HASP Student Payload Application for 2015

Payload Title: CRESS - Cosmic Radiation Exposure System for Seeds		
Payload Class: (check one) X Small ☐ Large	Institution: University of Florida	Submit Date: 12-18-2015

Project Abstract

The CRESS (Cosmic Radiation Exposure Seed System) project will expose wild-type *Arabidopsis thaliana* seeds to the unique cosmic ray environment of the Earth's stratosphere. At 120,000 feet, high altitude balloons are above 99% of the Earth's atmosphere and are therefore exposed to higher levels of various types of cosmic radiation than on the ground. High energy particles called HZE particles, mainly ionized carbon and iron, are of particular interest due to the damaging effects these particles inflict on genomic material. This project is an initial technology development for constructing a robust, reusable biological exposure platform for radiation exposure using balloons. It is understood that the current flight will see limited HZE compared to extended Antarctic flights planned for the future. Yet this flight opportunity allows design of flight payload and the development of handling procedures relevant to balloon flights. The biological targets are 100,000 seeds that fly dormant and without need for extensive life support during the flight. Though dormant, the seeds will capture the biological effects of any ionizing radiation that is encountered during the flight, and those effects will be revealed by post flight analysis of those seeds and the plants derived from those seeds.

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CRESS

(Cosmic Radiation Exposure System for Seeds)

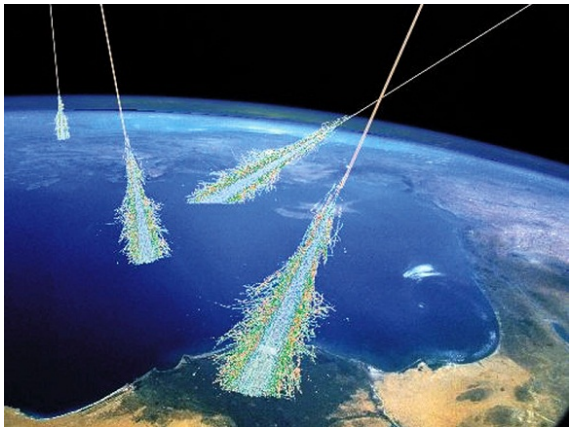

Image Credit: Simon Swordy/University of Chicago, NASA

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Abstract

The CRESS (Cosmic Radiation Exposure Seed System) project will expose wild-type *Arabidopsis thaliana* seeds to the unique cosmic ray environment of the Earth's stratosphere. At 120,000 feet, high altitude balloons are above 99% of the Earth's atmosphere and are therefore exposed to higher levels of various types of cosmic radiation than on the ground. High energy particles called HZE particles, mainly ionized carbon and iron, are of particular interest due to the damaging effects these particles inflict on genomic material. This project is an initial technology development for constructing a robust, reusable biological exposure platform for radiation exposure using balloons. It is understood that the current flight will see limited HZE compared to extended Antarctic flights planned for the future. Yet this flight opportunity allows design of flight payload and the development of handling procedures relevant to balloon flights. The biological targets are 100,000 seeds that fly dormant and without need for extensive life support during the flight. Though dormant, the seeds will capture the biological effects of any ionizing radiation that is encountered during the flight, and those effects will be revealed by post flight analysis of those seeds and the plants derived from those seeds.

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1.0 Mission Overview

1.1 Science Background

A. Why Arabidopsis?

The *Arabidopsis thaliana* (Arabidopsis) plant has been used as a model organism in plant biology for well over 100 years owing to a number of appealing innate characteristics. The most well-known are: short generation time (about 6 weeks) with high seed production, small seeds and small genome size, which enables relatively easy genome analyses. The CRESS payload will be housing Arabidopsis seeds for a number of experiment reasons. The small size of Arabidopsis seeds allows for approximately 100,000 seeds to fit into a volume of only 4mL. This high number will ensure plenty of replicates in the path of any incoming HZE to be available for post flight growth studies. Arabidopsis seeds, like any seeds, are robust and can endure the harsh conditions characteristic of high altitude balloon flights. This project relies heavily on post flight analysis of plant phenotypes so the short generation time will allow for quick analysis of the second generation in the case that a HZE impacted germ cells of the plant embryo.

