НАУКИ О ЖИЗНИ И КОСМОС

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СЕЛЬСКОЕ ХОЗЯЙСТВО В КОСМОСЕ: ЛЮДИ И СТРАНЫ, ЗАЛОЖИВШИЕ ОСНОВЫ

Аннотация. *Предметом исследования является анализ ключевых достижений и этапов в создании автономных систем жизнеобеспечения для долговременных космических полетов и обитаемых станций на поверхности планет с использованием живых организмов (микробных компонентов, растений, насекомых, птиц и животных). Ценность работы определяется тем обстоятельством, что его автор является непосредственным участником многих научных проектов и одним из ключевых специалистов в области космической биотехнологии Космического центра Кеннеди (США). Основное внимание уделено истории научного поиска и выработке основных технологических решений по использованию методов космической агрикультуры для контроля газового состава и при изменении давления внутренней атмосферы, утилизации продуктов жизнедеятельности человека и производству свежих продуктов питания в замкнутом пространстве. Для решения поставленных задач использованы общенаучные методы исследования - анализ, синтез, систематизация, формально-логический и другие. Исследование подводит промежуточный итог развития методов сельского хозяйства в космосе и внедрению их результатов в земном земледелии. На основе анализа научных публикаций (всего более 200) продемонстрирована специфика научного поиска и основные достижения ученых США, СССР и России, Канады, Японии, Китая и Европейского Союза. Разработанные методы, в частности, контроля освещенности, газового состава, атмосферного давления и обеспечения влажности растений, технологии вертикального земледелия, методы гидропоники для клубненосных овощных культур, способы контролируемой подачи воды в невесомости и системы переработки отходов, работающие в замкнутом цикле, создают необходимую технологическую основу для функционирования биорегенеративных систем жизнеобеспечения при космических полетах, на борту орбитальных станций и на внеземных базах.*

Ключевые слова: *Космос, Космическое сельское хозяйство, Биорегенеративный, Вертикальное земледелие, Передовое жизнеобеспечение, Фотосинтез, Агрономия контролируемой среды, Производство водорослей, Космические культуры, Утилизация отходов.*

Abstract. *Agricultural systems for space have been discussed since the works of Tsiolkovsky in the early 20th century. Central to the concept is the use of photosynthetic organisms and light to generate oxygen and food. Research in the area started in 1950s and 60s through the works of Jack Myers and others, who studied algae for O2 production and CO2 removal for the US Air Force and NASA. Studies on algal production and controlled environment agriculture were also carried out by Russian researchers in Krasnoyarsk, beginning in 1960s. NASA initiated its CELSS Program ca. 1980 with testing focused on controlled environment production of some plants. Related tests with humans and crops were conducted at NASA's Johnson Space Center in the 1990s. The European Space Agency MELiSSA Project began in the late 1980s and pursued ecological approaches for providing gas, water and materials recycling for space life support, and later expanded to include plant testing.As a result of these and other (Japan, Canada, China) studies for space agriculture novel technologies and findings have been produced. The theme of agriculture for space has contributed to, and benefited from terrestrial, controlled environment agriculture and will continue doing so into the future.*

Keywords. *Waste recycling, Algal production, Controlled Environment Agriculture, Photosynthesis, Advanced Life-Support, Vertical Farming, Bioregenerative, Space crops, Agriculture for Space, Space.*

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Introduction

In 1880, novelist Percy Greg wrote about a space traveler going to Mars and how he took plants with him to help with waste recycling (Greg, 1880) [71]. A few decades later in the 1920s, the Russian aerospace scientist, Konstantin Tsiolkovsky, described how humans and plants might co-exist inside closed environments in space by maintaining greenhouses with plants (Tsiolkovsky, NASA Translation, 1975) [185]. Tsiolkovsky envisioned agricultural modules that would gather sunlight and operate at reduced atmospheric pressure to reduce internal force and structure mass. He even included a sketch of a greenhouse module and talked of growing bananas and other crops (Tsiolkovsky, 1975) [185]. Decades later, in a book entitled Rockets and Space Travel, Willy Ley (1948) [106] noted that if the space journey is sufficiently long, growing plants would be an option to stowing oxygen, and suggested pumpkins as a candidate crop for this role, based on discussions he had with a botanist. This interest of plants and humans co-existing in space led to testing of algae for life support beginning with the work of Jack Myers and others during the 1950 and 60s for the US Air Force and the National Aeronautics and Space Administration-NASA (Myers, 1954; Krauss, 1962; Miller and Ward, 1966) [133, 100, 124]. The basis for space agricultural systems can be summarized by comparing the general metabolic equations for human respiration and plant photosynthesis, where plants or other photosynthetic organisms generate biomass (CH_2O) and oxygen (O_2), while removing CO_2 from the air (Myers, 1954; Gouleke and Oswald, 1964) [133, 68]. By choosing appropriate species, e.g., crops, a portion of this biomass can be food. A less obvious but perhaps equally valuable contribution is that waste water could be recycled to plants and the resultant transpiration condensed as clean water (Gitelson et al., 1976; Wolverton et al., 1983; Loader et al., 1999) [61, 202, 110].

In the paragraphs below I will try to identify some of the researchers, facilities, and findings that have been a part of this long-standing interest in space agriculture. I will certainly miss many contributors and researchers due to limited space, and many of my recollections will be somewhat biased toward NASA's work, since I am familiar with much of it. But space agriculture and bioregenerative life support have inspired talented researchers around the world for more than 50 years, and I salute the global bioregenerative life support community for their tireless and fascinating work in this field.

