Research Campaign

Plant and Phytopathogen Interactions in Altered Gravities: Integrated Research into the Stability of Plant-Based Crop Production Systems in Space

Chair: Dr. Andrew C. Schuerger (plant pathology & Mars astrobiology), Dept. of Plant Pathology, U. of Florida, Gainesville, FL 32611; ph: 321-261-3774; email: schuerg@ufl.edu

Dr. Natasha Haveman (plant physiology & omics), Dept. of Horticulture, U. of Florida, Gainesville, FL; nsng@ufl.edu.

Dr. Raymond Wheeler (plant physiology & BLSS crop production), NASA, Kennedy Space Center, FL; raymond.m.wheeler@nasa.edu.

Dr. Gioia D. Massa (plant physiology & flight experiments), NASA, Kennedy Space Center, FL; gioia.massa@nasa.gov.

Dr. Anna-Lisa Paul (plant physiology, omics, flight experiments), Dept. of Horticulture, Univ. of Florida, Gainesville, FL; alp@ufl.edu.

Dr. Doug Ming (Mars geochemistry & flight operations), NASA, Johnson Space Center, Houston, TX; douglas.w.ming@nasa.gov.

Dr. Jan Leach (plant pathology & flight experiments), Dept of Agricultural Biology, Colorado State Univ., Fort Collins, CO; jan.leach@colostate.edu.

Mr. David Reed (engineer, flight operations), TechShot, Inc, Space Life Sciences Lab, Merritt Island, FL; dreed@techshot.com.

Dr. Christopher McKay (planetary science & astrobiology), NASA, Ames Research Center, CA; chris.mckay@nasa.gov.

Dr. Christopher Brown (plant physiology & space flight experiments), Office of the Vice President for Research and Dept. of Biology Univ. of Alabama at Birmingham, Birmingham, AL; csbrown@uab.edu.

Dr. Jane E. Stewart (plant pathology & astrobiology), Dept of Agricultural Biology, Colorado State Univ., Fort Collins, CO; jane.stewart@colostate.edu.

Dr. Jamie Foster (space biology), Dept. of Microbiology and Cell Science, Univ. of Florida, Space Life Science Lab Merritt Island, FL; jfoster@ufl.edu.

Dr. Samuel J. Martins (plant pathology), Dept. of Plant Pathology, U. Florida, Gainesville, FL; sj.martins@ufl.edu.

Dr. Frank White (plant pathology), Dept. of Plant Pathology, U. Florida, Gainesville, FL; ffwhite@ufl.edu.

Dr. Robyn Roberts (plant pathology, virology), Dept of Agricultural Biology, Colorado State Univ., Fort Collins, CO; robyn.roberts@colostate.edu.

1. Abstract

The primary hypotheses (***Ha***) of the proposed Research Campaign (RC) are: (1) phytopathogen virulence and disease development are ***up-regulated*** (i.e., increased) in space, and (2) some plant resistance mechanisms are ***down-regulated*** (i.e., decreased) in space. These two hypotheses must be verified or refuted in a coordinated RC over the next 10 years to develop a fundamental understanding on how plant pathogens interact with plant tissues in space. The future design and operation of plant-based modules or bioregenerative life support systems (BLSS) depend on characterizing disease processes in altered gravity fields like interplanetary space (i.e., microgravity; μg), the Moon (0.17 g), or Mars (0.38 g).

2. Introduction

Humanity is entering a new era in space exploration in which resupply of spacecraft outside of low-Earth orbit (LEO) becomes problematic due to increased launch mass and energy constraints. In response to these problems, BLSS habitats have been proposed to recycle water, oxygen, and food stocks [6, 7, 18, 31]. Historically, small-scale plant-growth payloads have been utilized to conduct plant physiology, horticulture, and crop production research on the Shuttle, Mir, and International Space Station (ISS) (e.g., Biological Research in Canisters [BRIC]; Plant Growth Unit [PGU]; Advanced Plant Habitat [APH]; and the Multi-Use Variable-Gravity Platform [MVP]) [19, 34]. Few disease outbreaks occurred on plants in these self-contained aseptic systems because they were operated under tightly controlled conditions in which the payloads were returned to Earth for reprocessing, cleaning, and sanitizing before the next flight. However, during the last decade, crop production has shifted to 'open systems' like the Vegetable Production System (Veggie) in which ISS cabin air is circulated through the plant-production zone for cooling and relative humidity (RH) control [17].