Arabidopsis seeds contain a plant embryo composed of around 100 cells; about one half of the cells make up the proto-shoots while the other half make up the embryonic root (radicle) (e.g. Jurgens et al., 1995; Weijers, 2014). Of all of these cells, two are responsible for the development of the male and female germ cells. An impact from a high energy galactic cosmic ray with of any of these cells may cause irreversible genomic damage possibly leading to phenotypic changes during growth. An impact with embryonic somatic cells may cause changes in phenotypes and growth patterns in the first generations whereas impacts with germ cells may lead to phenotypic or growth pattern changes in the second generation. Again, the short generation time of Arabidopsis allows relatively quick high throughput, multigenerational screening.

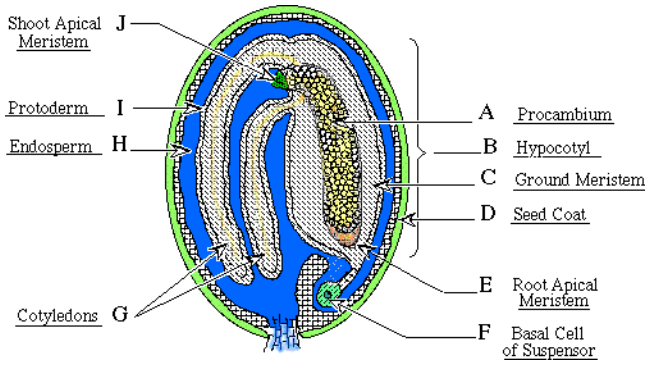

Figure 1. Anatomy of a dicot seed. An HZE impact on many of these structures could cause irreversible damage.

Image Credit: http://home.earthlink.net/~dayvdanls/plant_reproduction.html#Development

B. Space Radiation

The Earth is constantly bombarded by a number of different types of radiation such as gamma rays, x-rays, and galactic cosmic rays. For biological applications, galactic cosmic rays are of particular interest due in part to the bio-molecular damage these high energy particles can inflict of biological material. Galactic cosmic rays are mainly high energy protons and alpha particles but 1% are composed of heavier nuclei (atomic number > 2). These heavier nuclei are called HZE particles and have a high Linear Energy Transfer. Despite such a low relative abundance these high energy ionized carbon and iron have a high relative biological effectiveness (RBE). Relative biological effectiveness is a means of characterizing the damaging effects of ionizing radiation on biological molecules (DNA, RNA, proteins, and other bio-molecules). On Earth, natural HZE events are typically limited to stratospheric environments (Reitz, 1993)