Algal "Agriculture"

The initial studies of space agriculture in the 1950s and 60s focused largely on algae, and in particular *Chlorella* spp. for O_2 production and CO_2 removal (Sorokin and Myers, 1953; Krauss, 1962; Eley and Myers, 1964; Gouleke and Oswald, 1964; Miller and Ward, 1966; Taub, 1974) [162, 100, 52, 68, 124, 178]. *Chlorella* was hardy, very productive, and relatively easy to culture in reactors (e.g., chemostats) where light sources could be embedded directly in, or surrounded by the cultivation vessels, thereby providing near-total light absorption (Sorokin and Myers, 1953; Krall and Kok, 1960; Matthern and Koch, 1964; Miller and Ward, 1966; Taub, 1974) [162, 101, 120, 124, 178]. These studies provided predictions of electrical power requirements ranging from ~10 kW to 100 kW of electrical power for lighting, and 5 to 50 m^2 surface area to produce enough oxygen for one human (Miller and Ward; 1966) [124]. Other algae and cyanobacteria were also studied, including *Anacystis*, *Synechocystis*, *Scenedesmus*, *Synechococcus*, and *Spirulina* (Miller and Ward, 1966; Taub, 1974) [124, 178]. At the same time as these US studies were occurring, Russian researchers both in Krasnoyarsk, Siberia (Gitelson et al., 1975; 1976) [60, 61] and in Moscow (Gazenko, 1967) [57] were conducting human life support studies using algal bioreactors and plants to provide O_2 in closed habitats, and I will expand on this below.

Much of the early work with algae focused on O_2 production for programs like Mercury and Gemini (F. Taub, personal communication). Unfortunately, the mass and power requirements for photosynthetic systems for O_2 generation did not "trade" well for short duration missions; however, the notion of using photosynthetic organisms to produce both O_2 and food did gain attention. But converting the algae to palatable foods proved challenging (Krauss, 1962; Fong and Funkhouser, 1982; Averner et al., 1984; Karel et al., 1985) [100, 53, 6, 88]. Many algae were too rich in protein and nucleic acids for a balanced diet, and many contained large amounts of indigestible cell wall materials (Gouleke and

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Oswald, 1964; Karel et al., 1985) [68, 88]. Other studies found that some algae and cyanobacteria produced phytotoxic volatiles, which compromised some closed life support studies in the early BIOS projects in Russia in the 1960s and 1970s (Gitelson et al., 1975; 1976) [60, 61].

Plants for Space Agriculture

Plants (crops) have been used for food by humans for millennia, and of course provide the same atmospheric regeneration functions as algae (Myers, 1954) [133]. Not long after NASA was formed in 1958, a "Biologistics Symposium" was held at Wright Patterson Air Force Base, Ohio, which produced a list of crops for dietary supplements on space missions (Boeing Comp., 1962) [24]. Selection criteria included the ability to grow under relatively low light intensities, compact size, high productivity, and tolerance to osmotic stress from NaCl (from urine recycling). This list included: lettuce, Chinese cabbage, cabbage / cauliflower / kale, turnip, Swiss chard, endive, dandelion, radish, New Zealand spinach, tampala, and sweetpotato (Boeing Comp., 1962; Gouleke and Oswald, 1964) [24, 68]. Despite these recommendations, with a few exceptions (Mansell, 1968) [116], testing with crops for life support in the US space program lay dormant through the 1960 and 70s. But significant improvements in production approaches for plants of high intensity discharge lighting systems to achieve higher light intensities (Cathey and Campbell, 1980) [33], plant spacing approaches to reduce wasted light (Prince and Bartok, 1978; Davis, 1985) [145, 44], use of hydroponic cultivation to eliminate water and nutrient stress (Resh, 1989) [150], and the practice of CO_2 enrichment to increase photosynthetic rates and yields (Porter and Grodzinski, 1985; Grodzinski, 1992) [144, 72]. This led to steady increases in productivities with plants / crops making them competitive with algae.

Pioneering Studies in Russia

Throughout this time, bioregenerative testing flourished in Russia as part of the BIOS projects in Krasnoyarsk (Gitelson et al., 1975, 1976, 1989) [60-62] (Fig. 1). The BIOS studies also included tests with human crews living in a closed environment, where they grew much of their own food and provided atmospheric regeneration with crops like wheat, and in some studies recycled nutrients and water (from urine and laundry water) back to the plants. At one point, nearly 100 researchers and staff worked on this project at the Krasnoyarsk Institute of Biophysics (J.Gitelson, personal communication). Over a period of about 15 years, three closed life support tests were conducted with human crews (two or three people) in which crops were grown in up to three 20.4 m^2 "phytotrons" (plant growth chambers) to provide much of the food and all of the oxygen (Gitelson et al., 1976, 1989; Salisbury et al., 1997) [61, 62, 156]. Algae (*Chlorella*) cultivators were used in some tests, and could produce up to 1800 L O_2 day^{-1}. But when the atmospheres were connected between algal chambers and the plant chambers, wheat growth was stunted and heads became sterile, potato and tomato plants stopped growing, cucumbers stopped flowering and leaves turned yellow, and beet leaves showed high anthocyanin accumulation (Gitelson et al., 1976) [61]. This suggested that there was some unidentified toxic volatile(s) produced by the algae. Because of this, subsequent BIOS studies (the BIOS-3 phase) in the late 1970s and 1980s focused on plants for photosynthetic production (Gitelson et al., 1989; Salisbury et al., 1997) [62, 156].

Continuous lighting for each crop phytotron in BIOS-3 was provided by 20, water-cooled, 6-kW xenon lamps, which provided up to 1000 µmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) at the plant level. For some tests, the number of lamps was doubled providing even higher light intensity. To my knowledge, these were some of first controlled environment agricultural systems to push plant productivities beyond recorded field yields (Gitelson et al., 1976; 1989; Salisbury et al., 1997) [61, 62, 156]. Wheat covered most of the planted area, with beet, carrot, dill, turnip, Chinese cabbage, radish, cucumber, onion, and sorrel used in early studies (Gitelson et al., 1976) [61], and chufa (nut sedge), pea, carrot, radish, beet, onion, dill, tomato, cucumber and potato used in later studies (Salisbury et al., 1997) [156]. Full crop stands produced around 1000 L of O_2 day^{-1} per 20.4 m^2 phytotron, with an estimated 7-9% conversion efficiency of the incident photosynthetically active radiation (PAR) into biomass, and a combined crop assimilation quotient (CO_2 removed/O_2 produced)

