



Large-Scale Crop Production for the Moon and Mars: Current Gaps and Future Perspectives

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Poulet L, Engeling K, Hatch T, Stahl-Rommel S, Velez Justiniano Y-A, Castro-Wallace S, Bunchek J, Monje O, Hummerick M, Khodadad CLM, Spencer LE, Pechous J, Johnson CM, Fritsche R, Massa GD, Romeyn MW, O'Rourke AE and Wheeler RW (2022) Large-Scale Crop Production for the Moon and Mars: Current Gaps and Future Perspectives. Front. Astron. Space Sci. 8:733944. doi: 10.3389/fspas.2021.733944 In this perspectives paper, we identify major challenges for space crop production: altered convection in the microgravity environment, scheduling and logistics, crew time and the need for advanced automation, robotics, modeling, and machine learning. We provide an overview of the existing space crop production gaps identified by the Kennedy Space Center (KSC) space crop production team and discuss efforts in current development in NASA projects to address these gaps. We note that this list may not be exhaustive but aims to present the baseline needs for space crop production implementation and a subset of current solutions to the greater scientific community in order to foster further ingenuity.

Keywords: automation, microgreens, plasma agriculture, microbiology, nutrient delivery system, reduced gravity, hydroponic, aeroponics

1 INTRODUCTION

As humanity ventures into the Solar System and settles on other planetary bodies, movement towards independence from Earth to produce consumables such as food, oxygen, and water will be crucial. *In-situ* food production will depend on biological organisms such as plants and to a lesser extent, unicellular organisms. Large-scale crop growth systems included in bioregenerative life-support systems (BLSS) will allow for resource recycling and minimize resupplying from Earth. Several projects have aimed to identify the requirements for Lunar and Martian greenhouses, by assessing their feasibility, and developing concepts, theoretical designs, or prototypes at different scales and maturity levels (Wheeler and Martin-Brennan 2000; Stutte et al., 2009; Boscheri et al., 2016; Furfaro et al., 2016; Zeidler et al., 2017). KSC has a long history in controlled environment crop research. This began with the Biomass Production Chamber (BPC), extends to on-orbit platforms such as Veggie and the Advanced Plant Habitat (APH) and will continue with Ohalo III (a crop production chamber currently in development), with each platform deliberately selected and designed to lead to future crop production units destined for the Moon or Mars (Figure 1).



FIGURE1 | Examples of KSC's prior, current and future space crop production platforms selected and designed to lead to crop production units destined for the Moon or Mars.

Larger-scale platforms will bring new engineering challenges and needs. A set of logistical and physical obstacles have been identified as informed by the lessons learned from KSC's space crop production efforts on the International Space Station (ISS) using small scale platforms.

2 SPACE CROP PRODUCTION GAPS AND NEEDS

Space crop production efforts utilize the lessons learned from growing plants on the ground and in orbit. This knowledge, in combination with the expertise across NASA centers, academia, and industry serves to provide a framework for crop production systems for deep space travel (Johnson et al., 2021). Prior work conducted in plant chambers aboard the Shuttle and ISS has helped identify knowledge gaps and technology needs for space crop production (**Supplementary Table S1**). Throughout the remainder of this perspectives paper, we present a sampling of the current technologies being explored at NASA centers to address specific gaps, and we discuss their associated limitations and challenges.

2.1 Water and Nutrient Delivery: Challenges, Needs, and Current Research

Plant testing on the Shuttle and ISS revealed challenges with water and nutrient delivery. The lack (or reduction) of buoyancy-driven convection and the domination of surface-tension forces in reduced gravity leads to thick boundary layers of water (respectively air) forming around plant roots (respectively leaves) (Kitaya et al., 2003; Jones et al., 2012). Without appropriate water and nutrient delivery systems, roots suffer from hypoxia. Although substrate-based watering systems using capillary forces to guide water to the roots without suffocating them, are currently used on the ISS (e.g. Veggie and Advanced Plant Habitat (APH)) (Zabel et al., 2016), substrate-free or reduced-substrate water and nutrient delivery systems are being considered for future missions to reduce upmass and waste (Morrow et al., 2017). We present recent work investigating spaceflight testing of a hydroponics system and an Earth-based analog demonstration of aeroponics.

2.1.1 Substrate-Less Growth Systems

Space crop production efforts to date have focused on substratebased approaches mainly using arcillite for plant growth, for proof of concept demonstration of crop growth (Massa et al., 2018). The approach is similar to terrestrial methods, yet as discussed above, watering proves a challenge in the microgravity. Among water solutions for substrate-based growth methods is a passive watering system named Passive Orbital Nutrient Delivery System (PONDS) where plants will draw from a free-standing reservoir of water (Levine et al., 2021). Recently, NASA has tested on-orbit a substrate-less hydroponics approach known as Plant Water Management (PWM). The PWM uses capillary geometry to contain poorly wetting aqueous solutions (e.g., sugary or nutrient-rich water), and provide sufficient aeration and hydration for low-g hydroponics (Mungin et al., 2019). Initial low-g data analysis showed that this could be a viable method, and further testing will include the use of space crops rather than the current simulated plants.