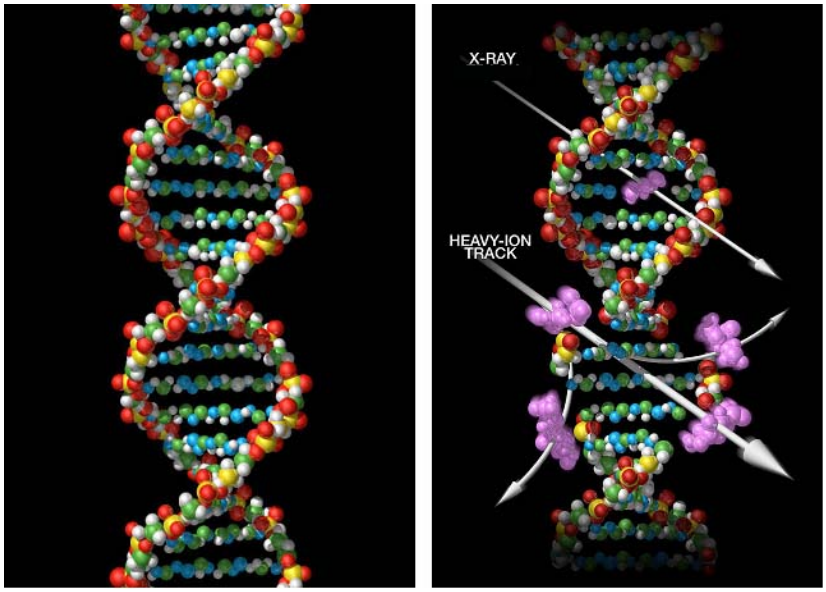

Figure 2. An illustration of a DNA helix interacting with two common types of radiation in the space environment. On the right, X-ray radiation passes through the spaces in the DNA helix while heavy ions like carbon and iron are too large and may disassemble the structure of DNA. Image credit: NASA

C. Early Plant Space Radiation Research

Some of the earliest plant biology experiments in space included seeds sent to study the effects of galactic cosmic radiation, mutation rates, and phenotypic changes due to this unique form of radiation. In 1972 the Biostack I and II experiments, onboard Apollo 16 and 17, respectively, in Russian experiments, and in the "Seeds in Space" project (Bucker, 1974; Kranz et al., 1990; Kranz et al., 1994). More recently, low Earth orbit studies and terrestrial analogs of galactic radiation have been conducted with both plants and animals (reviewed in: Arena et al., 2014). In the times when the environment outside of the Earth's magnetic field was accessible, the tools to analyze the effects of that environment at the genomic level were not available.

1.2 Science Goals

There are two interconnected goals for this proposal. The first aim of this project is to use modern genomic analyses, such as genomic resequencing (e.g. Metzker, 2010), to evaluate the effect of stratospheric radiation on Arabidopsis seeds. These will be the first seeds using whole genome analysis to completely define the genomic effects of actual space radiation. The second aim is to develop the flight hardware necessary to present Arabidopsis seeds to the radiation environment of the stratosphere, and integrate the payload into a high altitude balloon platform. These goals will be realized as we test the integration of all steps, including the evaluation of the processes required for post flight analysis of seeds.

Future mission to the Moon and Mars will require plants as a means of advanced life support (food production, air, water, waste recycling). Seeds are a naturally robust, low-weight, low maintenance method for bringing plants to the far-off destinations that we seek to explore and colonize. We must have a strong understanding of the effects of these damaging ionized particles on dormant seeds so astronauts are not surprised when they plant and harvest multiple generations of crops.

1.3 Principles of Operation

The primary goal is to develop the payload hardware, flight integration processes, and post flight analyses that will provide the first genomic evaluation of seeds exposed to the stratosphere environment. Each major principle will be kept simple with backup plans for any complex components.

Major principles

I. Plant seeds are dormant. Unlike living plants, the seeds will need limited life support during the flight. The goal is to have a payload of 100,000 seeds in a small cassette that is kept near 4C during the flight. Hardware will be designed to keep the 1U volume of the experiment in thermal and pressure regimes that are optimal for seed survival. In the event of hardware failure, the seeds can survive reasonably wide temperature swings, including freezing and hypobaria.

II. Radiation strikes will likely impact several seeds so even a single HZE event will produce multiple seed-revealed effects. The plan is to include film or other radiation sensors to map impacts within the seed cassette. But absent any radiation detection, the seeds themselves still record the biological impacts of events.

