpotato ( <i>Ipomoea batatas</i> )	TU – 155	Cuttings <sup>4</sup>	12/6-8	NA	7
Peanut ( <i>Arachis hypogaea</i> )	Pronto	Seed <sup>2</sup>	12/6-8	4	14
Rice ( <i>Oryza sativa</i> )	Ai-Nan Tsao	Seed <sup>1</sup>	~ 800	4	NA
Bean ( <i>Phaseolus vulgaris</i> )	Etna	Seed <sup>2</sup>	32/12-16	4	10
Supplemental Crops					
Lettuce ( <i>Lactuca sativa</i> )	Waldmann's Green	Seed <sup>5</sup>	40/20-24	4	10
Spinach ( <i>Spinacia oleracea</i> )	Nordic IV	Seed <sup>6</sup>	40/20-24	10	14
Tomato ( <i>Lycopersicon esculentum</i> )	Reimann Philipp	Seed <sup>5</sup>	20/10	6	10
Chard ( <i>Beta vulgaris</i> )	Ruby Red	Seed	40/20	4	10
Radish ( <i>Raphanus sativus</i> )	Giant White Globe	Seed <sup>5</sup>	50/40	4	7
Red Beet ( <i>Beta vulgaris</i> )	Ruby Queen	Seed <sup>6</sup>	40/20	7	14
Strawberry ( <i>Fragaria x ananassa</i> )	Oso Grande	Runners <sup>7</sup>	12-16	NA	14

<sup>1</sup> Dry seed was initially aerated in deionized (DI) water for 30 minutes. Following this, wet seed was wrapped in moist toweling and kept at room temperature for 1 day (rice) or refrigerated (~4°C) over night (wheat). Following sowing, seeds were sprayed daily with DI water and a translucent plastic cover was placed over the trays to maintain high humidity during germination and establishment. Recent work by Klassen and Bugbee, 2002 suggests 'Super Dwarf' as the preferred rice cultivar.

<sup>2</sup> Dry seed was aerated for 1 day and seed skin was removed (peanut only) prior to planting onto tray insert. Seeds were sprayed daily with DI water and a translucent plastic cover was used to maintain high humidity during the germination.

<sup>3</sup> Nodal explants were cultured on Murashigee and Skoog (agar solidified) medium without growth regulators. Plantlets were grown in vitro for 28-42 days under 25°C, a 12/12 photoperiod, with ~150 μmol m<sup>-2</sup> s<sup>-1</sup> CWF light. Plantlets were removed from culture vessels, agar was gently rinsed from the roots, and plantlets were transplanted to tray covers (white or black plastic film) and a translucent plastic cover was used to maintain high humidity during the 4-day acclimation process. Plantlets were supported in tray covers with pliable polyurethane (sponge) plugs by placing ~1/3 of the shoot above the plug and ~2/3 of shoot below to allow roots to contact the water.

<sup>4</sup> Stem cuttings were rooted in aerated DI water for 7-10 days. Cuttings were wrapped with a sponge (foam) plug and then positioned in hydroponic trays. No further acclimation was required.

<sup>5</sup> Dry seed was planted directly in contact with wicking material on tray insert. Seeds were sprayed daily with DI water and a translucent plastic cover used to maintain high humidity during the germination process.

<sup>6</sup> Dry seed was aerated for 6 days in DI water to initiate germination. Seedlings were then transferred to tray inserts and a translucent plastic cover was used to maintain high humidity during the 4-day acclimation process. For spinach, germination was carried out in a refrigerator at 4 to 5°C.

<sup>7</sup> Plantlets produced from mother plants (runners) were wrapped with sponge (foam) plugs and then positioned in hydroponic trays.

Table 4. Nutrient solution for ALS crops grown in NFT.

<i>Macro-nutrients</i>							
Salt	N	P	K (mmol L <sup>-1</sup> )	Ca	Mg	S	
KNO <sub>3</sub>	2.5		2.5				
Ca(NO <sub>3</sub> ) <sub>2</sub>	5.0			2.5			
MgSO <sub>4</sub>					1.0	1.0	
KH <sub>2</sub> PO <sub>4</sub>		0.5	0.5				
Startup Concentration	7.5	0.5	3.0	2.5	1.0	1.0	
Refill Concentration	70	10	56	12	10	10	
<i>Micro-nutrients</i>							
	Fe	Mn	Zn mmol L <sup>-1</sup>	Cu	B	Mo	
Fe-HEDTA	50						
MicroNutrients		7.4	0.96	0.52	9.5	0.01	
Startup Concentration	50	7.4	0.96	0.52	9.5	0.01	
Refill Concentration	134.0	96.0	12.5	6.8	123.5	0.13	

- Micronutrients were mixed in a combined stock solution using the following salts: H<sub>3</sub>BO<sub>3</sub>; MnCl<sub>2</sub>·4H<sub>2</sub>O; ZnSO<sub>4</sub>·7H<sub>2</sub>O ; CuSO<sub>4</sub>·5H<sub>2</sub>O; (NH<sub>4</sub>)<sub>6</sub>Mo<sub>24</sub>·4H<sub>2</sub>O.
- Fe-HEDTA mixed by adding FeCl<sub>3</sub>·6H<sub>2</sub>O and HEDTA.
- For sweetpotato, periods of nutrient (particularly N) depletion may be helpful for initiating storage root and limiting shoot growth. This can be achieved by only replenishing nutrients (EC set points) 1 or 2 times per week.
- Recent studies with potatoes in NFT suggest that lower EC set points with daily replenishment result in earlier tuber initiation and reduced shoot growth.
- For determinate crops such as wheat and some soybean varieties, reducing nutrients (e.g., lower EC set points) during seed filling might be considered.
- With nitrate being the sole source of N for this nutrient formulation, solution pH values tend to rise (go basic) during periods of high-nitrate uptake. This typically requires the addition of acid to control pH to a favorable range. A 0.4 M HNO<sub>3</sub> and 0.4 M KOH were typically used for pH control in KSC testing.

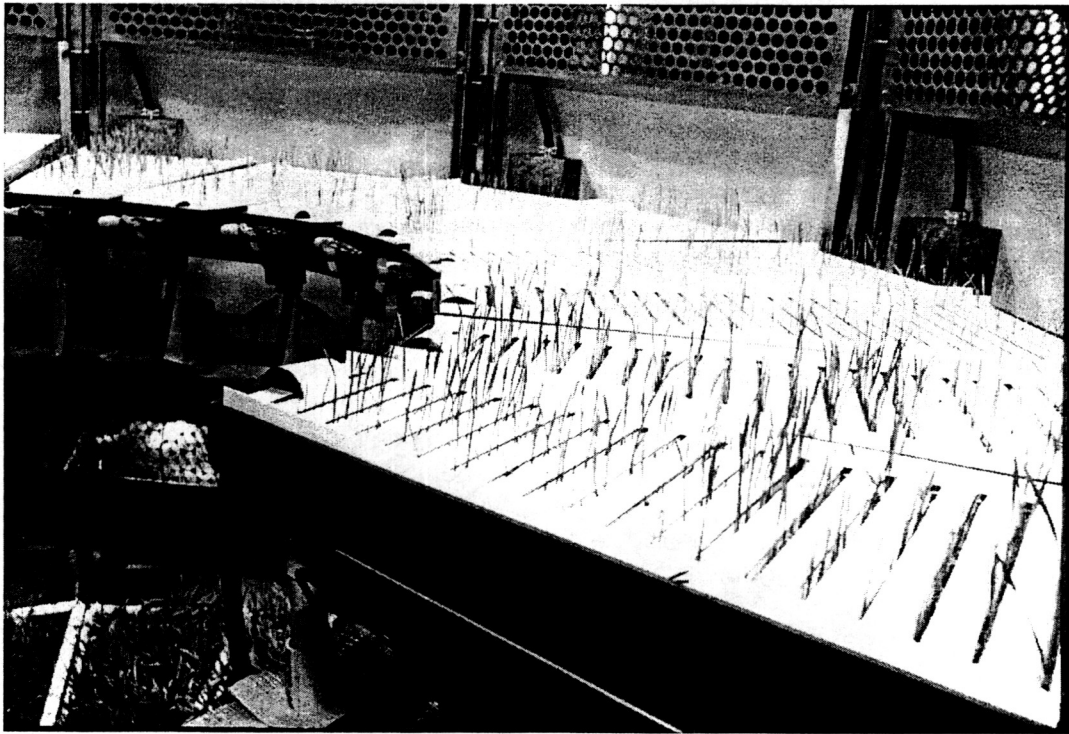


Figure 5. Tray covers for supporting wheat seedlings above a flowing nutrient solution.

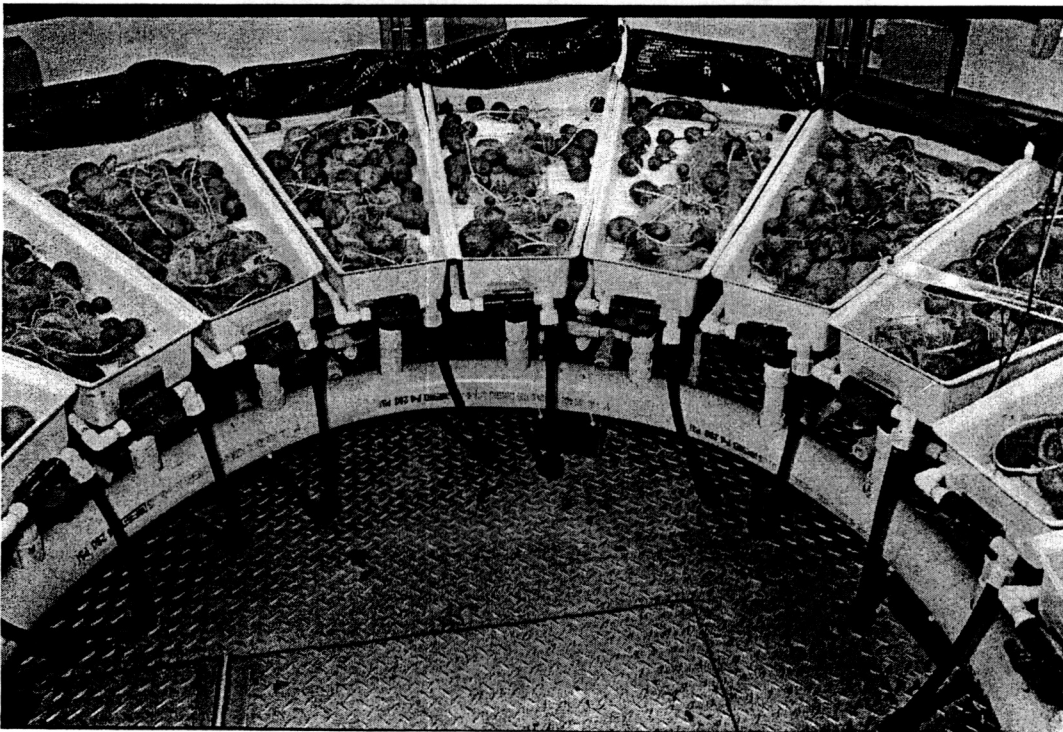


Figure 6. Potato tubers ready for harvest from NFT culture trays at 105 days after planting.

studied at KSC are shown in Table 3. Production cycles ranged from 18 days (radish) to 120 days (sweetpotato and peanut), while canopy heights ranged from ~20 cm (radish) to ~80 cm or more sweetpotato, potato, peanut and rice (Table 5). Because canopy heights varied among cultivars and were influenced by environmental conditions (e.g., Wheeler et al., 1991c), the values in Table 5 should only be used as a rough guide.

### 2.3 Measurements and data

Temperature, humidity, photoperiod, and CO<sub>2</sub> were monitored continuously using a computer and different versions of custom-developed software (Sager et al., 1988a; Bledsoe et al., 1993). Canopy level photosynthetic photon flux (PPF) was monitored manually above each tray weekly using a Li-Cor quantum sensor. Nutrient solution pH, EC, and volume were measured continuously for BPC studies or manually each day for most growth chamber studies (some growth chamber studies included continuous monitoring). This permitted a near-continuous tracking of plant water use, nutrient uptake, and acid requirements throughout each experiment. Nutrient solution samples were typically analyzed weekly or bi-weekly for elemental composition using Inductively Coupled Plasma (ICP) spectrometry. This provided guidance for making any adjustments to the replenishment stock solutions as well as helping assess potential plant deficiencies or toxicities. For BPC studies, evapotranspiration was monitored from water uptake from the nutrient delivery systems and from condensate recovery from the air handling system. Crop photosynthesis rates in the BPC were monitored using both closed and semi-closed approaches (Coombs et al., 1985), where chamber CO<sub>2</sub> drawdown rates were calculated immediately following dark cycles each day, or CO<sub>2</sub> mass flow additions were monitored during the light cycle (Wheeler and Sager, 1990; Wheeler, 1992). Crop respiration rates were monitored by calculating CO<sub>2</sub> increases during dark cycles (Wheeler and Sager, 1990). The accumulation of volatile organic compounds (VOCs), especially ethylene was also tracked for most BPC studies using gas chromatography/photo-ionization detector (GC/PID) or flame-ionization detector (FID) or mass spectrometry (GC/MS) (Wheeler, 1992; Batten et al., 1996; Wheeler et al., 1996b; Stutte and Wheeler, 1997).