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Figure 1. Academician Iosif (Joseph) Gitelson and Professor Genrich (Henry) Lisovsky inside BIOS-3 facility at the Institute of Biophysics in Krasnoyarsk, Siberia (ca. 1989). Gitelson and Lisovsky were two of the founding researchers behind Russian and worldwide research on bioregenerative life support systems. Note the vertically mounted, 6-kW water-cooled xenon lamps hanging from the ceiling. Crews of 3 people lived in the facility up to 4 to 6 months, during which the plants provided up to 70% of the food, 100% of O2, 100% of CO2 scrubbing, and 100% of the water regeneration (photo and information courtesy of Joseph Gitelson, Advisor of Russian Academy of Sciences SB, with permission of Dr. Alexander Tikhomirov, Executive Director of Intl. Center for Closed Ecological System Studies, Institute of Biophysics).

of 0.94 (Gitelson et al., 1976) [61]. During a 2-month period of testing in the 1970s, two BIOS-3 phytotrons ($41m^2$ total) produced about 117 kg of plant dry mass, with 37.4 kg of it being edible. This required 20.6 kg of fertilizer salts and acid (along with about 5 kg of water of hydration in the salts) to be added to the nutrient solution (Gitelson et al., 1976) [61].

In addition to the crops grown inside BIOS-3 (daily average of about 220 g of dry grain and 388 g fresh vegetables), the human crews ate some stowed foods, such as meat to augment their diets (Gitelson et al., 1976) [61]. Carbon dioxide levels inside the BIOS-3 tests varied from 6000 to as high as 24,000 ppm, with an average concentration over 10,000 ppm (1%) (Gitelson and Okladnikov, 1994; Salisbury et al., 1997) [63, 156], demonstrating the potential for reaching super-optimal levels for both human and plants in tightly closed systems. Interestingly, chronic exposure of humans to very high CO_2 is now an area of concern in space biomedicine (Law et al., 2014) [104], and the effects of super-elevated CO_2 on crops has been an area of interest for space agriculture (e.g., Wheeler et al., 1993a; Grotenhuis and Bugbee, 1997) [194, 74]. The potential for directly recycling human urine to the wheat crops was tested in later studies and showed sodium accumulation

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in the nutrient solutions, which stabilized between 0.90 and 1.65 g L^{-1}. This had little effect on the wheat growth productivity for the time period it was tested (Lisovsky et al., 1997) [109], and demonstrated the ability to directly recycle nutrients and water from waste streams back to crops (Lisovsky et al., 1997) [109]. But recycling urine for longer periods might require the use of Na separation technologies or halophytic plants to avoid excessive Na accumulation (Subbarao et al., 2000; Tikhomirova et al., 2005; Yamashita et al., 2007; Qin et al., 2013) [169, 182, 205, 148].

While the group at Krasnoyarsk pursued larger scale, ground testing for space agriculture, other Russian researchers, especially at the Institute for Biomedical Problems (IMBP) in Moscow began testing how agriculture might actually get started in space settings like the Mir Space Station or the International Space Station (ISS). This led to the development of the “Svet” plant chamber that was used on the Mir Space Station to study wheat and other plants through whole production cycles (Bingham et al., 1996; Levinskikh et al., 2000; Sytchev et al., 2001; Salisbury et al., 2003) [19, 107, 171, 157]. The Svet chamber principles were then used to develop the smaller “Lada” plant chamber for IMBP to fly on the ISS (Bingham et al., 2003) [21]. The Lada supported a number of studies with wheat, pea, barley, and mizuna, as well as some of the first attempts to understand food safety issues for space-grown crops (Sytchev et al., 2007; Hummerick et al., 2010; Sugimoto et al., 2014) [172, 83, 170]. The Lada hardware was also used to study interactions of water and gas in granular media (mineral substrates) for space (Heinse et al., 2007; 2009) [80]. In addition, Yuliy Berkovich and colleagues at IMBP developed innovative approaches for volume efficient, plant growth conveyors that could be used for continuous food production in μ-gravity settings such as the ISS or Mars transit missions (Berkovich et al., 1998; 2004; 2009) [16-18]. Spiral shaped systems such as Phytocycle and Phytoconveyor could accommodate small seedlings at one end and then have larger plants ready for harvest at the other end. As part of this testing, light emitting diode (LED) lighting systems were also incorporated due to their flexibility for different spatial arrangements (Berkovich et al., 2004, 2009; Avercheva et al., 2014) [17, 18, 4], and I will expand on LED research later.

NASA Research

NASA revived its bioregenerative research with the start of the Closed (or Controlled) Ecological Life Support Systems, or CELSS Program ca. 1980 (MacElroy and Bredt, 1985) [113] and convened several workshops to assess what crops might be studied (Hoff et al., 1982; Tibbitts and Alford, 1982) [82, 180]. Crop lists at this time targeted broader nutritional needs of humans (e.g., carbohydrate, protein, and fat) and considered harvest index, food processing, and horticultural requirements. Crops common to most of these lists included: wheat, soybean, potato, rice, sweet potato, lettuce, and peanut (Hoff et al., 1982; Tibbitts and Alford, 1982) [82, 180].

NASA’s CELSS program expanded rapidly in the 1980s and was based largely at universities, with some research at NASA’s Ames Research Center (e.g., Schwartzkopf, 1985) [160]. Researchers included Frank Salisbury and Bruce Bugbee (Utah State Univ.), Ted Tibbitts (Univ. of Wisconsin), C. David Raper Jr. (North Carolina State Univ.), Cary Mitchell (Purdue Univ.), Walter Hill (Tuskegee Univ.), Harry Janes (Rutgers), and others. Several of these investigators joined a contingent of NASA program managers to visit the Russian BIOS-3 facility in Krasnoyarsk in 1989 (Fig. 2).