III The major learning outcomes are technology demonstration, both biological and hardware. Hardware and payload development for biological scientists is a uniquely cross disciplinary action. In the event of complete failure, the development process itself is a valuable experience. Successful development of a 1U cubesat form factor payload for balloons opens the door for many biological experiments to consider balloon deployments. Fundamentally, a completely passive trip of seeds would produce exposures that would be biologically relevant. Seeds can survive freezing, low pressure and high *g*. Post processing of the seeds and the

resulting plants will require months of post flight effort, linking molecular genomics with the balloon exposure. Any seed with a genomic impact will be found and analyzed. The beauty of the system is that the seeds themselves act as the radiation capture system. They just need to get up there. As the HZE pass through them, the genomic disruptions remain as a biological footprint of the passing of the particle. That footprint will be painted by genome resequencing of the plants or plant parts showing mutational disruption.

2.0 Payload Design

2.1 Requirements

I. HASP Requirements

- Payload should not exceed 3 kg (6.6 lbs)
- The footprint cannot exceed 15 cm. by 15 cm
- The payload height should not exceed 30 cm
- Electrical systems must operate on 29-33 Volts
- Electrical systems must operate on 0.5 Amps @ 30 VDC
- Must operate in at temperatures of -70ºC to 40ºC

II. CRESS Payload Requirements

- Payload must fly 100,000 Arabidopsis seeds to the Earth's upper atmosphere

III. CRESS Payload Desirements

These elements are preferred but not required for a successful flight.

- Thermal control
- 1 atm. sealed container
- Film or electronic radiation detection system, ideally with some physical relationship to the seed cassette to enable physical mapping of the impacts.
- Record the internal and external temperature and pressure environment (e.g. HOBO data logger).

2.2 Biological Payload

100,000 wild-type Arabidopsis seeds will be organized in multiple thin sheets and stacked vertically in a cassette. Between each of these biological layers will be a layer of nuclear emulsion film to map and act as a second means of recording HZE particle impacts (beyond the seeds innate ability to record these events). This design follows that of the Biostack I and II biological payloads but has been simplified. Cellulose Nitrate was used in Biostack to determine exactly where on the seed the HZE particle impacted (Bucker, 1974). We are only concerned with the general area so as to reduce weight, cost, and expedite post flight screening. Post flight genomic analysis will reveal whether seeds sustained genetic damage from an impact. See section 2.2 for an illustration of the biological payload integrated with the payload chassis. Two

of these biological payloads will be assembled. One will go up in the CRESS payload while the other will be used as a ground control for post flight analysis comparisons.

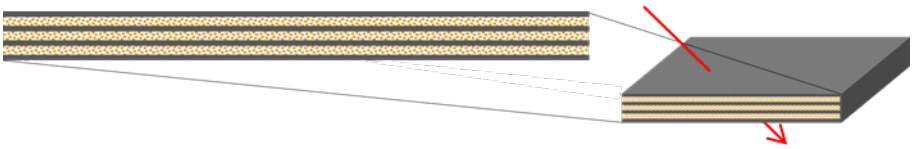

Figure 3. Example of how layers of nuclear emulsion film (dark grey) would surround layers of Arabidopsis seeds (speckled amber) to assist in the mapping of HZE particle impacts (red arrow).

Nuclear emulsion film, while an excellent, low cost, low weight means of recording HZE particle impact events, post processing and scanning can be a tedious and laborious process. Nuclear emulsion film will be included in the biological payload but improper development or time restrictions could limit full analysis. In which case the seeds themselves will exist as the primary records for HZE particle impact.

2.3 Mechanical Systems

Future missions for the CRESS payload include long-duration arctic balloon flight and spaceflight. To prepare for these missions the CRESS payload will follow the specifications for a 1-Unit cube satellite. A 1-U payload will measure 10.00 cm x 10.00 cm x 11.35 cm and weigh in under 1.33 Kilograms. The low cost, low mass, and standardization of U-class spacecraft will simplify downstream design and development of the CRESS project