Plant measurements for BPC and growth chamber studies typically included general observations on growth and development, fresh biomass (for certain species, e.g. tomato fruit, potato tubers), oven-dry biomass of different plant parts, and canopy height. Oven drying involved placing plant materials in paper bags and then drying in a forced-air oven at 60 to 70°C for 48 to 96 h. When possible, data were expressed as rates per unit area; for example g m<sup>-2</sup> d<sup>-1</sup>, rather than g m<sup>-2</sup> or g plant<sup>-1</sup>. This required dividing the yields by the available growing area and the time of growth. Growing area estimates were kept conservative to avoid over estimating productivities, and in some growth chamber studies, screening was used to reduce "side lighting" that would cause overestimation of productivities (Went, 1957; Bugbee, 1995a). For BPC plantings, an area of 20 m<sup>2</sup> was assigned based on estimates of area enclosed between two arcs defining the front and back boundaries of each growing shelf (Wheeler, 1992). Because "fixed" spacing was used from planting to harvest in most studies, area utilization was relatively inefficient for widely spaced crops (i.e., most of the light was not intercepted by crop foliage during early growth). Nonetheless, the entire space for the complete growth cycle was used to calculate final productivities. This kept productivity estimates conservative compared to what might be achievable in systems that incorporate

Table 5. Developmental characteristics of ALS candidate crops.

Crop ( <i>Genus species</i> )	Anthesis/ Flowering <sup>1</sup>	Duration of Grow-Out	Canopy Height at Maturity	Harvest Index
Staple Crops	(days)	(days)	(cm)	(%)
Wheat ( <i>Triticum aestivum</i> ) <sup>3</sup>	~35	85	~ 50	~ 30 - 45
Soybean ( <i>Glycine max</i> ) <sup>4</sup>	~28	90 - 97	~ 45 - 70	~ 40
Potato ( <i>Solanum tuberosum</i> ) <sup>5, 6</sup>	NA	84 - 105	~ 50 - 80 <sup>2</sup>	~ 60 - 80
Sweetpotato ( <i>Ipomoea batatas</i> ) <sup>6</sup>	NA	120	~ 50 - 80 <sup>2</sup>	~ 20 - 60
Peanut ( <i>Arachis hypogaea</i> )	~35	120	~ 60 - 80 <sup>2</sup>	~ 20 - 30
Rice ( <i>Oryza sativa</i> )	~45	105	~ 80	~ 30
Bean ( <i>Phaseolus vulgaris</i> ) <sup>6</sup>	~28	70	~ 50	~ 35 - 45
<b>Supplemental Crops</b>				
Lettuce ( <i>Lactuca sativa</i> )	NA	28	~ 20 - 30	~ 90
Spinach ( <i>Spinacia oleracea</i> )	NA	28	~ 20 - 30	~ 90
Tomato ( <i>Lycopersicon esculentum</i> ) <sup>7</sup>	~35	84	~ 35 - 45	~ 40 - 50
Chard ( <i>Beta vulgaris</i> )	NA	40 +	~ 40 - 50	~ 60 +
Radish ( <i>Raphanus sativus</i> )	NA	21 - 25	~ 20	~ 50
Red Beet ( <i>Beta vulgaris</i> )	NA	35 - 42	~ 40 - 50	~ 60 +
Strawberry ( <i>Fragaria x ananassa</i> )	~60	140	~ 20 - 30	~ 30 - 40

<sup>1</sup> Times to flowering and final harvest are dependent on environmental conditions. Values stated in this table are approximate for set points given in Table 2.

<sup>2</sup> Shoot growth in peanut, sweetpotato, and potato can be excessive in hydroponic culture with continuous EC and pH control. Control of nutrient levels may be important to control shoot growth and harvest index.

<sup>3</sup> Wheat stands in controlled environments are susceptible to lodging, and canopy supports should be considered. For BPC studies, wire grids were positioned about 30 cm above and parallel to the tray surface to support the stand. However, use of any canopy support systems will affect harvesting and materials handling. Harvesting and threshing are labor-intensive and produce dust and debris (also true for rice and soybean).

<sup>4</sup> No canopy support system required with Soybean cv. Hoyt, whereas support is required with indeterminate cultivars such as McCall. Lower canopy leaves tend to abscise during maturation and periodic removal of leaf litter provides good air circulation and tray surface reflection.

<sup>5</sup> Tuberization in potato in NFT can be suppressed when stolons are submerged in solution. NFT simplifies harvesting in comparison to solid media. Alternative methods for propagating potatoes (e.g., use of mini tubers) should be explored to eliminate the need for *in vitro* plants. Recent studies at KSC have shown that using the same recirculated nutrient solution for successive plantings caused a premature tuber initiation and stunted shoot growth, which resulted in lower yields due to incomplete canopy cover. The effect can be removed by passing the water through a charcoal filter (Wheeler et al., 1995). Management of recirculating systems with potatoes will require further testing to develop strategies for consistent yields.

<sup>6</sup> Oedema (intumescence or callus-like lesions) can occur on leaves of potato, tomato, and sweetpotato, and on stems bean under HPS lighting or low UV light environments.

<sup>7</sup> BPC tests with the tomato cv. Reimann Philipp have produced good fruit set and high yields without assisted pollination.

transplanting or automated spacing schemes to improve space utilization and light interception (Prince and Bartok, 1978; Knox, 1986). Portions of biomass from final harvests were analyzed for elemental composition using inductively coupled plasma (ICP) or direct current (dc) arc spectrometry (Alexander and McAnulty, 1981), and samples for several studies were sent to a commercial laboratory for proximate composition analysis using standard Association of Official Analytical Chemists (AOAC) procedures, including ash by muffle furnace, protein by Kjeldahl N (6.25 conversion factor), fat by ether extraction of acid hydrolysis, and carbohydrate by difference (Wheeler et al., 1993c). Some samples were also analyzed for total dietary fiber and nitrate composition (Wheeler et al., 1996c, 1997a).

## **2.4 Results and discussion**

**2.4.1 Horticultural procedures.** As with any effort in controlled environment agriculture, techniques and approaches for growing plants improved with experience. Some more significant horticultural observations from the large-scale testing include:

- Germination and establishment of seedling (e.g., wheat, lettuce) were most successful if high humidities and good wick contact with the solution were maintained for the first few days until roots had reached the flowing solution. Daily manual misting of seeds and seedlings under the tray covers was especially important to promote good seedling establishment.
- In early studies with lettuce, seeds were germinated using a single nylon wick, but seedling survival was sometimes poor. This was improved by using two adjacent wicks, which allowed seedling roots to grow between the wicks prior to reaching the flowing water below.
- Initial BPC tests with potato (BWP911 and 912) resulted in mediocre tuber yields (Tables 6 and 7). Nutrient solution temperatures during these tests were often 3 to 4 °C warmer than air temperatures (e.g., up to 24°C) due to inefficient cooling capabilities in the solution reservoirs. For subsequent potato studies (BWP921, 931, 941), auxiliary cooling was added using stainless-steel coils submerged in the reservoirs. This allowed solution temperatures to be held near 18 °C, which increased tuber yields (Burton, 1972).
- Providing adequate shoot support to prevent lodging (stand collapse) was important for some crops; an example of how lodging reduced stand photosynthetic rates in soybean study BSB891 is shown in Figure 13. For subsequent soybean studies, plant shoots were supported by wire mesh grids (fencing) positioned horizontally ~25-30 cm above the tray covers. Similar support techniques were used for wheat, potatoes, and some tomato studies. Smaller crops (e.g., lettuce and radish) and dwarf cultivars (e.g., Hoyt soybean) did not require additional supports.
- Planting of densely spaced crops (e.g., wheat) and harvesting of seed crops (e.g., wheat, soybean, rice) was labor-intensive. In addition, harvesting and threshing seed crops were dusty and required adequate ventilation or breathing masks for protection.

This suggests that both planting and harvesting operations of seed crops are important targets for mechanized or even automated procedures.

- With the exception of potato study BWP941 and several growth chamber studies (Mackowiak et al., 1989), KSC crop studies typically ran for one production cycle. Study BWP941 showed that four successive generations of potato could be sustained without exchanging the nutrient solutions, and that staggered plantings (e.g., two-tray blocks) provided a more continuous yield and photosynthetic gas exchange (Wheeler, 1996; Stutte et al., 1999). Creating gaps in the canopy by staggered harvests may have had a positive effect by adding some “side lighting” to adjacent trays (Stutte et al., 1999).
- Successive plantings of potatoes in the same nutrient solution during several growth chamber studies in 1993 and then in BPC study BWP941 showed that a growth regulating compound accumulated in the nutrient solution over time (Wheeler et al., 1995; Stutte and Yorio, 1998). This compound or factor resulted in reduced shoot growth and early tuber initiation, and methods for managing this factor in continuous production tests with potato are still under study (Stutte, Yorio, and Edney, unpublished).
- On several occasions, water pumps were inoperable due to losses of electrical power (e.g., thunderstorms, hurricanes). To prevent water stress, NFT trays were elevated at the drain end to “pond” nutrient solution in the tray and provide a water reserve (2 to 3 cm) for the plants.

**2.4.2 Crop yields.** Harvest results (dry mass) from BPC 20 m<sup>2</sup> stands of wheat, soybean, lettuce, potato, and tomato are shown in Table 6. Equivalent levels of CO<sub>2</sub> fixed and O<sub>2</sub> produced (based on biomass carbon content; Wheeler et al., 1996a), along with the total mass of water collected as condensate from each crop are also presented (Table 6). The highest biomass yields from BPC tests were obtained from wheat plantings, which received the most PAR (Table 6). The highest edible biomass yields were obtained from potato plantings, due in large part to the high harvest index of potato crops (Tables 6 and 7). In some studies, tubers accounted for > 80% of a mature potato plant’s biomass (i.e., harvest indices >80%). Biomass productivities (g m<sup>2</sup> d<sup>-1</sup>) along with daily photosynthetically active radiation (mol m<sup>-2</sup> d<sup>-1</sup>) for each BPC study are shown in Table 7. By dividing productivities by daily PAR, radiation conversion efficiencies (g biomass per mol PAR) can be calculated for each experiment (Table 7). These radiation conversion efficiencies for the C<sub>3</sub> crops in the BPC ranged from ~0.4 to 0.9 g mol<sup>-1</sup> for total biomass and ~0.2 to 0.6 g mol<sup>-1</sup> for edible biomass (Table 7). These results compare favorably with the 0.7 g mol<sup>-1</sup> conversion value listed for corn (C<sub>4</sub>) canopies under optimal field conditions (Norman and Arkebauer, 1991). If transplanting schemes had been implemented for widely spaced crops (e.g., potato, lettuce, soybean, and tomato), productivities and radiation conversion efficiencies could have been improved. For example, lettuce productivities based on the full use of 20 m<sup>2</sup> for 28 days were ~7 g m<sup>-2</sup> d<sup>-1</sup>. But by growing seedlings in smaller areas (e.g., nursery) for 10 to 12 days before transplanting to the final spacing, productivity and radiation use efficiency would have increased to ~10 g m<sup>-2</sup> d<sup>-1</sup> and ~0.6 g mol<sup>-1</sup>, respectively. An overall comparison of the influence of light (photosynthetically active radiation) and productivity are shown in Figure 7. The data show a near-linear response in productivity to PAR across the range of ~15 to 60 mol m<sup>-2</sup> d<sup>-1</sup> (Figure 7) and emphasize the importance of light in driving crop yields for life support systems (Sinclair, 1991).

Table 6. Life support outputs of crops grown in the BPC.

Crop/Date	Days of Operation (d)	Total Biomass (kg)	Edible Biomass (kg)	CO <sub>2</sub> <sup>1</sup> Fixed (kg)	O <sub>2</sub> <sup>1</sup> Produced (kg)	Water Collected (kg)
Wheat 881 <sup>2</sup>	77	23.06	9.24	35.5	25.8	3615
Wheat 882 <sup>3</sup>	64	26.14	early harvest	40.3	29.3	5700 <sup>4</sup>
Wheat 891	86	37.76	11.01	58.2	42.3	6903
Wheat 892	85	44.24	13.12	68.1	50.7	7809
Wheat 931	85	64.11	18.25	98.7	71.8	7500 <sup>4</sup>
Wheat 941 <sup>5,6</sup>	84	66.68	19.07	102.7	74.7	7600
Soybean 891	90	26.62	8.58	45.0	32.7	7758
Soybean 901	97	18.94	6.34	32.0	23.3	8211
Soybean 902	97	20.80	7.79	32.5	25.6	8450
Soybean 951 <sup>6,7</sup>	90	13.51	5.18	22.8	16.6	2594
Lettuce 901	28	----	sequential	harvest	study	----
Lettuce 902	28	2.84	2.60	4.2	3.1	976
Lettuce 911	28	3.54	3.24	5.2	3.8	998
Lettuce 921	28	3.57	3.36	5.2	3.8	1000 <sup>4</sup>
Lettuce 931 <sup>6</sup>	30	3.99	3.71	5.9	4.3	1074
Potato 911	105	45.58	14.89	68.4	49.7	8778
Potato 912	90	50.67	22.03	76.2	55.4	9361
Potato 921	105	55.42	37.64	83.1	60.5	7954
Potato 931	105	55.88	34.12	83.8	61.0	8546
Potato 941 <sup>6</sup>	418	272	167	409	296	28446
Tomato 951 <sup>6,7</sup>	84	11.03	5.15	16.6	12.1	3426
Tomato 961	87 <sup>8</sup>	33.87	17.06	50.9	37.0	12,700
Total	1991	880	409	1344	980	149390

<sup>1</sup> Estimated from total biomass and the percentage of carbon in tissue.