CELSS crop testing included wheat (Bugbee and Salisbury, 1988; Bugbee and Monje, 1992) [26, 27], soybean (Tolley-Henry and Raper, 1986) [183], lettuce (Knight and Mitchell, 1988; Barta and Tibbitts, 1991) [99, 10], potato (Wheeler and Tibbitts, 1986; Wheeler et al., 1991; Cao and Tibbitts, 1994) [191, 193, 35], sweet potato (Mortley et al., 1991, Bonsi et al., 1994) [129, 22], rice (Bugbee et al., 1994; Goldman and Mitchell, 1999) [28, 67], cowpea (Ohler and Mitchell, 1996) [139], peanut (Mackowiak et al., 1998; Mortley et al., 2000) [115, 132], tomato (McAvoy et al., 1989; Gianfagna et al., 1998) [121, 59], and various alliums (Jasoni et al., 2004) [86]. Experiments were typically carried out in growth chambers with electric lighting, using either hydroponics or solid growing media in pots. NASA researchers also studied the effects of CO_2 enrichment on crop growth and physiology (Wheeler et al., 1991; Bugbee and Monje, 1992; Mortley et al., 1996; Monje and Bugbee, 1998; Jasoni et al., 2004) [193, 27, 131, 125, 86]. In addition, extensive testing on crop responses to temperature, humidity, mineral

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Figure 2. NASA program managers and researchers visiting Krasnoyarsk, Siberia to meet with the BIOS-3 researchers in 1989. Back row, from left to right: Dr. Mel Averner (NASA Headquarter); Dr. Penny Firth (Lockheed Martin Corp), Dr. Ellen Baker (NASA Astronaut), Dr. Bill Knott (NASA Kennedy Space Center), Dr. Bob MacElroy (NASA Ames Research Center), Dr. Cary Mitchell (Purdue University), Dr. Herb Ward (Rice University), Dr. Tyler Volk (New York University). Front row: Dr. John D. Rummel (NASA Headquarters), Dr. Ted Tibbitts (University of Wisconsin), Vladimir Klimenko (interpreter), Dr. Frank Salisbury (Utah State University). Photo courtesy of John Rummel, East Carolina University, USA.

nutrition, PAR, photoperiod, even light spectral quality were conducted as part of the CELSS and subsequent Advanced Life Support programs (Bonsi et al., 1994; Bugbee and Monje, 1992; Cao and Tibbitts, 1991, 1994; Dougher and Bugbee, 2001; Frantz et al., 2000; Grotenhuis and Bugbee, 1997; Knight and Mitchell, 1988; Mortley et al., 1993; Wheeler et al., 1986; 1991) [22, 27, 34, 35, 49, 55, 74, 99, 130, 191, 193]. NASA funding to the University of Wisconsin's Center for Space Automation and Robotics (WCSAR) initiated testing of LEDs for use in the Astroculture plant chamber for the Space Shuttle (Bula et al., 1991; Barta et al., 1992) [31, 11]. This led to a patent for using LEDs to grow plants ca. 1990, and was followed by years of testing by Kennedy Space Center and other NASA funded groups (Tennessen et al., 1994; Tripathy and Brown, 1995; Goins et al., 1997, 2001; Schuerger et al., 1997; Kim et al., 2004, 2007) [179, 184, 65, 66, 159, 91, 92]. In the past 10 years, there has been a virtual explosion in the use of LED lighting in controlled environment agriculture, and this stands as an example of how research for space has benefitted terrestrial agriculture (Morrow, 2008; Massa et al., 2008; Avercheva et al., 2014; Mitchell et al., 2015) [128, 117, 4, 123].

As with the Russian BIOS studies, NASA's testing was most applicable to planetary surface settings, where gravity could assist water delivery

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and drainage (Bugbee, 1995) [29]. But NASA also funded testing of watering concepts for spaceflight (µg), such as the use of porous membranes or tubes for watering plants in space (Wright et al., 1988; Dreschel and Sager, 1989; Morrow et al., 1993; Heinse et al., 2007, 2009) [204, 50, 127, 80, 81]. These and other principles were considered in the design of a “salad machine” system that could be used to provide a source of fresh foods for astronauts on space stations or during Mars transit (Kliss and MacElroy, 1990; MacElroy et al., 1992; Kliss et al., 2000) [97, 114, 98]. The original “rack”- sized salad machine was never flown, but other smaller plant chambers such as the AstrocultureTM (ASC), Advanced Astroculture (ADVASC), Plant Generic Bioprocessing Apparatus (PGBA), Biomass Production System (BPS), and the current Veggie unit were flown by NASA, or NASA funded commercial groups (Zabel et al., 2016) [207]. Most of these chambers were used for gravitational research with plants, but all had capabilities for growing small amounts of food. Some studies were specifically focused on space agriculture, including the NASA collaborative testing with the Russians for growing wheat in the Svet chamber on Mir (Bingham et al., 1996; Levinskikh et al., 2000; Sytchev et al., 2001; Salisbury et al., 2003) [19, 107, 171, 157], the successful production of potato tubers using leaf cuttings in ASC on the Space Shuttle (Croxdale et al., 1997; Cook et al., 1998) [42, 38], and the growth of wheat in BPS to measure plant photosynthesis on the ISS (Monje et al., 2005; Stutte et al., 2005) [126, 168]. Expanded discussions of the Veggie plant unit for food production and crop research on the ISS are reviewed by (Massa et al. 2016) [118] in this issue.

Figure 3. NASA’s Biomass Production Chamber (BPC), which operated from 1988 to 2000 at Kennedy Space Center, Florida. Crops tested included wheat (upper left), potato (upper right), lettuce (lower right), soybean (lower left), tomato, rice and radish (not shown). All crops were grown using hydroponics (nutrient film technique) with higher pressure sodium and or metal halide lamps. NASA’s BPC was one of the first working examples of a vertical agriculture system. KSC researchers Neil Yorio and Lisa Ruffe are shown in the lower right panel. Photos provided by NASA.