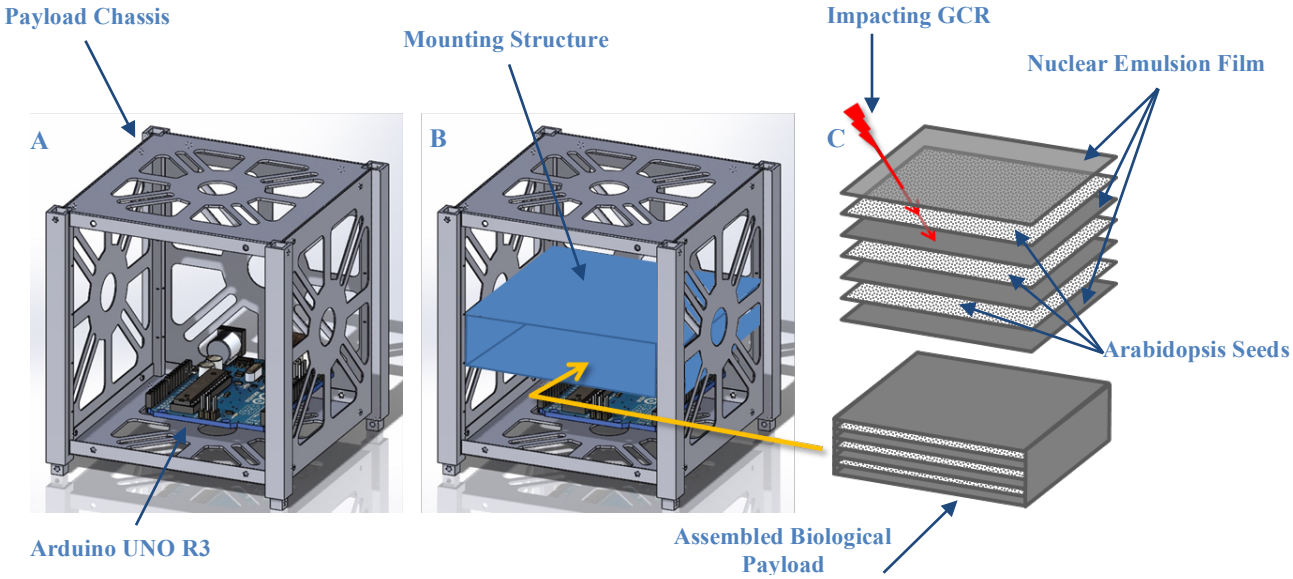

Figure 4. A) Mock-up of the 1-U payload with the Arduino Uno Microprocessor. One of the side plates has been removed to show the inner components. B) The 1-U mockup with the mounting structure that will hold the biological payload. C) The biological payload with layers expanded (top) to illustrate the layered configuration of the wafers: emulsion film (gray) interspersed with flat cassettes of seeds (speckled), and then in compressed configuration ready for flight (bottom).

2.4 Electrical Systems

The HASP power bus provides 0.5 A at 29-33 V of DC current. The main power requirements for the CRESS payload are the Arduino Uno R3, BME 280 Temperature and pressure sensors, and the resistive heating element. The Arduino operates on 5V and 100 mA, the BME280 sensor requires a maximum of 3.3 V and 3.6 μA (when using humidity, temperature, and pressure sensors). Power requirements for the resistive heating element have not yet been fully characterized as the desired COTS product must be modified to fit the payload. The power requirements will be minimal and will fall within the requirement of the HASP power bus.