<sup>2</sup> Only the upper half of the chamber used.

<sup>3</sup> 3/4 of available growing area used; plant harvest prior to maturity.

<sup>4</sup> Some missing data; totals estimated by interpolation of water use trend.

<sup>5</sup> Data collected from level four only; water estimated until final data compiled.

<sup>6</sup> Studies where half the plants were grown on recycled nutrients from an aerobic bioreactor.

<sup>7</sup> Simultaneous test with tomato (10 m<sup>2</sup>) in half of the chamber and soybean (10 m<sup>2</sup>) in the other half.

<sup>8</sup> Upper chamber harvested at 84 days; lower chamber harvested at 91 days.



Table 7. Yields and PAR levels for ALS crops.<sup>1</sup>

Crop/Date	Photoper. <sup>2</sup>		Daily PAR	Total Biomass			Edible Biomass		
	(h)/PPF	( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		( $\text{mol m}^{-2} \text{d}^{-1}$ )	( $\text{kg m}^{-2}$ )	( $\text{g m}^{-2} \text{d}^{-1}$ )	( $\text{g mol}^{-1} \text{PAR}$ )	( $\text{kg m}^{-2}$ )	( $\text{g m}^{-2} \text{d}^{-1}$ )
Wheat 881	24 / 666		57.5	2.31	31.6	0.55	0.92	12.6	0.22
Wheat 891	20 / 535		38.5	1.89	23.1	0.60	0.55	6.7	0.17
Wheat 892	20 / 691		49.7	2.21	27.3	0.55	0.66	8.1	0.16
Wheat 931	20 / 930		67.0	3.21	39.6	0.59	0.91	11.3	0.17
Wheat 941 <sup>4</sup>	20/1177		84.7	3.33	39.7	0.47	0.95	11.4	0.13
Soybean 891	12 / 815		35.2	1.33	15.5	0.44	0.43	5.0	0.14
Soybean 901	12 / 477		20.6	0.95	10.2	0.50	0.32	3.4	0.17
Soybean 902	10 / 644		23.2	1.04	11.2	0.48	0.39	4.2	0.18
Soybean 951 <sup>4</sup>	12 / 855		36.9	1.35	15.7	0.43	0.52	6.0	0.16
Lettuce 902	16 / 280		16.1	0.14	5.8	0.36	0.13	5.4	0.34
Lettuce 911	16 / 293		16.9	0.18	7.5	0.44	0.16	6.7	0.40
Lettuce 921	16 / 336		19.4	0.18	7.5	0.39	0.17	7.1	0.37
Lettuce 931 <sup>4</sup>	16 / 291		16.8	0.20	7.7	0.46	0.19	7.1	0.42
Potato 911	12 / 655		28.3	2.28	22.4	0.79	0.74	7.3	0.26
Potato 912	12 / 866		37.4	2.53	29.1	0.78	1.10	12.6	0.34
Potato 921 <sup>3</sup>	12 / 917		42.2	2.77	27.2	0.64	1.88	18.4	0.44
Potato 931 <sup>3</sup>	12-16 / 849		42.7	2.74	27.4	0.64	1.71	16.7	0.40
Potato 941 <sup>4</sup>	12 / 791		34.2	13.62	32.6	0.95	8.35	20.0	0.58
Tomato 951 <sup>4</sup>	12 / 615		26.6	1.10	13.1	0.49	0.51	6.1	0.23
Tomato 961	12 / 894		38.6	1.69	19.6	0.51	0.85	9.8	0.25

<sup>1</sup> Data based on an available growing area of 20 m<sup>2</sup>.

<sup>2</sup> Wheat, soybean, and lettuce seedlings covered for first 4 days with germination covers; potato covered for first 3 days; g m<sup>-2</sup> d<sup>-1</sup> calculations adjusted to reflect the respective number of days under full lighting.

<sup>3</sup> Photoperiod extension tests conducted throughout growth of potato crop 921; photoperiod switched from 12 to 16 h at 65 days for potato crop 931.

<sup>4</sup> Studies where half the plants were grown on recycled nutrients from an aerobic bioreactor.

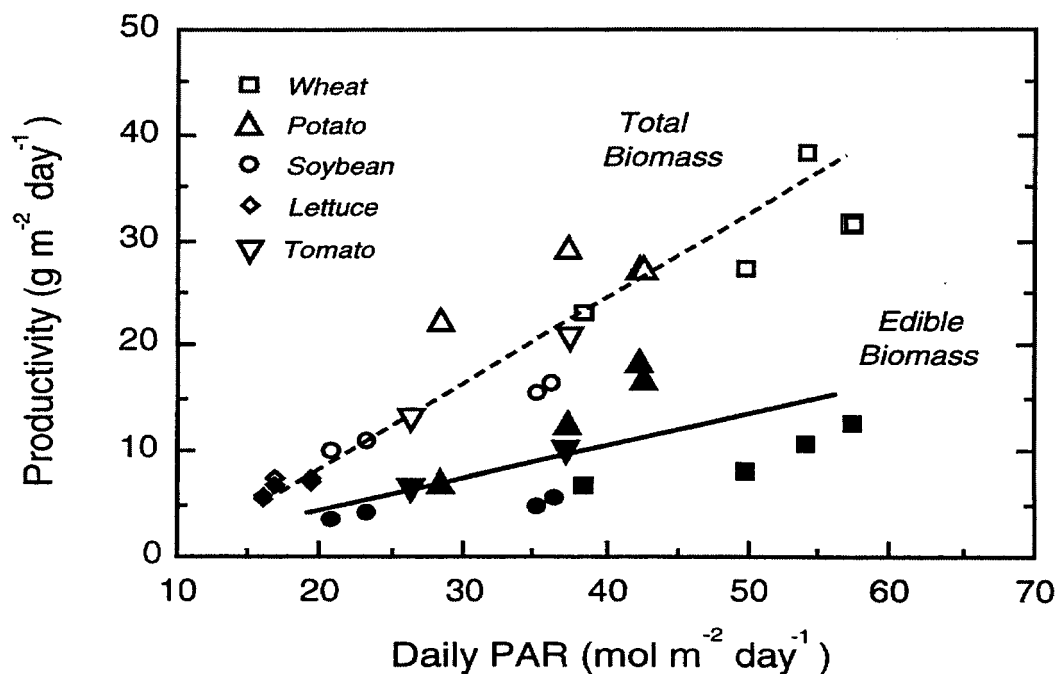


Figure 7. Productivity of total and edible biomass of different crops in the BPC (20 m<sup>2</sup> area) as a function of daily PAR. (Open symbols represent total biomass and closed symbols represent edible biomass.)

Comparisons of proximate composition of plant tissues grown in controlled environment tests (from ALS studies at KSC and Purdue University) with values reported for field grown plants (Duke and Atchley, 1986) are shown in Table 8. Because the analyses did not always include the same cultivars (genotypes), the comparisons should be taken cautiously. But in general, controlled environment grown crops tended to have higher ash content and protein (estimated from total nitrogen content). This suggests that the horticultural approaches and conditions used in the controlled environment testing (e.g., the hydroponic culture) promoted greater nutrient uptake (Wheeler et al., 1993c; 1997a).

**2.4.3 Water, nutrient and acid use.** Examples of water, nutrient (stock solution), and acid (pH control) additions to the NFT hydroponic system throughout the growth and development of wheat, soybean, lettuce, and potato crops are shown in Figure 8. Water use increased during early growth as the canopies "closed" and the total evaporating surface of foliage increased. Following this, water uptake rates remained relatively constant through stand maturation for wheat or declined slowly with age for soybean and potato stands (Figure 8). The particular example for soybean water use shows a large increase ca. 30 days, which coincided with a reduction in the humidity resulting from control system adjustments (study BSB902-upper chamber). Over the course of development, water use rates averaged 2 - 5 L m<sup>-2</sup> d<sup>-1</sup> (Table 9), while maximum rates ranged from 5 - 9 L m<sup>-2</sup> d<sup>-1</sup> depending on the species and the water vapor pressure deficit (Figure 8). Since most of the tests at KSC were conducted with "enriched" CO<sub>2</sub>

concentrations (e.g., 1000 or 1200  $\mu\text{mol mol}^{-1}$ ), water use rates would likely be higher at lower  $\text{CO}_2$  (e.g., 400  $\mu\text{mol mol}^{-1}$ ) or at super elevated levels (e.g., >5000  $\mu\text{mol mol}^{-1}$ ) (Wheeler et al., 1993b, 1999; Mackowiak and Wheeler, 1996).

Nutrient replenishment (stock solution addition) for wheat stands showed a rapid rise early in growth, whereas soybean, lettuce, and potato stands showed slow initial uptake rates followed by a rapid increase (Figure 8). In each case, the periods of rapid increase correlated closely with periods of rapid vegetative growth. With the exception of lettuce, nutrient replenishment declined as stands matured and shifted into reproductive growth (Figure 8). Because lettuce was harvested during rapid vegetative growth, there was no apparent drop in nutrient uptake at the time of harvest. The particular example of nutrient uptake data for soybean in Figure 8 showed a large overshoot in nutrient addition ca. 45 days, which corresponded to calibration adjustments in the EC monitoring and control system for that study. Average nutrient (total cation) addition rates ranged from 16  $\text{mmol m}^{-2} \text{d}^{-1}$  (lettuce) to 58  $\text{mmol m}^{-2} \text{d}^{-1}$  (wheat) (Table 9).

Nitric acid additions for pH control showed trends similar to nutrient replenishment with additions increasing during periods of rapid vegetative growth and rapid nutrient uptake (Figure 8). Comparisons of the acid and nutrient addition curves reveal some temporal differences, suggesting the balance of anion and cation uptake may shift throughout the growth and development of some crops (Marshner, 1995). The interesting bimodal peak of acid additions during early growth of wheat was observed in several studies and may indicate changes in the proportion of cation to anion uptake during this phase of development (Figure 8). Acid additions reached nearly 100  $\text{mmol m}^{-2} \text{d}^{-1}$  for wheat studies when daily PAR and crop growth rates were high (Table 9). Average acid addition rates through growth and development ranged from approximately 5 to 40  $\text{mol m}^{-2} \text{d}^{-1}$  depending on the crop and total lighting provided (Table 9). For wheat studies, nitric acid additions continued at a moderate level throughout mature growth, even though nutrient uptake had dropped (Figure 8). The reason for this is unclear but may be related to microbial denitrification in the rhizosphere under these conditions.

Plant tissues from all the BPC studies and many growth chamber studies were analyzed for elemental composition. Results from some of these analyses can be found in Wheeler et al., 1993c (wheat), Wheeler et al., 1994a (lettuce), and Wheeler et al., 1998 (soybean). The high ash levels from proximate analyses showed that some elements were high in leaves and stems. This luxuriant or excessive uptake of elements such as K and N may have been a consequence of maintaining a constant EC set-point throughout growth, and the use of nitric acid for pH control. Analysis of lettuce leaves and potato stems often showed K levels ranged of 10 to 15% of the tissue dry weight. The high K and  $\text{NO}_3$  levels in the tissue appeared to have no adverse effects on crop growth, but there was a concern in early testing that this was wasteful nutrient management for potential space applications. But concurrent resource recovery studies showed that many of these elements could be leached from the tissue by processing the inedible biomass in bioreactors (Garland and Mackowiak, 1990; Finger and Strayer, 1994; Mackowiak et al., 1997a). These retrieved nutrients could be then used to reconstitute more nutrient solution for growing subsequent generations of crops (Mackowiak et al., 1996, 1997a; Strayer et al., 1997).