Most of the NASA sponsored ground-based testing with crops was carried out in smaller growth chambers (e.g., ~1-4 m^2) with little testing conducted on a larger scale, like BIOS-3. This led to the development of the Biomass Production Chamber (BPC) at NASA's Kennedy Space Center, which operated from 1988-2000 (Prince and Knott, 1989) [146]. This was referred to as the Breadboard Project. The BPC provided 20 m^2 of growing area with a sealed atmosphere, similar to what might be encountered in space (Fig. 3). Plants in the BPC were grown hydroponically using nutrient film technique (NFT) on four shelves stacked vertically inside the 7.5 m high chamber. Unbeknownst to our group at the time, this was probably one of the first working examples of a vertical agriculture system (Prince and Knott, 1989; Goto, 2012) [146, 70]. Testing included four crops of wheat (about 86 days each), three crops of potato (about 105 days each), one test with four sequential potato crops that lasted for 415 days using the same nutrient solution; three crops of soybean (90 days each), four crops of lettuce (28 days each), two crops of tomato (85 days each), and exploratory tests with rice and radish (Wheeler et al., 1996a) [197] (Figs. 3). The sequential test of four potato crops in the same nutrient solution showed early tuber induction following the first planting, and confirmed observations from growth chamber studies, which showed the accumulation of an unidentified, tuberinducing or hormonal like factor in nutrient solutions (Wheeler et al., 1995; Stutte et al., 1999) [196, 167]. The BPC tests also allowed manipulations of light and CO$_2$ to assess transient changes on crop performance, measurements of light and CO$_2$ compensation points, and more (Wheeler et al., 1993b, 1996a, 2008) [195, 197, 201]. NASA BPC studies were some of the first to track whole canopy ethylene production rates by different crops, and showed that ethylene production occurred throughout normal growth and development, particularly during vegetative growth and rapid leaf expansion, as well as during climacteric fruit ripening with tomato (Wheeler et al., 1996b, 2004; see also Tani et al., 1996; Klassen and Bugbee, 2004) [198, 200, 176, 96]. The use of NFT hydroponic cultivation was also demonstrated on a large scale with potatoes (Wheeler et al., 1990; 1996a) [192, 197], and related NASA studies showed the NFT approach could work with other subterranean crops like sweet potato and peanut (Mortley et al., 1996; Mackowiak et al., 1998) [131, 115]. The NASA team of about 30 people supporting the BPC was led by Dr. William (Bill) Knott and included plant physiologists, horticulturists, microbiologists, chemists, agricultural / biological engineers, mechanical engineers, and computer scientists (Fig. 4).

Although yields from the BPC tests were good, they were typically less than the best yields measured from studies using smaller chambers (Wheeler et al., 1996a). This was an important observation and could have been related to several things: First, smaller plantings often have more pronounced edge effects from side lighting, which can increase yields. Second, the ability to provide close attention to individual plants typically diminishes with increasing system size due to time and logistics demands. Third, the effects of closure and build-up of volatile organic compounds likely had some negative effects on the crop yields in the BPC (Batten et al., 1995; Wheeler et al., 2004; Klassen and Bugbee, 2004) [15, 200, 96].

As with the Russians, NASA developed integrated, bioregenerative life support test capabilities for humans in closed systems. These tests were conducted at NASA's Johnson Space Center showed the one human's O$_2$ needs could be provided by as little as 11 m^2 of wheat grown at high light intensity (1500 µmol m^{-2}s^{-1}) (Edeen et al., 1996) [51]. This test was followed by a series of tests with four humans living in a closed chamber to test different life support technologies (Barta and Henderson, 1998) [12]. During a 91-day test, O$_2$ was produced, and CO$_2$ was removed by the 11 m^2 of wheat grown in a chamber that was atmospherically connected to the living habitat; this supported the air regeneration needs of one human, while the needs of the other three crew members were supplied by physico-chemical life support equipment (Barta and Henderson, 1998) [12]. In addition, a small plant growth chamber was placed in the human living habitat to allow the crew to grow fresh lettuce to supplement to their stowed foods (Barta and Henderson, 1998) [12]. This test also recycled nutrients recovered from inedible biomass of previous plantings using stirred-tank bioreactors (Strayer et al., 1998) [166]. The staggered planting approach revealed some challenges for growing different aged crops hydroponically on the same nutrient solution, where older plants tended to remove K and P quickly, causing nutrient deficiencies

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Figure 4. NASA's Advanced Life Support research team working at Hangar L at Kennedy Space Center, Florida in 1994. The Biomass Production Chamber is visible behind the group. Dr. Bill Knott, founder of Kennedy Space Center's life science research is the 12th person from the right side of the photo (see white arrow), directly behind Lisa Ruffe and Dr. Cheryl Mackowiak, horticultural researchers. The author is the fourth person from the right. Photo provided by NASA.

in younger plants (Barta and Henderson, 1998) [12]. The next step in this test sequence was to build a larger facility that could ultimately supply most of the life support needs for human crews using crops (Barta et al., 1999) [14]. This facility was called BIO-Plex and included two large agricultural modules (~80 m^2 each) with an efficient volume to area ratio of 2.3 m^3, m^{-2} = 2.3 m (Barta et al., 1999) [14]. For comparison, NASA's Biomass Production Chamber had 113 m^3 / 20 m^2 = 5.6 m. But BIO-Plex was never completed and NASA's large scale bioregenerative life support testing came to a halt ca. 2000.

NASA also supported efforts to develop concepts for greenhouse structures that might be deployed or connected to human habitats on planetary surface setting (e.g., Fowler et al., 2000; Wheeler and Martin-Brennan, 2000; Sadler and Giacomelli, 2002; Bucklin et al., 2004; Rygalov et al., 2004; Kacira et al., 2012) [54, 199, 154, 30, 152, 87]. Such concepts could use electric lighting, or sunlight captured directly by structures, or by collectors and then delivered by fiber optics to protected habitats (Cuello et al., 2000; Nakamura et al., 2009) [41, 134]. Related testing with plant growth systems inside isolated settings such as the US Antarctic South Pole Station were also conducted, which provided a good analog for isolated settings in space (Sadler, 1995; Patterson et al., 2008) [153, 142].

During the period of active research with regenerative life support systems, including space agriculture, the journal Life Support and Biosphere Science (ca. 1994-2002) was published as an outlet for

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various life support and space related articles with Dr. Harry Janes of Rutgers University as the Editor. The name of the journal was later changed to Habitation. Although these journals are no longer published, they provide a valuable archive of bioregenerative and controlled environment agricultural research from the 1990s and early 2000s.