Once open crop production systems were utilized, the risks of disease outbreaks increased significantly. For example, the opportunistic fungus, *Fusarium oxysporum*, was identified as an aggressive phytopathogen on *Zinnia hybrida* plants grown in the ISS/Veggie 01C flight experiment in late 2015 (**Fig. 1**). The phytopathogen was shown to be more aggressive in μg than during ground (GND) pathogenicity tests suggesting that disease development progressed more rapidly during space flight (FLT) than on Earth [26]. Similarly, Ryba-White et al. [26] observed significantly more disease and increased accumulation of phytopathogen reproductive structures (i.e., oospores of the phytopathogen *Phytophthora sojae*) in infected soybean roots grown onboard a Shuttle experiment in 1996 than in GND controls. Furthermore, several studies have shown that clinorotation increases the severity of plant diseases including crown gall on carrot discs (i.e., a tumor-like proliferation of cells caused by the bacterium, *Agrobacterium tumefaciens*) [14, 30], bean rust development [5], and stunting of tall fescue seedlings infected with the fungus *Neotyphodium* sp. [29].

Fig. 1. Aerial mycelia on *Zinnia hybrida* from the opportunistic phytopathogen, *Fusarium oxysporum*. [Adapted from Schuerger et al., 2021a.]

In other studies [see reviews 20, 21, 28, 33], microbial virulence appears to be up-regulated (i.e., increased) when non-plant microbial/host pathosystems are exposed to μg. For example, both spaceflight and simulated μg increased the virulence of *Serratia marcescens* in the

fruit fly *Drosophila melanogaster* [10]; μg enhanced the pathogenicity of *Escherichia coli* and *Salmonella enterica* compared to Earth *1g* controls [24]; and the fungus, *Aspergillus fumigatus* recovered from the ISS crew quarters, was significantly more lethal to zebrafish than similar isolates from ground crew quarters [15].

In contrast, numerous studies have reported that structural components of plants that are involved in host resistance are compromised in μg [see reviews 8, 11, 13, 23, 32]. For example, cell wall rigidity, cell wall thickness, cellulose and matrix polysaccharides, lignin, and peroxidase can be impaired in space [3, 4, 8, 11, 13, 16]. And lastly, two μg studies [22, 35] showed that host-resistance genes in the transcriptome of *Arabidopsis thaliana* were down-regulated in μg, including genes that involve pathogen defense and environmental stressors.

An alarming consequence of reduced plant defenses would be that plants in space might be more susceptible to infection by microbes, even those that normally do not cause disease. In a telling example, wheat plants grown on an 8-day space Shuttle mission were heavily diseased despite rigorous seed surface sterilization protocols applied prior to flight [1]. The fungal organism damaging the wheat was *Neotyphodium chilense*, which is typically found as a common endophyte of wheat seed that under normal conditions, causes no disease. Even organisms that are beneficial to plants, such as growth promoting bacteria, may have opposite or pathogenic effects on plants during growth in space [9].

3. Knowledge Gaps in Space-Based Plant Pathology

The following 17 *Knowledge Gaps* (KGs) are submitted as the top priorities in the *Research Campaign* described herein. This is not an exhaustive list (due to space constraints).

3.1. Plant-based *KGs* relevant to the development of plant diseases in space:

KG-1: Are plant-resistance mechanisms down-regulated in altered gravity environments including μg (LEO or Earth/Mars transit), 0.17 *g* (Moon), or 0.38 *g* (Mars)? Is there a gravity threshold at which a nominal response in host resistance to infection is observed?