Table 8. Proximate composition of controlled environment (CE) and field-grown crops.<sup>1</sup>

Crop (plant part)	Growth Setting	Protein	Fat	Ash	Carbo- hydrate	No. of Studies
		(%)	(%)	(%)	(%)	
Wheat (seeds)	CE	19.1	3.1	2.0	75.9	4 <sup>2</sup>
	Field	14.3	2.3	2.2	81.1	5 <sup>3</sup>
Soybean (seeds)	CE	37.1	20.0	7.4	35.3	4 <sup>2</sup>
	Field	38.2	18.2	5.4	38.2	4 <sup>3</sup>
Lettuce (leaves)	CE	27.4	4.6	21.8	46.1	4 <sup>2</sup>
	Field	22.8	4.6	10.4	60.0	8 <sup>3</sup>
Potato (tubers)	CE	15.2	0.7	7.6	76.5	4 <sup>2</sup>
	Field	10.4	0.6	5.0	84.0	5 <sup>3</sup>
Sweetpotato (roots)	CE	9.6	0.5	9.6	80.2	1 <sup>4</sup>
	Field	5.4	0.9	2.6	90.9	6 <sup>3</sup>
Peanut (seeds)	CE	30.3	43.3	3.3	23.1	2 <sup>4</sup>
	Field	26.7	42.0	2.6	28.7	2 <sup>3</sup>
Tomato (fruit)	CE	18.9	3.6	10.2	67.2	8 <sup>5</sup>
	Field	16.6	3.8	8.1	72.3	6 <sup>3</sup>
Radish (roots)	CE	25.9	3.1	16.2	54.7	4 <sup>6</sup>
	Field	14.9	1.5	11.3	72.2	4 <sup>3</sup>
Spinach (leaves)	CE	33.4	2.3	23.5	40.8	1 <sup>4</sup>
	Field	29.1	5.3	20.9	44.7	6 <sup>3</sup>
Rice (seeds)	CE	15.3	3.8	3.6	77.2	2 <sup>4,6</sup>
	Field	7.6	2.2	3.4	86.9	4 <sup>3</sup>
Strawberry (fruit)	CE	11.9	1.0	7.0	80.1	1 <sup>4</sup>
	Field	7.7	4.0	5.8	82.5	4 <sup>3</sup>

<sup>1</sup> Data expressed on a dry weight basis; carbohydrate determined by difference. Additional comparisons of field and CE-grown crops of the same genotype can be found in Grant et al., 1993; McKeehen et al., 1996; Nielsen et al., 1996; Jurgonski et al., 1997; see also, Wheeler, 2000a.

<sup>2</sup> Data from NASA's Biomass Production Chamber (Wheeler et al., 1996a).

<sup>3</sup> Field data taken from Duke and Atchely, 1986.

<sup>4</sup> Data from growth chamber studies at Kennedy Space Center.

<sup>5</sup> Data from Wheeler et al., 1997a.

<sup>6</sup> Data from McKeehen et al., 1996a, b.

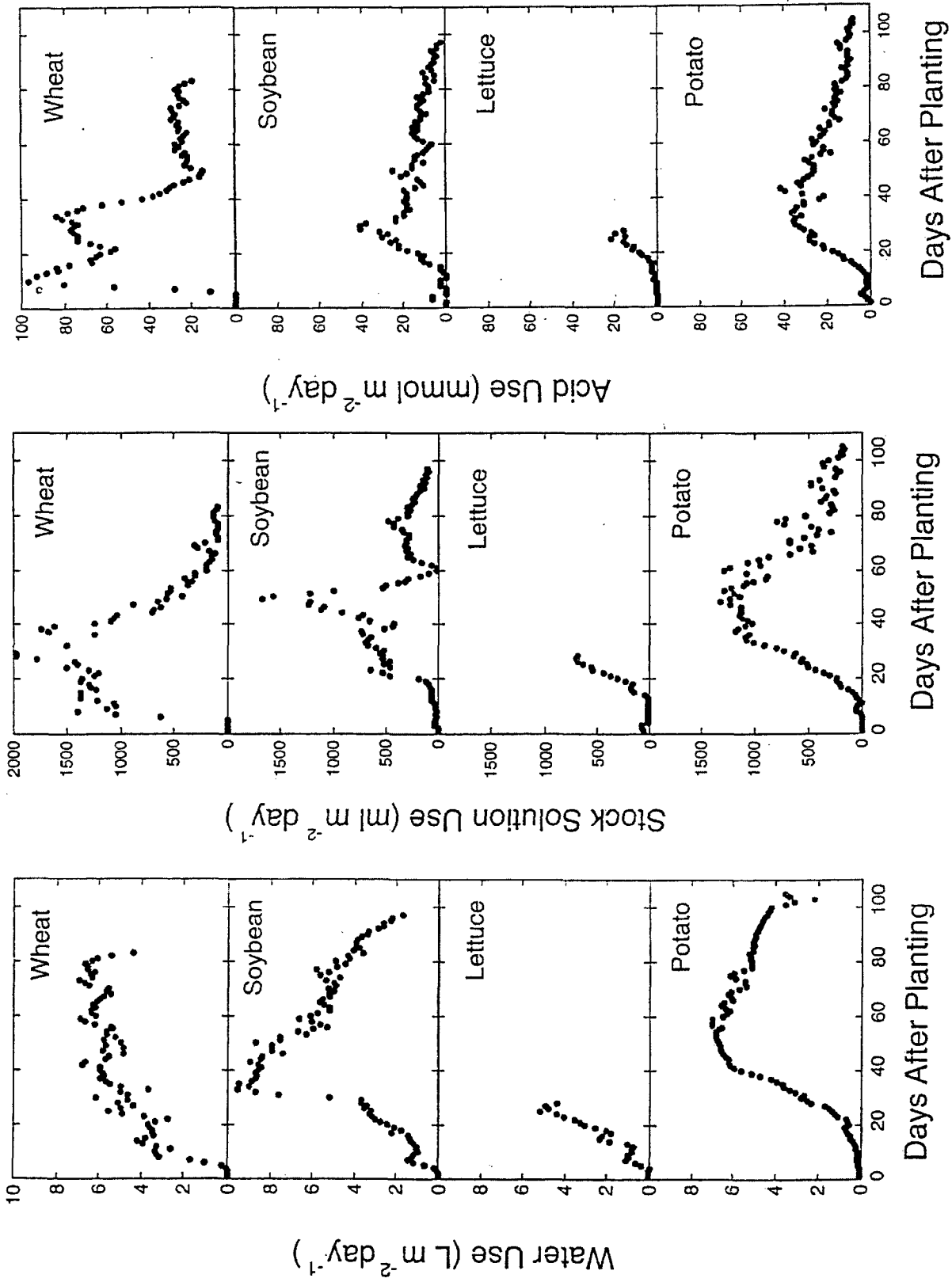


Figure 8. Examples of water use, nutrient uptake (stock solution use), and acid use (for pH control) for 20 m<sup>2</sup> crops of wheat (BWT931), soybean (BSB902--HPS), lettuce (BLT911), and potato (BWP921) over time.

Table 9. Water, nutrient (cation), and acid (for pH control) requirements for crops grown in recirculating hydroponic systems.

	Soybean	Wheat	Potato	Lettuce
Water Use <sup>1</sup> (L m <sup>-2</sup> d <sup>-1</sup> )	4.7	4.7	4.0	2.1
Nutr. Use <sup>2</sup> (mmol m <sup>-2</sup> d <sup>-1</sup> )	29.2	58.3	44.7	16.3
Acid Use <sup>3</sup> (mmol m <sup>-2</sup> d <sup>-1</sup> )	12.5	41.6	18.0	6.1
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g DM / L Water	3.1	7.7	6.7	2.9
g DM / mmol K, Ca, Mg	0.49	0.60	0.59	0.38
g DM / mmol Acid	1.14	0.85	1.47	1.02

<sup>1</sup> Water use includes volumes from stock solution and acid (HNO<sub>3</sub>).

<sup>2</sup> Nutrient use expressed as mmol of K, Ca, and Mg.

<sup>3</sup> Acid use expressed as mmol H<sup>+</sup>.

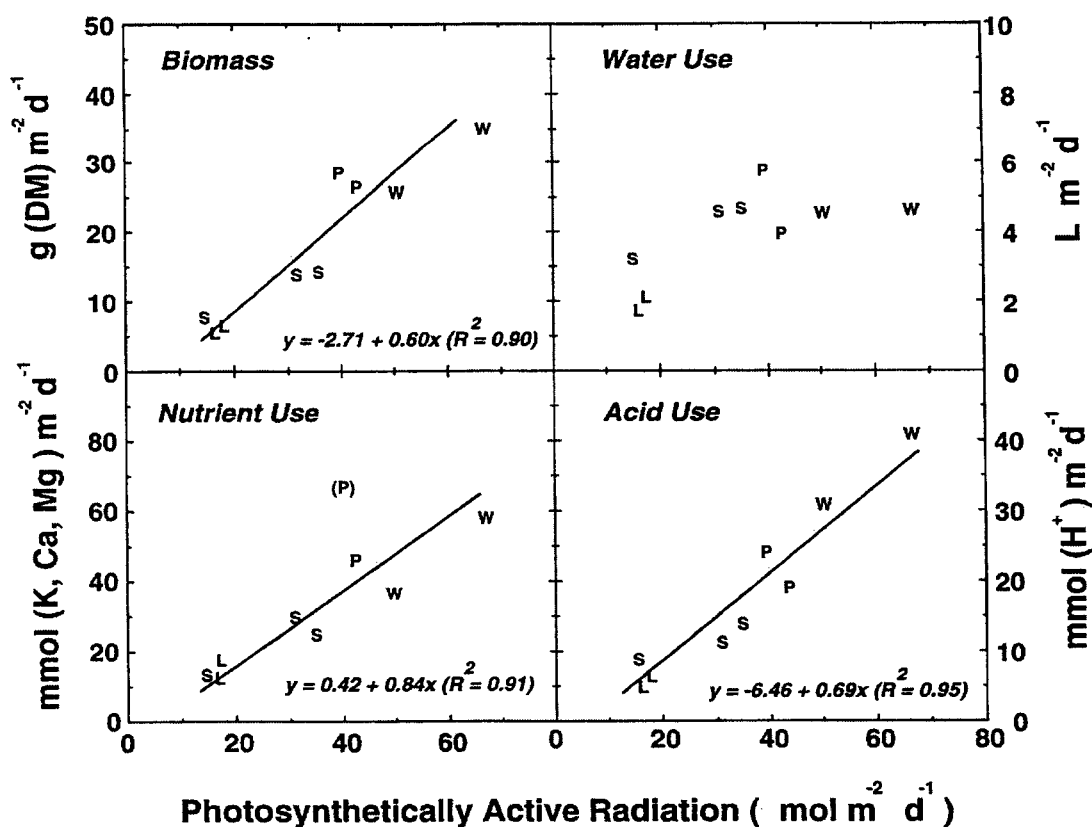


Figure 9. Biomass production (g), water use (L), cation uptake (mmol), and acid addition (mmol) as a function of PAR provided to different crops of wheat (W), soybean (S), lettuce (L), and potato (P) grown in the BPC. (Potato study BWP911 resulted in low tuber yields and excessive vegetative growth; consequently, nutrient uptake was high and was not included in the nutrient use regression equation. No mathematical relationship is shown for water use due to differing vapor pressure deficits among studies.)

Water use efficiencies for the different crops ranged from ~3 to 8 g DM L<sup>-1</sup> water used (Table 9). Nutrient (cation) use efficiencies averaged from 0.4 to 0.6 g DM mmol<sup>-1</sup> cations (K<sup>+</sup>, Ca<sup>++</sup>, and Mg<sup>++</sup>) (Table 9). Average acid use efficiencies ranged from 0.85 g DM mol<sup>-1</sup> acid (wheat) to ~1.5 g mol<sup>-1</sup> for potato (Table 9). Comparisons of PAR provided to the crops and the various nutrient solution management parameters indicate that radiation use efficiency for nutrient uptake was 0.84 mmol cations mol<sup>-1</sup> PAR (excluding one outlier study with potato), while radiation use efficiencies for acid averaged 0.69 mmol acid mol<sup>-1</sup> PAR (Figure 9). Water uptake rates are also plotted as a function of daily PAR in Figure 9, but no clear correlations existed; this was likely due to the more influential effects of different water vapor pressure deficits (VPDs) among the different studies.

**2.4.4 CO<sub>2</sub> exchange rates.** Carbon dioxide exchange rates by plant stands in the BPC were calculated by tracking the rates of change during nocturnal increases in CO<sub>2</sub> concentration (dark period respiration) or by the “morning” drawdown rates (net photosynthesis) after the lamps came on each day (Figure 10) (Wheeler, 1992). These measurements could only be done while the chamber was closed without humans inside, using what is traditionally called a closed-system approach (Coombs et al., 1985). Measurements of CO<sub>2</sub> addition rates to maintain set points throughout the light period gave similar photosynthetic rates, in this case using a null-balance or semi-closed approach. For some experiments, changes in oxygen concentration were tracked simultaneously using a closed approach (Figure 10) (Wheeler, 1992, 1996). The closed atmosphere of the chamber allowed direct manipulations of the environment, including deliberately shutting off the CO<sub>2</sub> supply and allowing the concentration to “drawdown” to a compensation point where net photosynthesis was zero (Figure 11). By plotting the photosynthetic rate as a function of CO<sub>2</sub> concentration, a complete Pn vs. [CO<sub>2</sub>] response could be determined for the plant stand at that stage of development (Wheeler, 1992). Because only C<sub>3</sub> type photosynthesis species were tested, net photosynthesis rates typically saturated between ~1000 – 1500 parts per million (ppm) CO<sub>2</sub> (Coombs et al., 1985; Wheeler et al., 1996a), while compensation (i.e., zero net uptake) typically occurred between 50 to 100 ppm CO<sub>2</sub>. By tracking the diurnal changes in CO<sub>2</sub> each day, the stand CO<sub>2</sub> exchange rates could be monitored through growth and development, as shown in Figure 12 (wheat), Figure 13 (soybean), Figure 14 (lettuce), and Figure 15 (potato). In each case, net photosynthesis rates were low early in growth, prior to full canopy cover and maximum light interception. Rates typically peaked soon after canopy closure as the height of the stand increased and the incident PPF increased (Figures 12, 13, and 15), i.e., as the stand grew closer to the lamp bank. After this, stand net photosynthesis and respiration rates gradually decreased with time as the stand matured and upper leaves began to senesce. Because lettuce stands were harvested soon after canopy closure during vegetative growth, they do not show the gradual decrease in photosynthesis and respiration with age (Figure 14).