Furthermore, KSC's ongoing collaborative work with the German Aerospace Center (DLR) Institute of Space Systems in the analog extreme environmental setting of the EDEN ISS greenhouse facility at the Neumayer Station III site in Antarctica, has provided precedence for using an aeroponics approach (Zabel et al., 2017). Aeroponic nutrient delivery and a modified ebb/flow technique for plant growth systems in microgravity will also be evaluated using the eXposed Root On-Orbit Test System (XROOTS) (Morrow et al., 2017). In addition, the Porous Tube Nutrient Delivery System (PTNDS), which utilizes suction to hold plants seeded onto a porous ceramic water delivery tube, is in current development with additional watring approaches under development by university and industry researchers (Monje et al., 2019).

2.1.2 Fertilizer/Nutrient Supply: Plasma Activation of Water

In the realm of nutrient delivery, a recent and interesting development in plasma agriculture (use of plasma, the fourth state of matter, to generate reactive species that interact chemically) is the plasma activation of water (PAW). For PAW, the introduction of plasma to water is able to change its properties (Foster et al., 2018). Plasma research is underway at KSC to explore the use of low temperature plasma to treat water, serve as a microbial sanitization method for hardware, and to explore plasma introduction of NOx groups and changes in pH for fertilizer-like applications. There is yet to be a complete understanding of plasma water chemistry, with prior and ongoing research at UC-Berkeley (Graves 2012) and recent modeling efforts at the University of Michigan (Kruszelnicki et al., 2019).

2.2 Crop Cultivation: Challenges, Needs, and Current Research at KSC

Volume is a limited resource in space, and future crop production systems will need to use available growth space effectively. In microgravity, plants can grow in all dimensions, thus making use of the entire available volume. Trade-off studies between monocrop and multicrop cultivation will help to determine if growth units will benefit from one or multiple crop species (Boscheri et al., 2016; Zeidler et al., 2017). To use volume efficiently, crop scheduling is also key. Depending on the growth cycles of individual species, crop planting could be staggered to meet dietary needs of the crew (Zeidler et al., 2017).

Together, NASA and the United States Department of Agriculture (USDA) are investigating the use of microgreens, plants that are small in size, nutrient-dense, and quick to grow with little crew time. Ready-to-eat plants like microgreens are easy to maintain and the rich nutrient and phytochemical composition of microgreens can help mitigate spaceflightinduced health risks (Anderson et al., 2017; Cooper et al., 2017). Microgreens have a high-volume optimization potential, and efficiently use light, fertilizer, and water, all of which are important selection criteria for plants grown in space (Romeyn et al., 2019). These crops are harvested one to 3 weeks after planting, when the cotyledons are fully expanded, and the first true leaves have emerged (Xiao et al., 2012; Xiao et al., 2016). The short growth cycle of microgreens adds flexibility to crop systems, especially if disruptions occur. Microgreen production would require more seeds than full size crop production, but seeds are small and light, and the mass of seeds is largely offset by the reduced mass of fertilizer and their small volume requirements. An upcoming project at KSC will work to quantify this trade-off.

2.3 Environmental Monitoring Platform: Stress Detection and Food Safety Technologies

Studying plant growth in simulated and actual space flight environments, will enable the development of plant growth mechanistic and knowledge models. Such models can account for the combined physical, biochemical, and morphological phenomena involved; a necessary tool to accurately control and predict plant growth in life-support systems (Hézard et al., 2010; Poulet et al., 2020). In addition, we aim to achieve an advanced level of autonomy by implementing an integrated machine learning approach to monitor and coordinate space crop production systems, including the plant compartment atmospheric conditions, plant and microbiome genomic and metabolomic trends, water system microbiome and chemistry, and biomass recycling. Crew time for crop cultivation in future missions will be limited, as the primary activity of astronauts will be to accomplish mission tasks (Russell et al., 2006). Future space crop systems will need to be less crew time-intensive than current systems (Poulet et al., 2021; Zeidler et al., 2021) and utilize automation (e.g., watering or health monitoring). Small space crop production chambers on ISS, such as Veggie and the APH, can be used to validate and inform automation efforts, such as disease detection by imaging.