KG-2: What are the mechanisms for the down-regulation of plant resistance in altered gravities (e.g., structural alterations in plant tissues, thinner cell walls, lower deposition of lignin or callose in response to infection, etc.)?

KG-3: Are there genomic, transcriptomic, proteomic, and/or metabolomic alterations in plants in space that explain the down-regulation of host resistance?

KG-4: How do other space-stressors besides μg affect disease development in crops (e.g., space radiation, planetary regoliths, spacecraft microbiomes, high CO_2 levels, artificial illumination, hardware used for crop production, etc.)?

KG-5: Is plant nutrient uptake, incorporation, and energy source-sink relationships impacted by space conditions, and if so, how does this alter host-resistance?

KG-6: Can *Growth Promoting Bacteria* be used in space crop production systems to boost host-resistance against the indigenous microbiomes of spacecraft or space habitats?

KG-7: Will the growth of plants in Moon or Mars regoliths alter plant nutrition, horticultural productivity, and host-resistance to phytopathogens?

3.2. Phytopathogen *KGs* relevant to the development of plant diseases in space:

KG-8: Are virulence factors in phytopathogens up- or down-regulated in altered gravities? Is there a gravity threshold at which nominal virulence factors are maintained?

KG-9: Are up- or down-regulation processes for virulence the same for fungal, bacterial, or viral phytopathogens?

KG-10: What are the *interactive effects* of space radiation, microgravity, closure, artificial illuminations, the ISS microbiome, etc. on plant disease development in space?
KG-11: Are there metagenomic, transcriptomic, proteomic, and/or metabolomic alterations in phytopathogens in altered gravities that explain the up-regulation of virulence and/or disease development in space?
KG-12: Can phytopathogens in space evolve over time, shifting their host ranges to non-target crops in space crop-production systems?
KG-13: Will opportunistic phytopathogens emerge from the endemic microbiomes in spacecraft or space habitats?
KG-14: How will crop production in planetary regoliths affect the expression of virulence genes in phytopathogens and development of plant diseases in crops?
KG-15: Can an *Integrated Disease and Pest Management* (IDPM) program be developed for space-based plant-growth systems to mitigate crop diseases to acceptably low levels?
KG-16: Can a *Rapid Disease Detection* system be developed for space-based crop production?
KG-17: Can microgravity simulators (e.g., HARV, RPM, etc.; see below) accurately mimic the responses to spaceflight in plant/phytopathogen interactions?

4. Research Campaigns to address KGs [*Timelines & budget estimates are in brackets*.]

There are only three papers on diseases development in μg and only five papers on clinostat experiments on Earth that pertain to disease development in space-based plant production systems. The paucity of data on plant/phytopathogen interactions makes it very difficult to properly predict risks for using BLSS modules for crew life support. The following RC will briefly outline both GND and FLT experiments that are required to advance the nascent field of space-based plant pathology. The goal will be to answer as many *KGs* as possible within a 10-year time frame to allow coordinated integration of plant production into Moon and Mars BLSS modules as soon as possible in the 2030's. *The primary KGs and assumptions for the RC are: (i) pathogen virulence and disease development appear to be* ***up-regulated*** *in space while (ii) plant resistance mechanisms (and other hosts) appear to be* ***down-regulated*** *in space.*

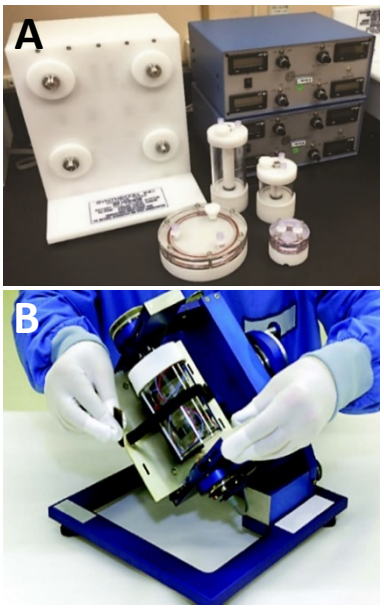

Fig. 2. (**A**) High-Aspect Ratio Vessels (HARVs). (**B**) Random Position Machines (RPMs).