As with CO<sub>2</sub> concentration, short-term manipulations with PPF were studied and showed a near linear response in photosynthetic rate to incident PPF (Figure 16) (Wheeler and Sager, 1990). The light compensation points for the stands at that stage of development (in this case, just following canopy closure) could then be determined from the x-intercept, where net photosynthesis was zero, while the stand respiration rates could be determined from the y-intercept where there is no photosynthetic activity. Interestingly, light compensation points for crop stands in BPC studies were near 200 μmol m<sup>-2</sup> s<sup>-1</sup> during mid-term growth; in other words, these crop stands required at least 200 μmol m<sup>-2</sup> s<sup>-1</sup> of PAR to offset their background respiration. This may seem high but it is important to note that the stands were grown at ~700 to 800 μmol m<sup>-2</sup> s<sup>-1</sup> PPF and thus their standing biomass and canopy architecture adapted to

that environment (Figure 16). For stands grown at higher PPF levels, the light compensation points tend to be even higher because of the greater standing biomass and increased background respiration rates (Bugbee, 1995a). On the other hand, photosynthetic rates of single leaves typically show much lower light compensation points but then tend to saturate at lower PPFs in comparison to whole stands (Figure 16).

For most BPC studies, photosynthesis and respiration rates were also measured in response to short-term changes in temperature set points (Wheeler et al., 1993b). For wheat stands grown at 20°C/16°C (light/dark), increasing the temperature to 24°C increased respiration and reduced net photosynthesis, while decreasing the temperature to 16°C decreased respiration but had little effect on net photosynthesis (Wheeler et al., 1993b).

**2.4.5 Ethylene and volatile organic compounds.** The closed atmosphere of the BPC also permitted tracking of volatile organic compounds through growth and development (Batten et al., 1996; Wheeler et al., 1996b; Stutte and Wheeler, 1997). Examples of ethylene gas concentrations in the BPC throughout growth and development of wheat, soybean, lettuce, and potato are shown in Figure 17. Ethylene accumulation in the BPC varied depending on the species, the amount of standing biomass, and the stage of development for the crop (Figure 17). By dividing the ethylene concentrations in the chamber by the standing biomass, a relative rate of ethylene production over time can be plotted, which showed that ethylene production rates by the wheat, soybean, lettuce, and potato stands were generally highest during early, vegetative growth (Figure 17). Based on observations from wheat tests on the Russian Mir space station (Salisbury, 1997; Bingham et al., 1999), the relatively low harvest indices of wheat (~30% study BWT881, 882) in the BPC may have been due in part to deleterious effects of ethylene on pollination and seed set (Figure 17).

Ethylene measurements were also taken throughout growth and development of two tomato crops (BTM951, BTM961) and in each case showed relatively low concentrations during vegetative growth, followed by sharp increases during fruit ripening, reaching levels of 400 to 500 parts per billion (ppb) prior to the periodic fruit harvests (data not shown). Tomato fruits go through a so-called climacteric ripening sequence, which is typically accompanied by increased respiration and elevated ethylene production (Abeles et al., 1992). Tomato study BTM961 included a comparison of ethylene filtered (using potassium permanganate coated pellets) and unfiltered air in the upper and lower halves of the chamber. Although the ethylene scrubbing efficacy was intermittent, the results showed that reducing the amount of ethylene in the atmosphere slowed tomato fruit ripening. These results emphasize the potentially critical role of managing ethylene in ALS crop production systems. The influence of chronic ethylene exposure to ALS crops is currently being studied at Utah State University (e.g., Klassen and Bugbee, 2002).

In addition to ethylene, many other volatile organics can accumulate in the atmosphere above plant growing systems (Table 10). Some of these compounds emanate from the materials in the chamber (e.g., plastics, glues, paints, etc.), while others appear to be biogenic, (i.e., from the plants) (Batten et al., 1996; Stutte and Wheeler, 1997). In addition, the rates of production and types of compounds can vary depending on environmental conditions and the stage of development (Charron et al., 1996; Batten et al., 1996).



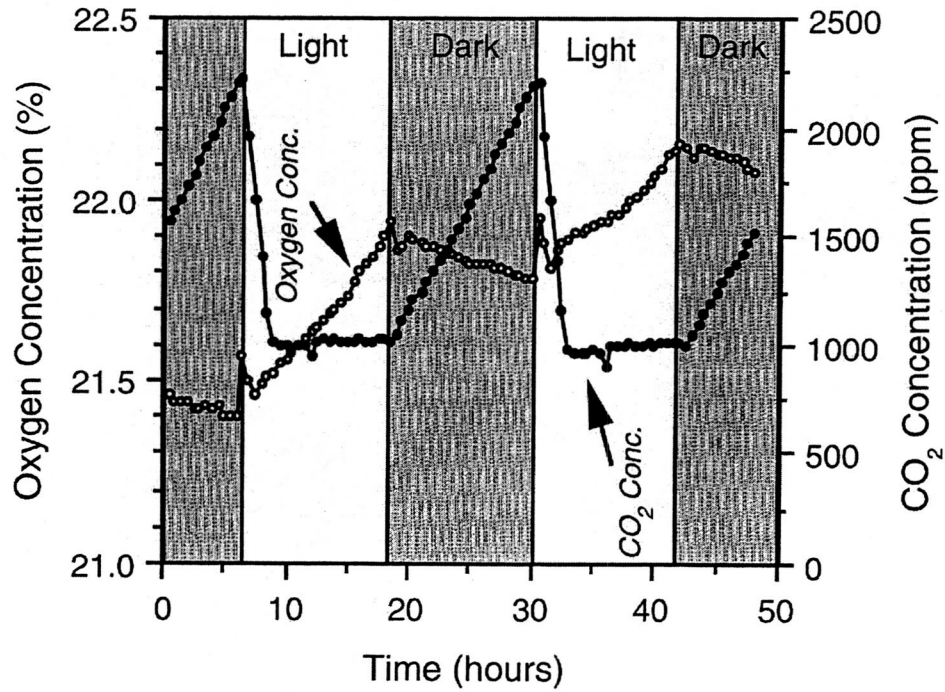


Figure 10. Repeating CO<sub>2</sub> drawdown from photosynthesis (light period) and CO<sub>2</sub> increase from respiration (dark period) of a 20-m<sup>2</sup> soybean stand in the BPC. (Simultaneous measurements show an increase in oxygen during the light period and decrease during the dark.)

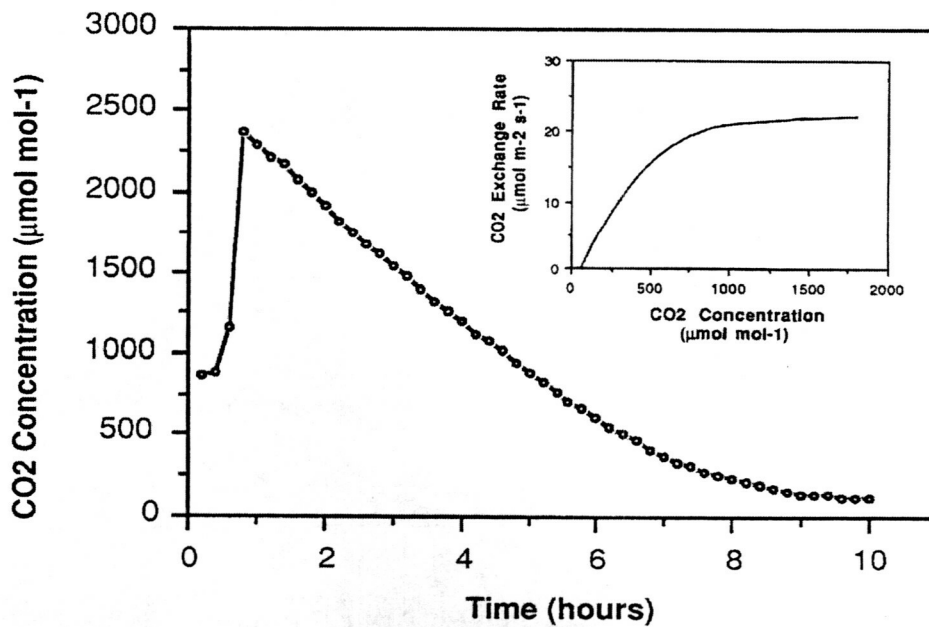


Figure 11. CO<sub>2</sub> drawdown by a 20-m<sup>2</sup> stand of wheat in the BPC. (Data were recorded by raising the CO<sub>2</sub> and then deactivating any further CO<sub>2</sub> addition. By taking the first derivative of the drawdown rate and plotting as a function of CO<sub>2</sub>, stand net photosynthesis vs. CO<sub>2</sub> is obtained [inset]).

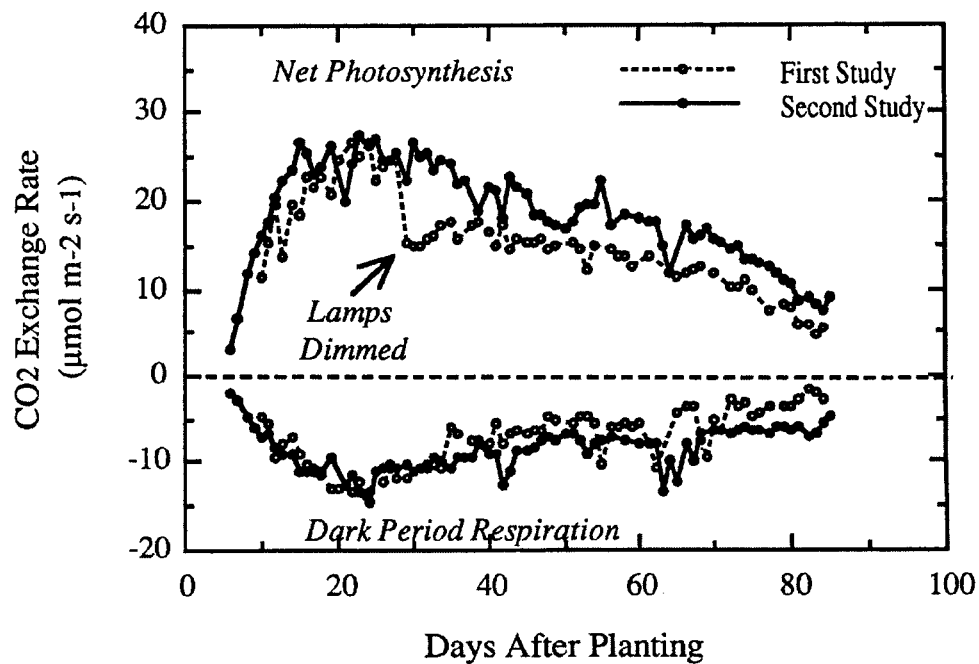


Figure 12. CO<sub>2</sub> exchange throughout growth and development of a 20-m<sup>2</sup> stand of wheat (cv. Yecora Rojo). (Stands were grown at  $\sim 700 \mu\text{mol m}^{-2} \text{s}^{-1}$  for the first study but reduced to  $\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$  at 28 days in second study.)