Biosphere 2

Certainly one of the most impressive efforts ever to study humans and closed ecological systems was the privately sponsored Biosphere 2 facility, designed and constructed near Tucson, Arizona, US in the late 1980s and early 1990s (Alling et al., 2005) [1]. The atmospherically closed structure was nearly 1.2 ha in area and contained human living quarters, multiple ecosystems with a wide range of plants and animals, complex environmental management and control capabilities, including sophisticated pressure damping systems to reduce leakage, and a large agricultural area of approximately 2000 m^2 with 2720 m^3 of soil, which provided about 80 percent of the food for the eight humans living inside the facility for 2 years (Alling et al., 2005) [1]. The scale and complexity of Biosphere 2 was larger than what most space agencies might envision for early missions, but their goals of understanding closed ecological systems and bioregenerative approaches for human life support provided insights into the challenges for agricultural and biological approaches for space life support. The Biosphere 2 group still continues their testing with smaller, laboratory scale modules and have studied crops such as pinto bean, cowpeas, sweet potato and wheat in closed systems (e.g., Nelson et al., 2005, 2008) [135, 136], and their undertaking has been discussed and emulated by various groups around the world.

Space Agriculture around the World

Japan

At about the same time as planning for NASA's BIO-Plex facility was taking place, Japanese researchers working with Dr. Keiji Nitta began development of the Closed Ecological Experiment Facility (CEEF) in Aomori Prefecture (Ashida and Nitta, 1995; Nitta et al., 2000) [5, 138] (Fig. 5).

CEEF was part of the Institute for Environmental Sciences (IES) developed to track radio isotopes through closed ecosystems. When not in use for their primary studies, the facilities could be utilized for

Figure 5. Japanese Closed Ecological Experiment Facility (CEEF) team in 2005. Dr. Keiji Nitta, group founder and lead is second from the right in the front row, and Dr. Yasuhiro Tako, CEEF plant research lead is third from the left in the front row. CEEF was used to conduct a series of human life support tests where crops provided the O2 and water, and nearly all of the food for the two humans and two miniature goats (Tako et al., 2008, 2010). Photo courtesy of Yasuhiro Tako, CEEF.

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studies of controlled environment agriculture and human life support (Nitta et al., 2000; Tako et al., 2001, 2008, 2010) [138, 173-175]. CEEF researchers designed complete diets from crops grown in 150 m^2 of the plant cultivation modules (Masuda et al., 2005; Tako et al., 2010.) [119. 175]. Some areas used natural sunlight with supplemental electric lighting, while others only used electric lamps, such as high pressure sodium (Tako et al., 2010) [175]. Two-person crews lived inside the facility for 1-week, 2-week, or 4-week tests, eating foods grown inside the facility (Masuda et al., 2005; Tako et al., 2008, 2010) [119, 174, 175]. Rice, soybean and peanut were some of the major crops used for these studies (Fig. 6).

In addition to the humans, two miniature goats were enclosed in the system and were fed inedible parts (leaves and stems) of the crops (Tako et al., 2008, 2010) [174, 175]. Findings from CEEF documented differences in assimilation or photosynthetic quotients (CO_2 fixed/O_2 produced) of carbohydrate crops like rice (AQ = 0.95) and fat/protein producing crops like soybean (AQ = 0.87) (Tako et al., 2010) 175]. Such differences had been known for many years from algal studies (Krall and Kok, 1960; Miller and Ward, 1966) [101, 124] but were never clearly measured for plants. As with the Russian and NASA controlled agriculture testing, the Japanese CEEF used gravity dependent watering concepts that were targeted for planetary surface settings.

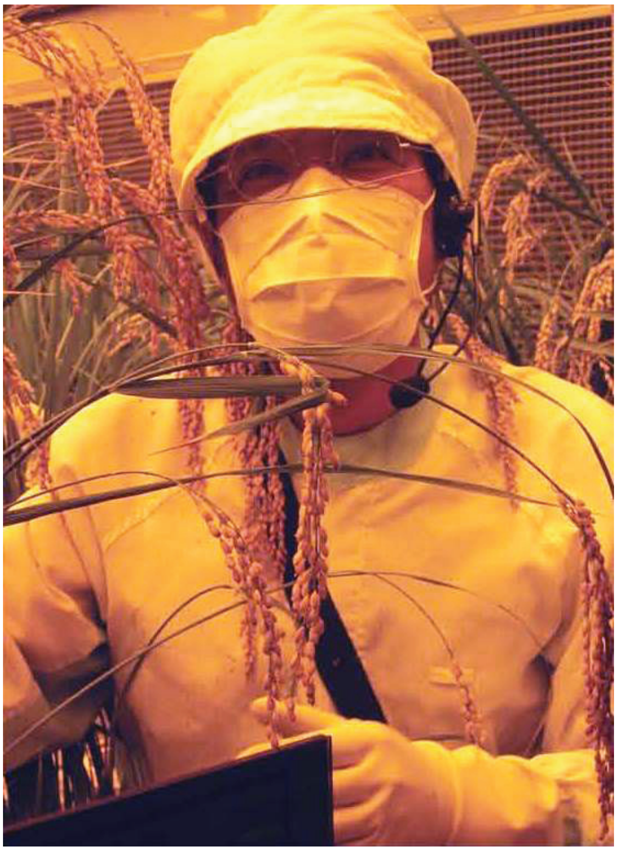

Figure 6. “Econaut” crew member of the Closed Ecological Experiment Facility (CEEF) team in Japan tending rice plants inside the facility (2007). Two Econauts and two miniature goats lived inside the facility for periods of up to 4 weeks, where ful5l life support, including a near-complete diet was provided by plants. Notice the yellowish orange light from the high-pressure sodium lamps used for crop light (photo taken by the author).