Our overall recommendations are as follows: (1) add text to annual Space Biology RFPs to call specifically for Plant Pathology research; the extra topical bullet point for requested proposals needs to be separated from Plant Microbiome studies, (2) both GND and FLT research opportunities should be called out in order to determine if microgravity simulators can help speed up the characterization of plant/phytopathogen interactions in altered gravities, (3) conduct parallel research into plant/phytopathogen interactions in space, and (4) *phytopathology has to be an integral part of ongoing plant biology research in space if plant BLSS are to be used as life-support systems in near-term space missions to the Moon or Mars*.

4.1. Ground research with simulated altered gravities [2023-2032; $15 million/10 years].

A key research goal should be to conduct coordinated GND and FLT experiments in which ground-based microgravity simulators (**Fig. 2**) are used. If a specific type of GND-

simulator can mimic the flight environment accurately, then more progress can be achieved by supporting GND research into plant, phytopathogen, and microbiome interactions. In addition, new protocols for both GND and FLT experiments are required for inoculating plants, remote sensing for tracking disease progression, harvesting, and processing samples to identify the effects of simulated gravity on bacterial, viral, fungal, and oomycete phytopathogens, and tissue fixation protocols applicable to space flight to preserve plant + phytopathogen tissues for Earth-return. An effort is needed to increase both launch and Earth-return mass capabilities to support these experiments. Furthermore, the Microgravity Simulation Support Facility at the Kennedy Space Center, FL should be expanded to include 3 dedicated plant pathology growth chambers, six additional HARV and six additional Random Position Machines (**Fig. 2**), and one new scanning electron microscopy system.

4.2. New flight hardware to accelerate plant pathology research in space [2023-2028; $25 million/5 years]. Currently, only one ISS flight experiment is scheduled over the next few years to characterize host resistance when challenged by a phytopathogen (e.g., Schuerger et al.; new NASA grant #80NSSC22K0209). The pace of future plant pathology ISS flight experiments should be increased to 2-3 ISS flight experiments per year. There are too many *KGs* related to disease development in space to continue at the pace that is currently funded. We recommend that a specific topic be added to all future Space Biology flight experiment RFPs to request "plant pathology studies in support of future crop production in space." To support the increased rate of ISS flight experiments, one additional APH, two additional Veggie units, two additional MVP units, and all support equipment (**Fig. 3**) are recommended for deployment on the ISS.

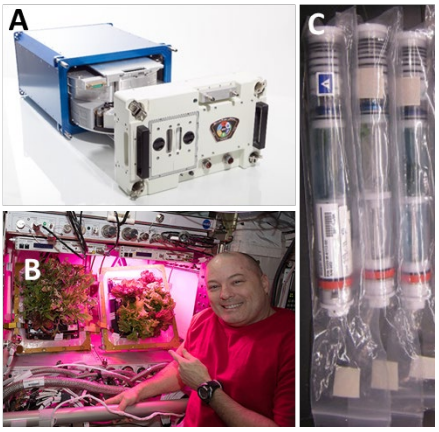

Fig. 3. (**A**) Multi-Use Variable-Gravity Platform (MVP), TechShot, Inc. (**B**) Veggie onboard the ISS. (**C**) KSC Fixation Tubes.

4.3. Plant pathology flight research with variable-*g* payloads [2023-2032; $15 million/10 years]. A key *KG* is whether there is a gravity threshold for the normal development of phytopathogens in μg. Thus, the availability of variable-gravity flight hardware (like the MVP; **Fig 3**) is critical. We recommend that variable-g GND and FLT experiments be coordinated and enhanced to answer whether BLSS habitats in μg, on the Moon, or on Mars are possible given the human-associated microbiomes likely to occur in these space habitats.