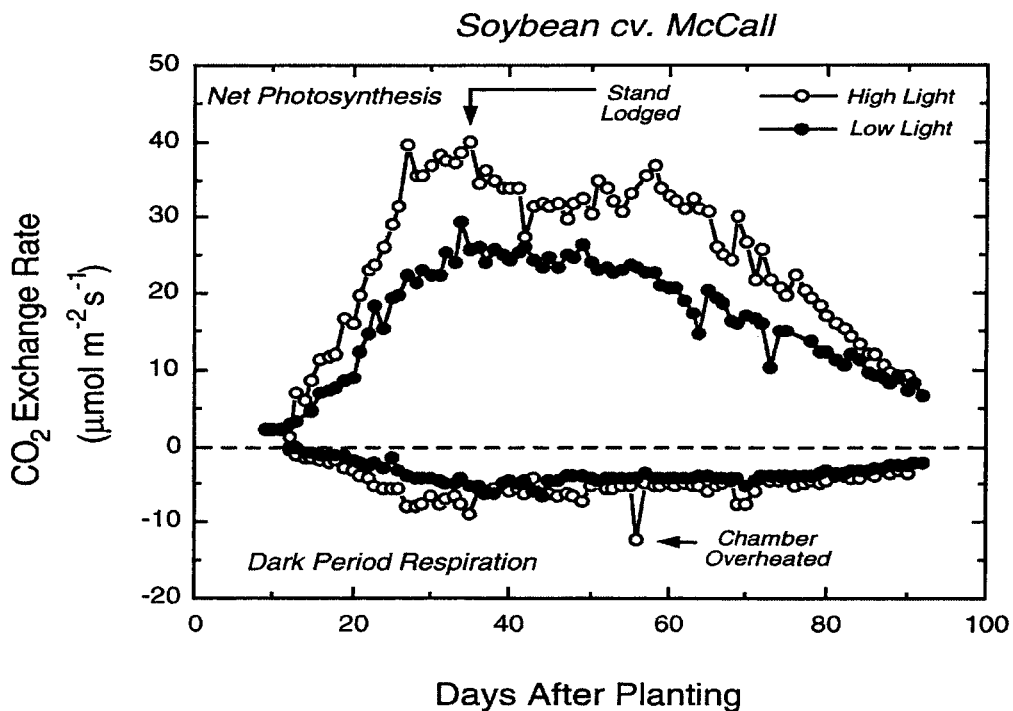


Figure 13. CO<sub>2</sub> exchange throughout growth and development of a 20-m<sup>2</sup> stand of soybean (cv. McCall). (Stands were grown at high light [ $\sim 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ] or low light [ $\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ].)

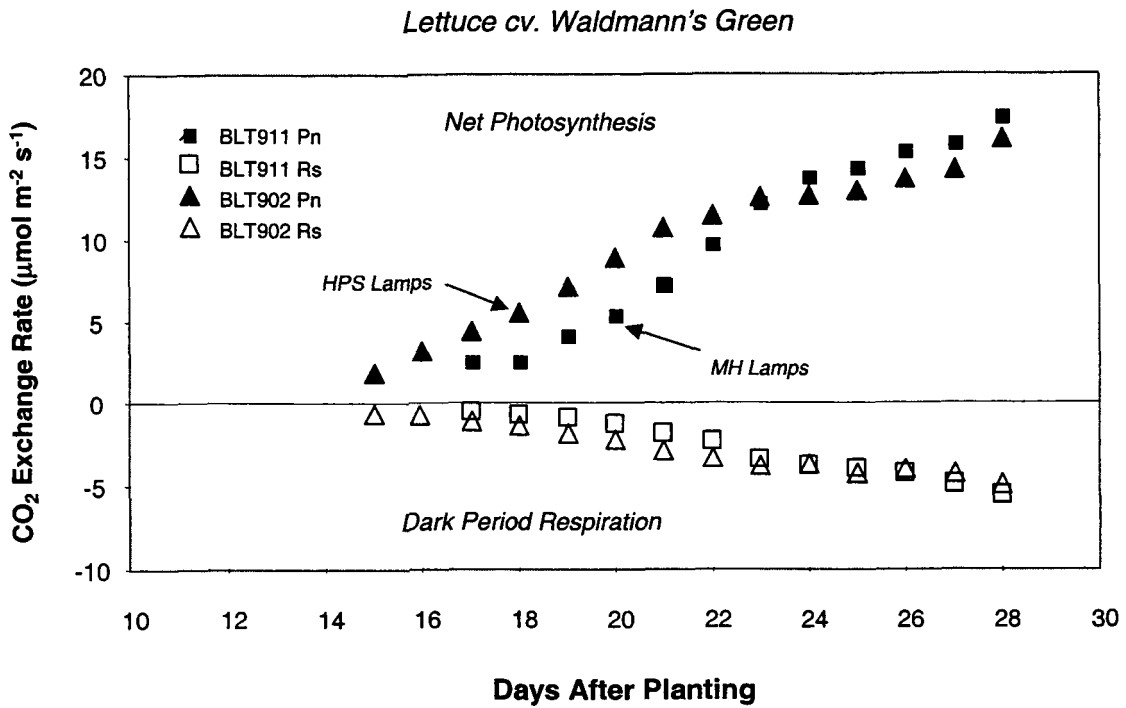


Figure 14. CO<sub>2</sub> exchange throughout growth and development of a 20-m<sup>2</sup> stand of lettuce (cv. Waldmann's Green). (Stands were grown at ~300 µmol m<sup>-2</sup> s<sup>-1</sup> under HPS (BLT911) or MH light (BLT902). HPS grown plants tended to stretch more during early growth resulting in more rapid canopy cover.)

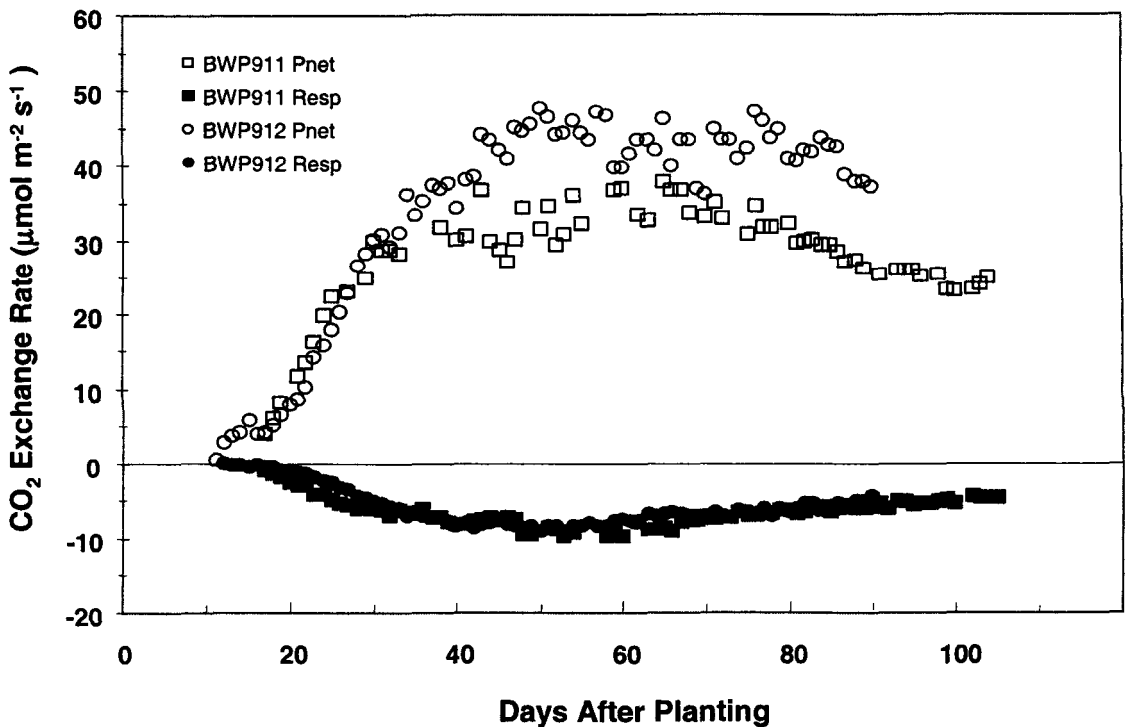


Figure 15. CO<sub>2</sub> exchange throughout growth and development of a 20-m<sup>2</sup> stand of potato. (Study BWP911 used cvs. Denali and Norland grown under HPS (upper chamber) or 2/3 HPS and 1/3 MH (lower chamber), and study BWP912 used cv. Norland grown under HPS.)

### Canopy vs. Single Leaf Photosynthesis

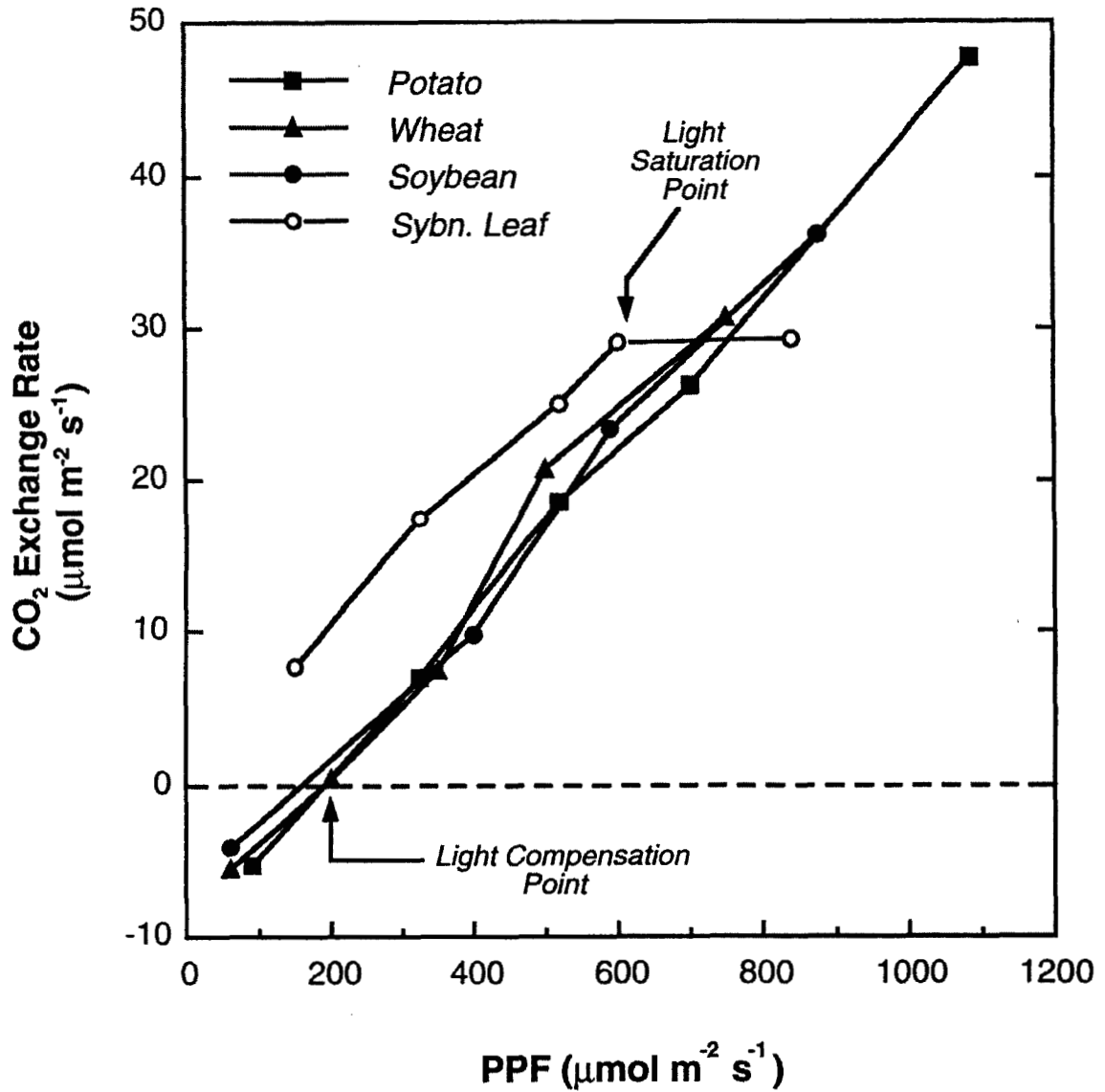


Figure 16. Plant stand gas exchange rates as a function of PPF. (Measurements were taken soon after full canopy cover for each species. PPF compensation point for stands was ~200 μmol m<sup>-2</sup> s<sup>-1</sup> for all three species, which were grown at maximum lighting in the BPC. Note that a single leaf (e.g., soybean) saturated at a lower PPF than the whole stands [data measured with ADC portable photosynthetic system].)