In addition to CEEF, there was widespread interest in Closed Ecological Life Support Systems (CELSS) research throughout Japan (Nitta and Yamashita, 1985) [137], and for many years the CELSS Journal (1989-2001) served as an outlet for regenerative life support and space agriculture research (Kibe and Suzuki, 1997) [90]. The journal name was later changed to the Eco-Engineering (2001-present) to broaden its scope of topics. Japanese studies related to space agriculture included plant responses to hypobaric pressures (Goto et al., 1996, Iwabuchi et al., 1996, 2003) [69, 84, 85], CO_2 and trace gas management (Tani et al., 1996) [176], lighting and air movement (Kitaya et al., 2003; Kitaya and Hirai, 2008) [93, 94], innovative cultivation approaches (Kitaya et al., 2008) [94], studies of salt tolerant plants (Yamashita et al., 2007) [205], and the potential for using insects such as silk worm or termites to convert inedible biomass to foods (Katayama et al., 2008; Wada et al., 2009) [89, 186], just to mention a few. I would encourage the readers to go through the many research articles related to space agriculture in the Japanese CELSS Journal and Eco-Engineering.

Europe

In Europe, there were pioneering studies to grow crops in atmospherically closed chambers to quantify crop photosynthesis, respiration, and transpiration (Gerbaud et al., 1988; Andre et al., 1989) [58, 2], and some of the first studies of plant growth under hypobaric conditions (Andre and Massimino, 1992; Daunicht and Brinkjans, 1992) [3, 43]. In 1987, the European Space Agency initiated its Micro-Ecological Life Support System Alternative (MELiSSA) project to test life support concepts based on ecological principles for materials cycling (Mergeay et al., 1987)

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[122]. Much of the initial MELiSSA testing focused on waste processing using microbial systems, with photosynthetic bacteria or cyanobacteria for biomass production (Lasseur et al., 1996; Godia et al., 2004) [102, 64]. Over the following years, MELiSSA expanded to include plants for a controlled environment agriculture compartment, which could be coupled to the microbial and cyanobacteria compartments (Waters et al., 2002; Godia et al., 2004) [189, 64]. MELiSSA studies also tested remote sensing to monitor crop stress (Chaerle et al., 2007; Lenk et al., 2007) [37, 105], crops such as beet and durum wheat, comparisons of soybean cultivars in controlled environments (Stasiak et al., 2003, 2012; De Micco et al., 2012; Paradiso et al., 2012) [164, 165, 45, 140], tests of hydroponic cultivation techniques (Paradiso et al., 2014) [141], and studies on recycling of plant wastes in collaboration with the Institute of Biophysics in Krasnoyarsk, Russia (Tikhomirov et al., 2003; Gros et al., 2004) [181, 73]. As with many other space agencies, ESA also developed strategies to transition ground based testing of agriculture into actual spaceflight settings, such as the International Space Station (Wolff et al., 2013) [203], and is currently planning upgrades to their European Modular Cultivation System (EMSC) to support biogenerative testing on the ISS (A-I. Kittang Jost, personal communication).

European companies such as Aero Sekur (Rossignoli, 2016) [151] and Thales Alenia have also enthusiastically supported space agriculture through the biennial AgroSpace Workshops held in Sperlonga, Italy, and through their own internal research and development efforts (Lobascio et al., 2006, 2008) [111, 112]. More recently, the German Space Agency (DLR) life sciences team at Bremen has become an active and energetic group in the space agriculture arena (Schubert et al., 2011; Zabel et al., 2016) [158, 207]. Their efforts along with a consortium of University and Industrial partners through the European Union funded EDEN ISS project are focused on deploying a plant growth system chamber to the Antarctic Neumayer Station III to grow fresh food for the crew. In addition, they are designing a rack-based plant growth system for possible use on the ISS. Among other things, analyses by this DLR led group have pointed out the many similarities between intensive space agriculture systems and terrestrial approaches that might be used for vertical agriculture (Schubert et al., 2011) [158].

Canada

As noted earlier, several groups in Europe and Japan conducted studies on the effects of atmospheric pressure on plants, as did several NASA researchers (Schwartzkopf and Mancinelli, 1991; Corey et al., 1997, 2002; Rygalov et al., 2004; He et al., 2007; 2009) [161, 39, 40, 132, 78, 79]. Pressure is not a typical concern for terrestrial agriculture, yet it is critical for space settings. It is not a "given" that human space habitats will operate at 1 atmosphere pressure (~101 kPa) and NASA's Gemini and Apollo spacecraft, and NASA's Skylab Space Station of the 1970s operated at 34 kPa pressure (Lange et al., 2005) [103]. This allowed quicker access for extravehicular activities (EVAs or space walks) with no "pre-breathing" or acclimation period. For planetary missions where EVAs might be frequent, reduced cabin pressures could also save on gas loss during air lock events. This unique niche of space environmental control led to the development of a highly specialized facility at the University of Guelph in Ontario, Canada in the late 1990s (Dixon and Schmitt, 2001; Chamberlain et al., 2003; Bamsey et al., 2009) [46, 36, 7]. The facility includes multiple 1.5 m^2 chambers for growing plants under a wide range of pressures, light, temperature, humidity, and CO_2 (Fig. 7), and numerous smaller hypobaric chambers. Pressures can go as low as 1-2 kPa with plants and water inside, while still holding temperature and humidity, and the closed atmosphere of the chambers allows close tracking of whole canopy gas exchange rates at the different pressures (Chamberlain et al., 2003) [36]. Testing included studies of pressure effects on plant gene expression (Paul et al., 2004) [143], plant biochemical responses (Levine et al., 2008) [108], whole plant growth and development studies (Wehkamp et al., 2012) [190], and more.