2.3.1 Hyperspectral Imaging

Monitoring plant growth and health during the entire life cycle of a crop is needed to ensure food safety of crops eaten by astronauts during cis-lunar, lunar and Martian missions (Anderson et al., 2017; Monje et al., 2019). Hyperspectral imaging can collect relevant spectral data for developing suitable vegetation indices, providing non-destructive and autonomous estimates of plant health with minimal crew time (Gowen et al., 2007; Araus and Cairns 2014; Huang et al., 2018; Zeidler et al., 2019). Early identification of plant responses to nutrient deficiencies, drought, flooding, or microbial/fungal infections, will provide the crew with enough time to mitigate these problems (Kim et al., 2001; Gowen et al., 2007). To this end, a prototype Plant Health Monitoring (PHM) system was developed by the USDA Environmental Microbial and Food Safety Laboratory (EMFSL, Beltsville, MD) and is in operation at KSC (Monje et al., 2021). The PHM is being used to develop a database of images from plants exposed to abiotic (e.g. drought) or biotic (e.g. fungal infection) stresses. This database will be used to develop suitable

vegetation indices for autonomous early stress detection and for training future AI algorithms for plant health monitoring and to ensure food safety. In the future, miniature imaging systems will be deployed for monitoring plant health and microbial composition remotely in spacecraft such as ISS, Gateway, and Deep Space Transport.

2.3.2 Microbial Sequencing

Molecular methods have been essential for understanding plantassociated microbiomes (Bulgarelli et al., 2012; Chaparro et al., 2014; Knief 2014) and plant pathogens (Pecman et al., 2017; Díaz-Cruz et al., 2019; Piombo et al., 2021). Monitoring the microbes present in the spaceflight environment has been important since Apollo, but with short mission durations, not practical to perform during flight. For the ISS, requirements were generated to ensure that microbial contamination was periodically assessed in the ISS air, water, and on surfaces (Castro et al., 2004; Yamaguchi et al., 2014). From the beginning of plant growth on ISS, plants and plant growth hardware have been routinely sampled to understand the plant microbiome interaction and pathogen control in supplemental food production (Hummerick et al., 2010, Hummerick et al., 2011, Hummerick et al., 2012; Massa et al., 2017b; Khodadad et al., 2020). Veggie plant samples have been evaluated by culture-based isolation and identification, as well as microbiome analysis using Next Generation Sequencing on the Illumina MiSeq platform upon return to Earth (Khodadad et al., 2020). Such assessments of the microbiological food safety of crops grown on orbit has led to the development of a hazard analysis critical control point plan (HACCP) for space crop cultivation (Hummerick et al., 2011; 2012). The goal for spaceflight microbiology is to move beyond the need to culture potentially harmful microorganisms and to this end, culture-independent, swab-to-sequencer processes using the Oxford Nanopore MinION are now conducted onboard the ISS (Stahl-Rommel et al., 2021).

Future paths to assess plant-microbe interactions in the space crop production environment can benefit from the development of system level approaches where the power of automated sensing through imaging, omics (genomics, transcriptomics) and spectrometry (metabolomics, nutrients, volatiles) is realized. This surveillance, in combination with correlative and machine learning techniques, will allow for the detection of stress indicators, plant and human pathogens, reduced nutrient content, or the formation of health-hazardous volatiles, respectively.

2.4 Microbiome Ecosystem-Related Needs 2.4.1 Microbial Control Solutions for Watering Systems

Microbial biofilms have been a cause for concern in ISS biodeterioration, and as such, represent a risk to safe long-term human space exploration (Landry et al., 2020). Research into the effects of spaceflight on microbes has unveiled changes in virulence and biofilm formation under microgravity conditions (Kim et al., 2013), further underlining the need to develop robust biofilm management solutions (Landry et al., 2020). NASA continues to explore treatment methods such as biocides,

antimicrobial surfaces, nutrient filters, applied shockwaves, thermal and UV treatments (Velez-Justiniano et al., 2020). Biofilm management studies explore the use of other biological agents (such as bacteriophages or plasmids) to balance the ISS Water Processor Assembly (WPA) ecosystem (Sillankorva and Azeredo 2014) and even consider plasma-based approaches. The identification of the best solution for microbial control can be tailored to the relevant platform, be this a space crop production chamber, or the water processing system used for downstream irrigation.

2.4.2 Plasma for Seed Sanitization

To avoid contamination by plant or human pathogens in space crop production platforms, seed surface sanitization is carried out before flight. Traditional methods of seed sanitization on the ground include alcohol soaking and chemical gas fumigation (Khamsen et al., 2016; Massa et al., 2017a), but these methods are not effective for all seeds. Plasma treatment of seeds, surfaces, or containers inoculated with fungi or bacteria have demonstrated significant log reductions after exposure (Filatova et al., 2009; Takemura et al., 2014; Puligundla et al., 2017a, Puligundla et al., 2017b; Zahoranová et al., 2018). The effectiveness of seed or surface sanitation may vary greatly based upon the plasma source and plasma gaseous medium being employed. Preliminary work is being conducted at KSC to test space crop production seed sanitization using different plasma sources and their effects on the associated seed microbiome.