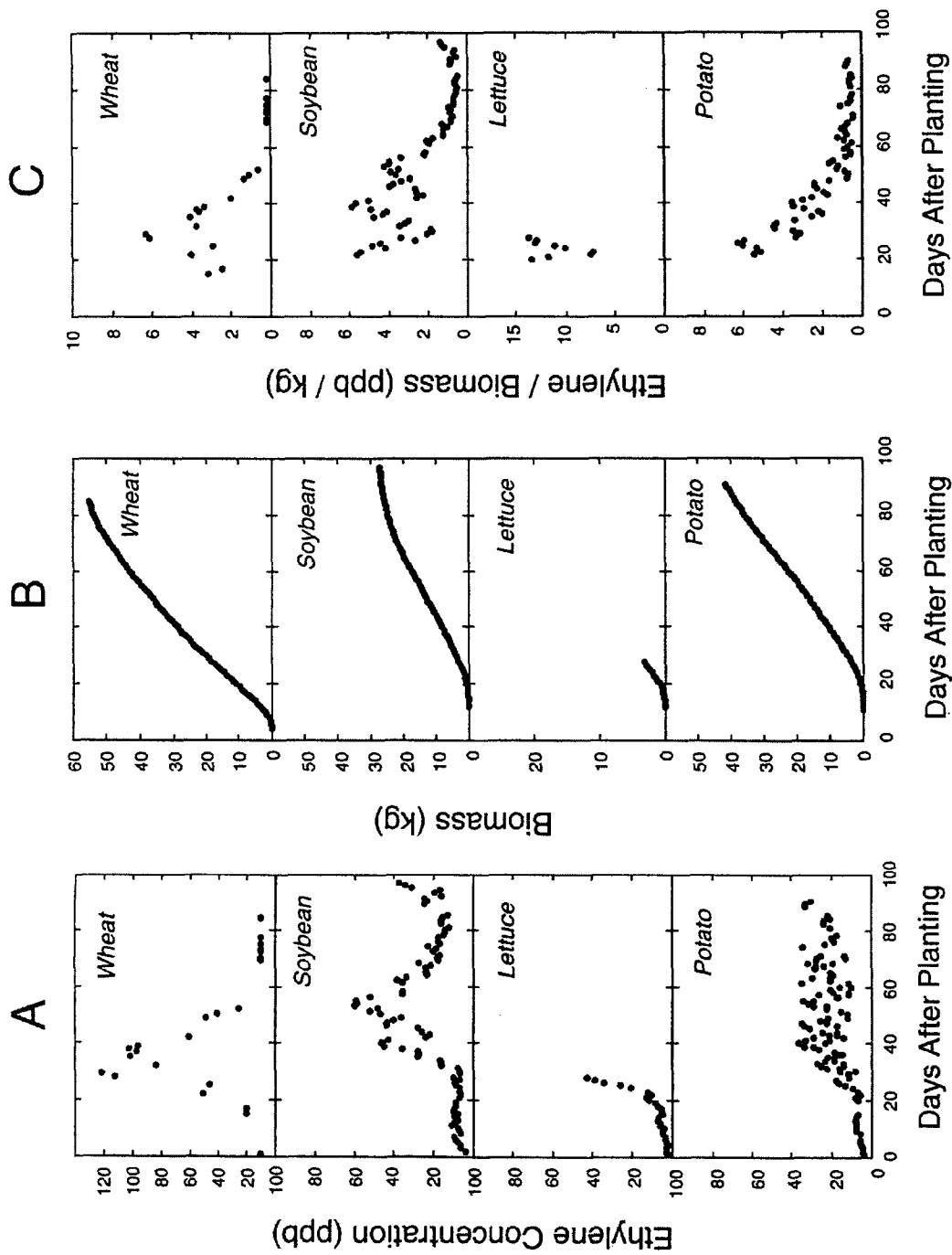


Figure 17. Ethylene concentrations (A) in the Biomass Production Chamber over time for crops of wheat, soybean, lettuce, and potato. (Panel B indicates a running total of biomass for each study (as calculated from CO<sub>2</sub> uptake). Panel C indicates the relative abundance of ethylene per amount of standing biomass.)

**Table 10.** Some volatile organic compounds from humans and plants.

Humans <sup>1</sup>	Plants <sup>2</sup>
acetaldehyde	benzaldehyde
acetone	2-butanone
ammonia	carbon disulfide
n-butyl alcohol	ethylene
carbon monoxide	2-ethyl-1-hexanol
caprylic acid	heptanal
ethanol	hexanal
ethyl mercaptan	2-hexen-1-ol acetate
hydrogen	isoprene
hydrogen sulfide	limonene
indole	2-methylfuran
methanol	nonanal
methane	ocimene
methyl mercaptan	$\alpha$ -pinene
propyl mercaptan	$\beta$ -pinene
pyruvic acid	$\alpha$ -terpinene
skatole	tetrahydrofuran
valeraldehyde	tetramethylurea
valeric acid	thiobismethane

<sup>1</sup> Reed and Coulter (1999).

<sup>2</sup> Stutte and Wheeler (1997); Stutte (1999).

## 2.5 Other observations

**2.5.1 Hydroponic system operations.** For many of the smaller growth chamber studies, water, nutrient, and acid (for pH control) replenishments of the hydroponic solutions were carried out manually each day. For BPC studies these activities were automated, but the larger reservoirs of stock solution and acid still required periodic manual preparation and filling (also, before 1990, condensate and hydroponic tanks were manually emptied and filled, respectively). Further automation may help reduce labor requirements somewhat but fundamentally different approaches for hydroponic system management may be required. Similarly, engineering upkeep and maintenance of pumps, sensors, and filters of the hydroponic systems, as well as other aspects of the plant chambers (lamps, fans, electrical components, etc.) will require

various levels of crew time, which must be considered in overall mission costs and demands. (Note that many engineering aspects of crop production for life support have not been included in this document and it is our hope that a similar document on engineering issues for bioregenerative life support will follow at a future date.)

**2.5.2 Materials handling.** Most of the planting and harvesting activities for crop studies in the BPC and smaller growth chambers were carried out manually and were labor intensive. For example, wheat seed sowing took several "person-hours" for the 20-m<sup>2</sup> BPC. Likewise, harvesting required manually removing the tray covers and crop materials from the chamber and then clipping or picking seed heads (wheat), pods (soybeans), or tubers (potatoes) by hand. To save time, threshing of wheat heads was done mechanically. Clearly further mechanization or even some automation should be considered for more sophisticated life support systems, thereby reducing crew time requirements. Regardless of whether threshing was manual or mechanical, cleaning wheat and soybeans seeds was a dusty process. These operations were carried out in open, ventilated settings (e.g., outside behind Hangar L), but such options will not exist in closed life support systems and dust control will be a serious challenge. Following harvest, inedible biomass (leaves, stems, fibrous roots) was typically oven-dried to obtain dry mass data. This was required for research purposes but would not be needed for a functional bioregenerative system. Likewise, post-harvest processing of inedible materials typically used the oven-dried biomass (Finger and Strayer, 1994). Direct processing of fresh biomass would be more energy efficient but will require additional studies, e.g., wet grinding/chopping methods, use of alternative dehydration/water retrieval methods, etc.

Most of the BPC studies used batch plantings where the entire chamber was either planted or harvested in a single day. This was done to accommodate the research mission but resulted in episodic, labor-intensive events. In addition, this approach required dedicated storage capabilities for keeping the biomass. A more manageable approach would be to stagger the plantings and harvests over more frequent intervals, which would reduce the single-day labor requirements and allow a reduction in the processing and storage system components (Drysdale et al., 1994b). Moreover, by staggering the crop harvests, a portion of the system would always be photosynthetically active, thereby providing continuous O<sub>2</sub> production, CO<sub>2</sub> scrubbing, and water recycling (Drysdale et al., 1994; Wheeler, 1996; Stutte et al., 1999).

**2.5.3 Sanitation.** Plant pathogens are a constant concern in terrestrial controlled environment agriculture (CEA), but most of these systems are relatively open to the surrounding environment, e.g., ventilated greenhouses. Because the BPC's atmospheric system was relatively closed, many of the risks were reduced. Despite this, early plans for operation of the BPC considered sterilization protocols for much of the materials handling. The impracticality of this approach was quickly realized and sterile procedures were abandoned in favor of sanitation and avoiding overt risks. For example, seeds and plant materials were obtained from certified or reputable sources, seeds were surface sterilized for many studies, transplants (potato plantlets) were taken from sterile in vitro cultures, "tacky" mats were positioned at each entrance of the chamber to remove shoe dirt, and plant growing trays, tray covers, germination wicks, and plumbing systems were cleaned between crops. In addition, extraneous plant materials (including lunch vegetables and cigarettes) and soil were not permitted in or near the chamber. Initial studies included disinfecting the hydroponic systems with either a hypochlorite (bleach) solution or nitric acid between plantings, but this was later abandoned in favor of physically cleaning and flushing the system. High-efficiency particulate air (HEPA) filters were used in the air ducts for early studies to remove airborne microbes and spores, but these were

removed with little consequence on atmospheric and surface microbial counts (N. Fields, personal communication).

With one exception, no widespread pathogen outbreaks were detected in the BPC crop studies over the ~12 years of operation. There were isolated examples of decay spots on some potato tubers or fungal growth on the crowns of isolated wheat plants, but none of these appeared to spread to other plants. The exception to this was a root zone pathogen on cv. Apogee wheat plants grown in a study in 1997. The infection appeared as "water-soaked" areas in the roots and continued to spread slowly among trays, resulting in decreased plant vigor and poor yields. The symptoms were typical of a *Pythium* organism, which was supported by evidence from some water-agar assays (M. Stanghellini, Univ. of Arizona, personal communication). The origin of the infestation was never determined and resolution to the problem involved terminating the study and sanitizing the trays and hydroponic systems. Potatoes were growing in the chamber during the same study but in separate hydroponic systems and showed no adverse symptoms. In another wheat study (BWT941), extensive fungal growth occurred on the dead leaves and stem sheaths following a high temperature event (air temperature briefly reached ~50°C). Plants were harvested about 10 days after this event and the saprophytic fungus probably had little effect on the crop, but numerous spores were released to the air as the plant trays were removed from the chamber, which may present a health concern for humans if they were forced to work in a closed environment with these materials.

The relative infrequency of obvious pathogens in the BPC studies was encouraging and suggests that using clean procedures and maintaining a diverse and stable microflora in the root zones (Strayer, 1994) may avoid many pathogen problems. Yet the serious consequences of an aggressive pathogen, as noted in the one wheat study indicate the pathology must be taken seriously and counter measures or contingencies should be part of the mission planning (Nelson, 1987; Schuerger, 1998). This might include the use of interplantings with multiple species and/or isolating different growing environments to reduce overall system risks, or implementing disinfection procedures if recirculating nutrient solutions are used (e.g., filtering nutrient solutions or treating solutions with hydrogen peroxide or ozone). Another possibility would be the use of fungicides, but few fungicides are cleared for use on hydroponic crops, particularly those that have edible structures that directly contact the nutrient solution.

Related to the topic of plant pathogens are concerns that the crop production systems may act as havens for human pathogens. This becomes even more important when waste recycling systems are used to supply nutrients and water to the crops. Studies in which human-associated organisms were deliberately added to crop hydroponic systems (small growth chamber studies) showed that counts of the human-associated bacteria dropped off quickly in the nutrient solutions and rootzones, suggesting that these organisms were generally not competitive in root environments (Morales et al., 1996). Nonetheless, further testing with other organisms, different loading rates, and different crop management practices is needed. For example, if plants are co-utilized in a wastewater processing scheme, it may be prudent to only use seed-producing crops for this function, where there is little risk of direct water contact with the edible portions of the crops.

### **3. FUTURE CONSIDERATIONS**

With the exception of a 416-day continuous production study with potatoes (four 104-d generations), all of the BPC studies were single generation plantings (Table 6). Tests during the Russian BIOS projects were also limited to 4 to 6 months maximum (Gitelson et al., 1989; Salisbury et al., 1997). In contrast, use of bioregenerative life support for long-duration



missions will require continuous operation of crop production systems and hence a thorough understanding of system performance over long durations is required. The potential risk of pathogen incursion, nutrient imbalances, and mechanical failures will likely increase over time, resulting in lower productivity. Additionally, bioregenerative systems will likely have multiple species cultured simultaneously, which may require combining different crops in a common environment. If all of the crops are grown in a common environment, allelopathic interactions may occur, particularly if crops share a common root-zone and/or a common nutrient solution. This might even include interactions between different aged plants of the same species, where for example older plants might outcompete younger plants for certain nutrients, e.g., potassium and phosphorus (Barta and Henderson, 1998). In addition, if the crops are grown in a common environment, it is unlikely that the temperature, CO<sub>2</sub>, PPF, and photoperiod can be optimized simultaneously for all the species, and the consequences of such environmental compromises should be studied. This should include developing more complete environmental response surface data for modeling crop growth and yield across a range of possible conditions. As resources become available, it would also be prudent to expand the number of species tested for bioregenerative systems to increase the range of foods for internationally and culturally diverse crews.

A number of significant horticultural and operational questions also remain unanswered and should be studied over long-duration tests. For example, can seed viability and vigor be maintained over time, and can vegetative propagation be used repeatedly for some crops (e.g., sweetpotato, potato, strawberry)? How long can growth media (solid or solution systems) be used for successive crop production? What are the operational life spans and spare parts issues for mechanical support systems, e.g., pumps, electric lamps, power supplies, and sensors (Fortson et al., 1994b)? What are the crew time requirements for operating crop production systems? (Gathering accurate data on this will be especially important for assessing overall system costs). What types of mechanization and automation are needed to reduce crew time requirements? Can the mass, volume, and power requirements of the mechanical support equipment be reduced? What types of failures (biological and physical) can occur and what is their frequency? What contingencies and safeguards are needed for minimizing these failures? Eventually various life support subsystems will need to be integrated and assessed from a larger system perspective. Projects such as the Russian BIOS tests, the Japanese Institute of Environmental Science's (IES) Closed Ecology Experiment Facility (CEEF), the European Space Agency's Micro-Ecological Life Support System Alternative (MELISSA) Project, and the proposed BIO-Plex facility by NASA will be needed to assess these integration issues (Gitelson et al., 1989; Tako, 1997; Barta et al., 1999; Lasseur and Savage, 2001). These integration studies should continue testing of solid and liquid waste processing that is linked with crop production (e.g., Finger and Strayer, 1994; Mackowiak et al., 1996a,b; Subbarao et al., 2000b) to assess the consequences on crop yield, mass savings through nutrient recycling, and potential food safety issues.