Results showed that radish plants could grow at pressures as low as 10 kPa provided pO_2 was kept above 7 kPa (Wehkamp et al. 2012) [190], which were consistent with findings of He et al. (2007) [78]. This demonstrates the potential for using reduced pressure systems for space agriculture. These findings emphasized the need for unique research capabilities such as those at the University of Guelph to support space agriculture research. The chambers could also be used for rapid decompression tests with plants to assess system risks and failures. Studies showed that wheat, radish, and lettuce could withstand decompression down to ~1.5 - 2.0 kPa for up to 30 min

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Figure. 7. Hypobaric plant test chamber plant test chambers located at the University of Guelph, Ontario, Canada (left). Dr. Mike Staskiak (right) of the University of Guelph removing trays of radishes grown at 33 kPa total pressure. Photos courtesy of Dr. Tom Graham, University of Guelph.

with no apparent damage (Wheeler et al., 2008) [201] Thus, plants could survive a catastrophic pressure loss that would be lethal to humans. The Guelph studies also examined new crops and cultivars for space agriculture (Waters et al., 2002; Stasiak et al., 2003, 2012) [189, 164, 165], innovative light sources and light delivery concepts (Stasiak et al., 1998) [163], ion specific sensors for controlling hydroponic systems (Bamsey et al., 2012) [8], plant CO_2 responses (Grodzinski, 1992) [72], and plant production systems in harsh, high latitude settings such as Devon Island (Bamsey et al., 2012) [8].

China

One of the most recent developments in the space agriculture community has been the construction and testing of the Chinese Lunar Palace 1, located at Beihang University in Beijing. Under the leadership of Prof. Hong Liu, her team (Fig. 8) designed and constructed a closed ecological life support system containing human, plant, insect, and microbial components. Compared to the closed system tests in Russia, USA, and Japan, Lunar Palace 1 was perhaps to most complex in terms of its biological components, integrating plant cultivation, animal protein production, and microbial bioconversion of solid wastes into soil-like substrates for growing plants. During a 105-day test period in the Lunar Palace 1 (Fig. 9), 100% oxygen and water, and 55% of the food requirements for three crewmembers were regenerated using the controlled environment agriculture, surpassing the duration and overall system closure of any prior, related tests with bioregenerative systems (Fu et al., 2016) [56]. The agricultural module used state of the art red and white light emitting diodes (LEDs) (Dong et al., 2014a) [47] with a growing area of 69 m^2, spread across vertically arranged shelves with hydroponic growth systems - another use of vertical agriculture (Fu et al., 2016) [56].

As with other groups, fundamental testing of crop responses to light, CO_2, and mineral nutrition were conducted prior to the closed Lunar Palace 1 study by the Chinese (e.g., Wang et al., 2015a, 2015b; Dong et al., 2014b) [187, 188, 48]. In addition to Lunar Palace 1, other groups in China have been involved in controlled environment testing for space agriculture, including tests of salad crops (Qin et al., 2008) [147], the effects of salt stress on crops (Qin et al., 2013) [148], crop growth under LED lighting (Ren et al., 2014) [149], effects of hypobaric pressures on crops (Tang et al., 2010) [177], and a test (180 days) with four humans and crops in a hermetically sealed environment has just recently been completed by another group (http://english.cctv.com/2016/12/14/VIDEvMLAnbgqUGqAdZnis9lU161214.shtm).

Figure 8. Lunar Palace 1 research team, Beihang University, Beijing, China. Prof. Hong Liu, team lead is at the center of the photo, second row wearing a red ribbon and medal. The Lunar Palace 1 supported three humans for 105 days using bioregenerative life support technologies, with crops grown under LEDs providing the air, water, and most of the food. Photo courtesy of Hong Liu, Beihang University.

Figure 9. Dr. Chen Dong of Beihang University inspecting wheat plants inside the Lunar Palace 1 facility in Beijing, China. Note the pinkish colored light from high output red and white LEDs to grow the crops that supported three crew members for 105 days. Photo courtesy of Prof. Hong Liu, Beihang University.

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Some Concluding Thoughts

The use of agriculture for human life support in space has been one of the longest standing areas of space research, and has provided an intellectual and collegial bridge between the aerospace and agricultural communities. Numerous ground studies have shown that crops can regenerate air, recycle water, and produce much of the needed food for humans living in closed systems. But to succeed, space agricultural systems must be highly closed and efficient, where energy use is minimized, and air, water, and nutrients are recycled as much as possible. Understanding the complexities of diverse cropping systems and their associated microbiomes will also be required. This has created a productive synergy between terrestrial and space CEA systems. Studies for space agriculture have documented crop yields far greater than yields reported from the even most productive field settings, suggesting there is still untapped potential from our field crops. Recirculating hydroponics with efficient water use and minimal nutrient discharge have been demonstrated for multiple crops, including crops like potato and sweet potato. Measurements of photosynthesis, respiration, and transpiration rates for whole crop communities, as well as the production of volatile organic compounds like ethylene, have generated novel data, including the effects of transient and long-term perturbations to crops. Because lighting and energy conversion are key for growing the crops, space systems pioneered the use of LEDs for crops, which have now achieved remarkable electrical conversion efficiencies and are being used for CEA around the world. Volume constraints of space have driven selection and development of shorter crops with high harvest indices, which along with the hydroponic advances and use of energy efficient LEDs have applications for vertical agriculture and plant factories on Earth. The ability to control CO_2 and closed systems has provided insights into what the future might hold for terrestrial agriculture with rising CO_2. This decades-long effort has come from a global community of dedicated and enthusiastic researchers, who will one day literally have the seeds and fruits of their labor growing on other planets.

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Dedication: Professor Frank B. Salisbury (Utah State University) died on 26 December 2015. Frank was an avid researcher, accomplished photographer, and eloquent writer who authored 100s of research papers and numerous books, including plant physiology textbooks used by botany students around the world for nearly two decades. He was curious about many things in nature, and especially plants and their environments. This led Frank and colleagues to propose building a test facility for space agriculture at Colorado State University in the late 1950s (personal communication). But NASA was not ready for it at the time and the grant was never awarded. That didn't deter Frank from pursuing his interests in space agriculture, including leading the first efforts to grow wheat crops on the Mir Space Station. I would recommend reading Frank's paper on "Lunar Farming" in HortScience magazine (Salisbury, 1991) [155] to appreciate his unique skills as a writer and scientist. Frank was my graduate research advisor and it was through Frank that I had the good fortune to connect with the space agriculture community. We will all miss Frank's insights and contributions to space biology, and to all of plant physiology.

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