3 DISCUSSION

3.1 Hyperspectral Imaging Current Challenges and Prospects

Although hyperspectral imaging enables precision plant health monitoring and early detection of diseases, it is currently limited by several factors. Data collection and analysis is dependent on the conditions in which measurements were performed, such as leaf orientation, illumination, or even leaf texture (Mahlein, 2016; Zeidler et al., 2019). Currently, spectral vegetation indices (SVI) are tied to specific wavelengths and obtained with specific environmental conditions and plant species, which means data to define SVI need to be collected in a high-fidelity environment. It is hard to apply SVIs to different conditions and plant types without risking data misinterpretation (Lowe et al., 2017; Thomas et al., 2018). The amount of data produced is large and therefore requires the development of efficient data handling and analysis methods (Mahlein, 2016; Thomas et al., 2018). For space applications, this raises the issue of telemetry limitations, which translates into the need for in situ processing. However, deep learning algorithms seem to be a promising solution for hyperspectral data analysis and interpretation (Thomas et al., 2018; Nagasubramanian et al., 2019), provided that they can be trained on a big enough dataset (Zeidler et al., 2019).

3.2 Substrate-Free vs. Substrate-Based

Alongside substrate-less systems presented in section 2.3.1, substrate-based systems using lunar or Martian regolith are

being investigated (Wamelink et al., 2019; Eglin and Guinan, 2020; Eichler et al., 2021; Fackrell et al., 2021). Plant growth experiments have been conducted with returned Moon material (Ferl and Paul, 2010) and Wamelink et al. (2019) have successfully grown nine different species in JSC-1A regolith simulant mixed with organic material and further projects, such as the Mars Gardens, have compared this regolith simulant approach to hydroponic growth methods (Eglin and Guinan, 2020). Among the concerns raised when cultivating plants in regolith was the fact that it cannot be used as is; harmful compounds (e.g., perchlorates) need to be removed from Martian regolith and organic materials need to be added (Eichler et al., 2021). Furthermore, geomicrobiological methods have the potential to modify regolith to enable its utilization for plant growth. In this scenario, microorganisms are used to create soil from Lunar and Martian regolith (Verseux et al., 2016). Previous studies on plant growth in regolith simulants may have overlooked some important chemical composition aspects. However, the recent development of agriculturally relevant Martian regolith will enable more accurate studies (Fackrell et al., 2021). For the near term, hydroponic systems may prove a better option that is more amenable to a BLSS infrastructure. Hydroponic systems provide efficient absorption of nutrients as roots are directly in contact with the nutrient solution (Eglin and Guinan, 2020) and easy monitoring for subsequent control of the root zone microbiome.

3.3 Plasma Technology Development and Cost Reduction

Plasma applications in agriculture have largely focused on 1) plasma activated water, 2) seed germination, growth, and development, and 3) seed sanitization and biofilm sanitation. Uunderstanding of the chemistry and uses of plasma activated water is far from complete, but there are potential benefits in terrestrial and off-Earth controlled environment agriculture (Besten, 2019; Ranieri et al., 2020). As resupply is costly, plasma based applications and technologies provide an avenue of investigation worth researching to reduce the reliance on conventional methods of sanitation. The plasma source and gas requirements can be small, potentially even using breathable air. On Earth, use of plasma activated water is not widespread due to the problem of scaling for industrial use. Plasmas can be expensive outside of a laboratory setting and conventional methods are cheaper and within regulatory requirements. As advancements in electronics are rapidly decreasing costs, and regulations become stricter on particulate contaminants, plasma becomes a more favorable option. Additionally, new research is providing insight into plasma interactions with water and plasmawater chemistry that allow for system optimization of tasks. Although the application of plasma in various water systems is in its infancy, it should be considered as an aid or potential replacement to some current methodologies.

4 CONCLUDING REMARKS

As NASA and its commercial partners are getting closer to Gateway and robotic and crewed lunar surface missions, it is imperative to plan for large-scale crop production systems that will be deployed on the Moon and Mars. These present numerous challenges, and significant technology development and knowledge gaps need to be resolved prior to successful implementation. We have presented select considerations currently under investigation at NASA centers that can be integrated into large-scale crop production systems design to address these gaps. While not exhaustive, these state-of-the-art technologies include new methods for space crop production, environmental monitoring, water processing, and microbial containment approaches. Future crop production systems must be resilient, but also evolvable, so that new advances can be implemented as innovations occur. It will require contributions from numerous disparate disciplines to feed future exploration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

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AUTHOR CONTRIBUTIONS

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