There are many more questions that could be added to this list, including what are the human psychological implications of having plants present in a confined living space, and what, if any, are the benefits of working with the plants and consuming fresh foods (as opposed to stowed foods) on long-duration missions (Flagler and Poincelot, 1994; Waters et al., 2002). Answers to these and other questions will require long-term commitments by NASA and other space agencies around the world. Facilities such as the Biomass Production Chamber at Kennedy Space Center have been valuable tools for addressing some of these questions, and it is our hope that research on bioregenerative life support concepts will continue so that plants will one day be used as sources of food, oxygen, and CO<sub>2</sub> removal for humans as they explore the solar system and beyond.

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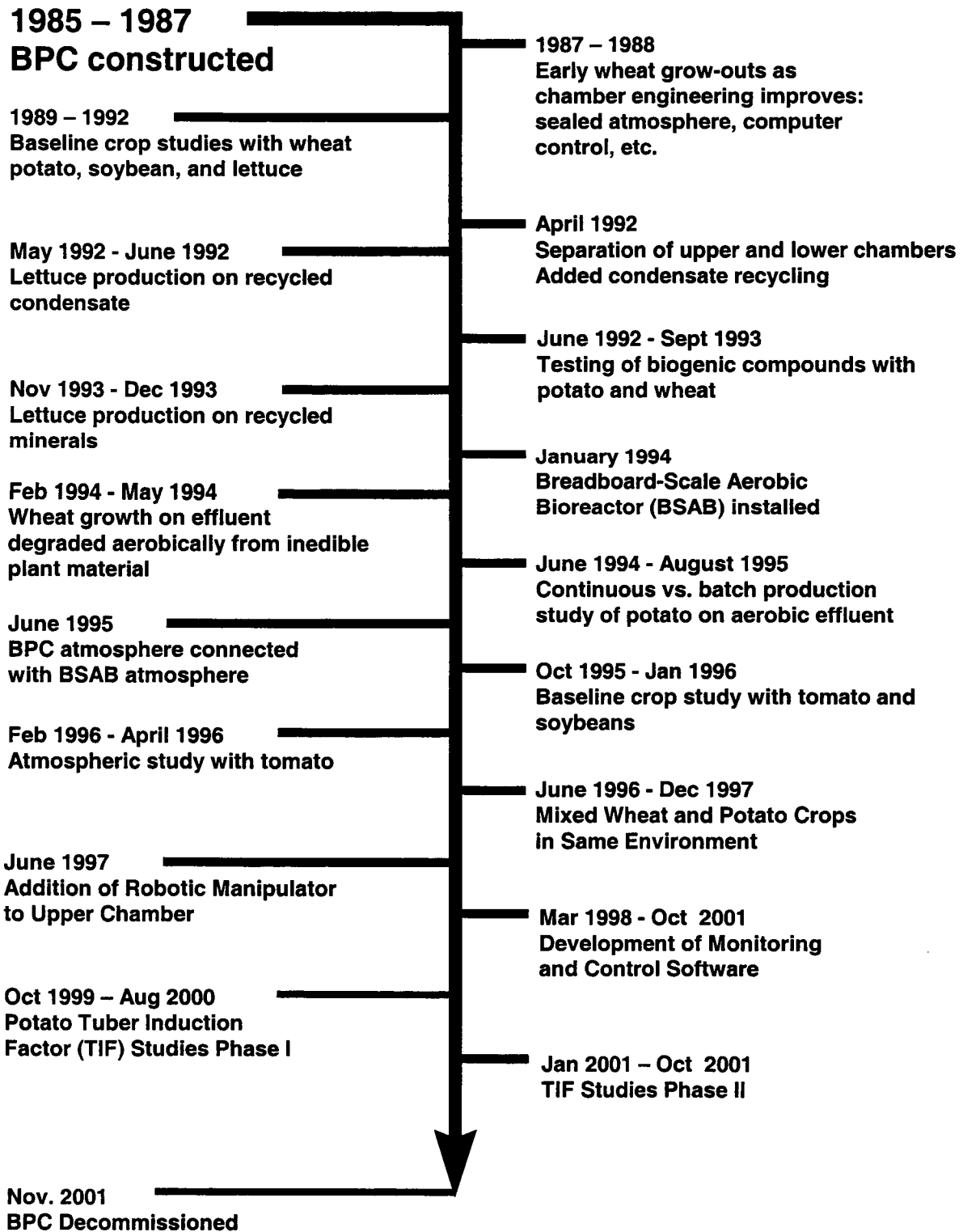
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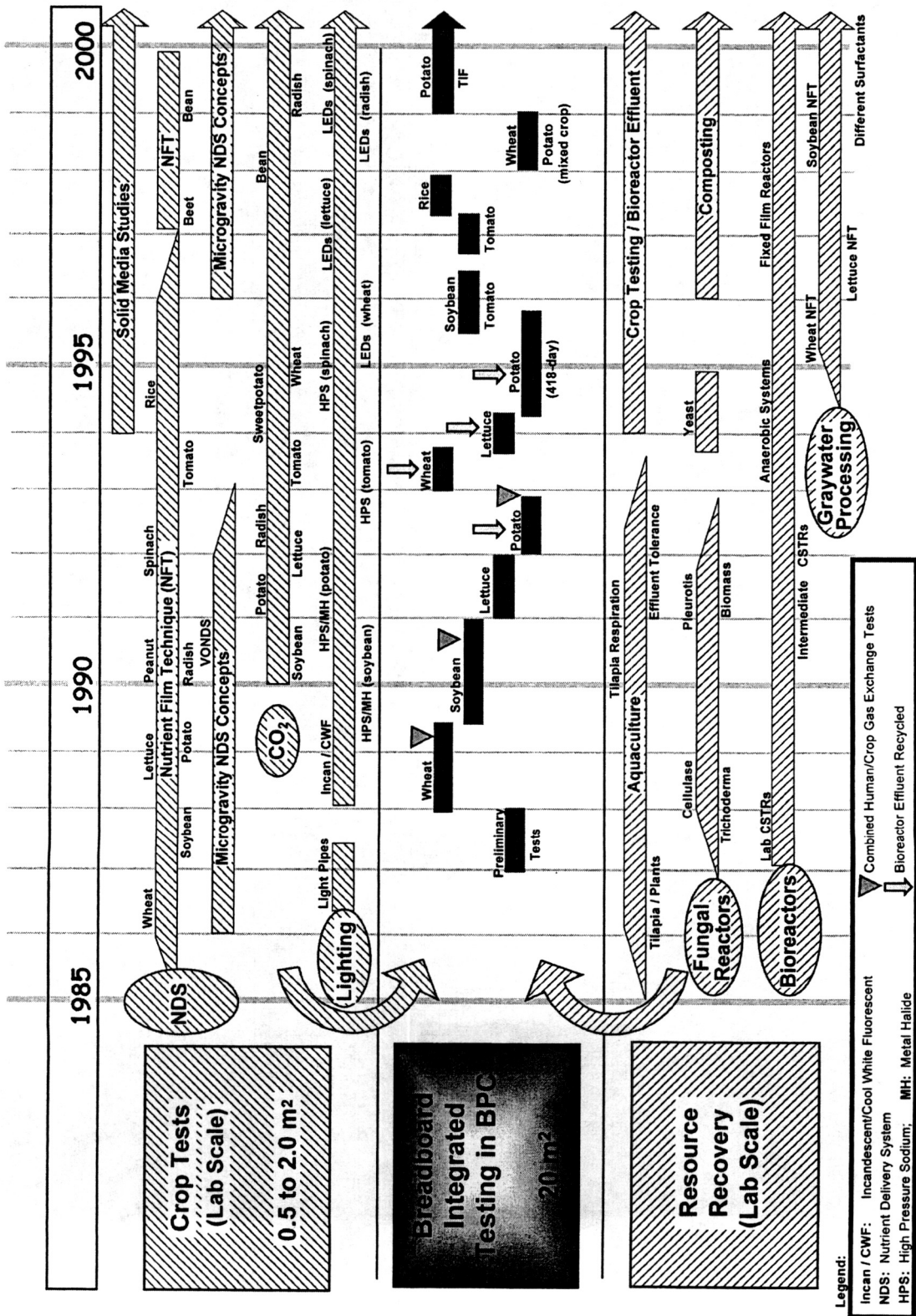
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**Appendix A: Timeline of developments and engineering modifications to the Biomass Production Chamber (BPC) at Kennedy Space Center, FL.**

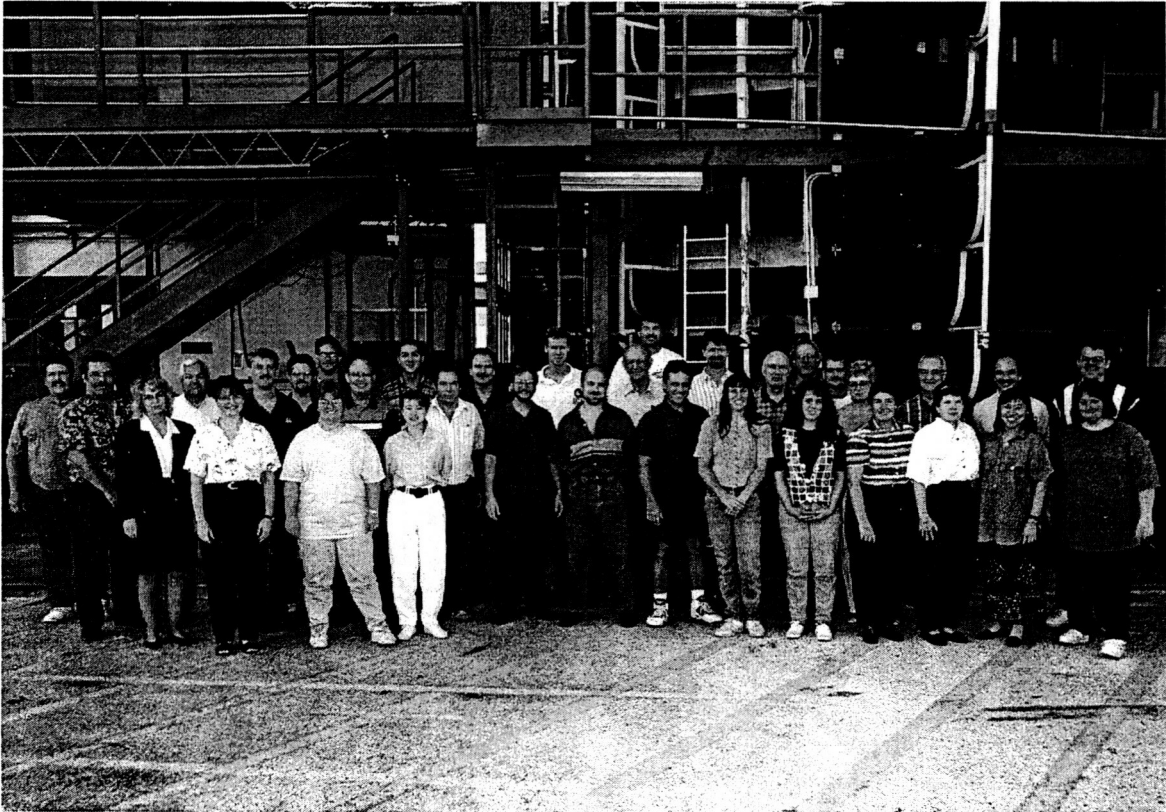


**Appendix B: Historical timeline for crop and resource recovery research for Advanced Life Support (ALS) activities at Kennedy Space Center. (Lab-scale testing typically preceded “breadboard” scale, integration tests using the Biomass Production Chamber.)**





**Appendix C.** Members of the Kennedy Space Center CELSS/ALS Research Team.





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<b>13. ABSTRACT (Maximum 200 words)</b> Advanced Life Support studies at NASA's Kennedy Space Center began in 1985 with the initiation of the CELSS Breadboard Project. These studies focused on testing with plants (crops) in a large, atmospherically closed test chamber called the Biomass Production Chamber (BPC). This document gives an overview of activities leading up to the Breadboard Project, a description of the BPC, and summaries of crop test data, including: (1) environmental and horticultural approaches, (2) observations on growth and development, (3) crop biomass and oxygen yields, (4) proximate composition of biomass, (5) whole stand photosynthesis and respiration rates and responses, (6) nutrient solution measurements, including water use, nutrient uptake, and acid additions for pH control, and (7) ethylene and other volatile organic compound production by plants. Because of the extent and diversity of crop testing at KSC during the 1985 to 2002 time frame, results and discussion are limited primarily to BPC studies with wheat, potatoes, soybeans, lettuce, and tomato. An expanded bibliography of plant-related CELSS/ALS research is included in the document. As our knowledge on growing plants for life support expands, the specific set points and outputs from ALS cropping systems will continue to be improved. But more testing is needed so that plants can one day be used as routine components for space life support.				
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