4.4. Omics GND and FLT research in parallel with directed plant pathology Exps. [2023-2032; $15 million/10 years]. Thorough omics analyses of plants and phytopathogens in spaceflight, simulated microgravity, and 1-g controls will provide holistic views of functional pathways engaged during host infections under spaceflight relevant conditions. The results generated from these analyses will provide insights into altered metabolic states or gene expression changes that may be used to prevent the development of plant pathogenesis in future space-based crop production.

4.5. ISS microbiome studies. [2023-2032; $10 million/10 years]. The ISS is an excellent platform to examine the movement, survival, evolution, and succession of microbiomes among the diverse modules. Special emphasis should be placed on tracking phytopathogens between and among individual ISS modules in relation to source niches and crop production

hardware. Results will inform our understanding of plausible risks to BLSS crop production from indigenous or introduced phytopathogens. Metagenomics studies are essential to tracking the flow of microbiomes and phytopathogens in the ISS modules, and these are now feasible during spaceflight [2]. However, we also propose that efforts should be made to (1) return viable cultures of phytopathogens to Earth, and (2) return all plant tissues that develop disease signs and symptoms for proper diagnosis on the GND.

4.6. Dedicated Flight RC with an astronaut core trained in the operation of all plant biology hardware during one, 6-month mission (2028-2030) [2028-2032; $30 million for dedicated RC mission]. Towards the end of this decade, we propose that a dedicated ISS mission be flown that utilizes all available plant-production hardware to characterize multiple plant/phytopathogen interactions during one, 6-month mission. The coordinated experiments will inform our community on how multiple phytopathogens develop in their hosts in space and can be used to cross-check the *KGs* listed above. If there are key trends that cut across all plant/phytopathogen interactions, then broader and more comprehensive conclusions–and predictions–can be drawn.

4.7. Develop an *Integrated Disease and Pest Management* (IDPM) program for all crop production and BLSS habitats [2023-2032; $12.5 million/10 years]. Each stage in the utilization of crops in advanced BLSS missions will require a comprehensive IDPM program to ensure crop safety and minimal loss of edible biomass [27]. Developing such an IDPM program is an evolutionary process in which the IDPM program becomes more complex as the plant-production capabilities become more complex. To achieve this goal, one senior plant pathologist position should be filled at the KSC/Plant Biology program, and the KSC labs should be expanded to accommodate isolated plant pathology research away from FLT hardware and GND-support horticultural research (i.e., isolated growth chambers and lab space).

4.8. Rapid Diagnostic Detection system for plant diseases in space-based crops [2023-2028; $10 million/5 years]. A plant disease rapid-detection system that is deployed on ISS, Gateway, Moon, and Mars missions needs to be developed. The report by Schuerger et al. [2021a] on the diagnosis of the *Fusarium oxysporum* phytopathogen on ISS-flown zinnia plants indicated that approx. 6 months was required to fully identify the causal agent of the disease "after" infected host tissues were returned to Earth. An unacceptable time-delay for a BLSS module on the Moon or Mars. We recommend that a rapid detection and diagnosis system be developed for plant diseases in space to achieve a probable diagnosis within 24-48 hrs. Although such a system has been tested on the ISS [2, 12], significant additional development is required.

5. Summary

The primary ***Ha*** for this RC are *(1) phytopathogen virulence and disease development are **up-regulated** and (2) some plant resistance mechanisms are **down-regulated** in space*. Relevant to solving these hypotheses, a comprehensive Research Campaign is proposed that will focus on GND and FLT research into the interactions among plants, phytopathogens, and the resident microbiomes in space-based crewed habitats. We propose here at least 17 *KGs* that are relevant to the topic. Furthermore, we propose eight main RC programs that must be developed concurrently over the next 10 years to place humanity in the position to select the best approach to using BLSS modules and habitats for crewed missions to the Moon and Mars by the mid-2030s. Plant Biology and Plant Pathology research must be a coordinated and evolutionary approach to optimize our understanding of how phytopathogens will affect plants in BLSS habitats. The schedules and budgets for each RC topic are aggressive but achievable. Results will dramatically improve the utility of biological life support systems for future missions.

6. References

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