Electric Flow Diagram

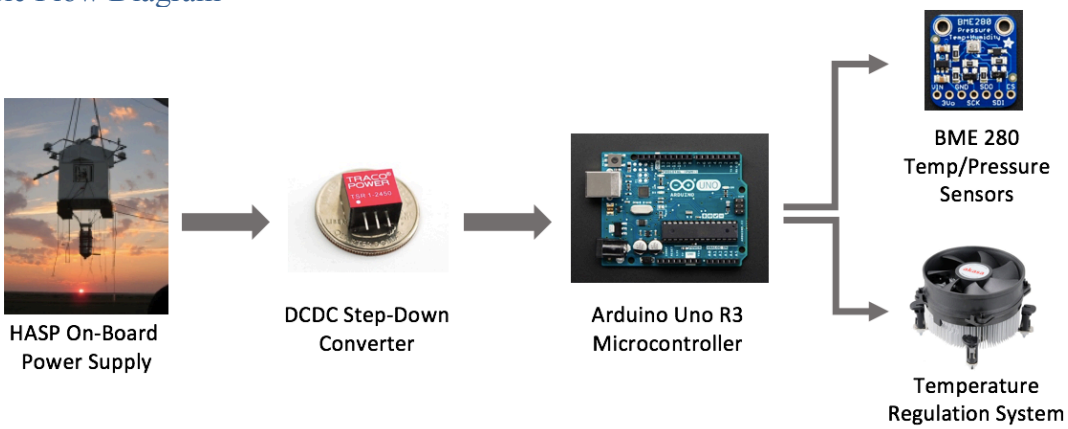

Figure 5. The CRESS payload will be powered by the HASP onboard power supply which will be attenuated with a DCDC Step-Down Converter and then distributed by the Arduino to all of the temperature/pressure sensors and to the thermal regulation system.

2.5 Data Collection

Data from the internal and external temperature and pressure sensors will be automatically recorded and stored to a micro SD card in-flight. Data stored in flight will only require manipulation post flight.

Figure 6. Information from the BME280 Temperature/Pressure sensors will be send to the Arduino and subsequently stored to the onboard micro SD chip.

Data collection is a low risk component of the system as it is not absolutely essential for a successful flight. These systems also have reputation of success on many previous high altitude

balloon flights thereby reducing some risk of failure. Extensive environmental testing will be conducted to ensure data collection and storage is still operational in stratospheric conditions.

2.6 Software Systems

The CRESS payload software will utilize the well-known and commonly used Arduino microcontroller and integrated programming language. The nature of this payload requires only Arduino's simplest microcontroller, the UNO R3. Data received from the temperature and pressure sensors will be output directly to a micro SD flash memory card. This system requires no in-flight manipulation.

2.7 Thermal Management

Although seeds are considerably resistant to thermal fluxes a consistent thermal environment is preferred. To ensure the thermal environment remains at 4ºC ± 10ºC a resistive heating pad, and fan assembly will be used. This thermal system will be powered by the HASP power output and controlled by the Arduino Uno R3 and Temperature sensor. A program will activate the thermal system when temperatures drop below the recommended range and will turn-off when the correct temperature has been reached. The fan will homogenize the air temperature within the payload to reduce the occurrence of hot spots.

2.8 Pressure Management

Ambient ground pressure will be maintained by a locking top door mechanism that is air tight. This system can be sealed on the ground and will ensure ground atmospheric conditions are present for the duration of the flight. This element poses the largest risk for the HASP payload as an immediate depressurization could cause damage to elements surrounding the CRESS payload. Rigorous testing will be conducted to ensure that the payload chassis is capable of maintaining 1 atm of internal pressure in 0.001 mbars of external pressure without catastrophic failure. Special care will be taken to ensure that if depressurization occurs, the process is slow and controlled through the use of purge valves and/or other means.

2.9 Mass Budget

HASP Component Mass Budget			
Component	**Quantity**	**Mass**	**Total Mass**
Payload Chassis	1	700g ± 100g	700g
BME280 Pressure Sensor	2	1.3g	2.6g
Temperature Regulation System	1	12.53g	12.53g
Arduino Uno R3	1	27.95g	27.95g
Wires + additional auxiliary electronics	1	50.0g	50.0g
Biological Payload + Seeds	1	75.0g	75.0g
Total			**868.08g**

3.0 Testing and HASP Integration

3.1 Thermal Testing

The thermal environment of the HASP flight has been well recorded over past years. Temperatures will range from -70ºC at altitude to 40ºC on the ground. This large flux poses the potential risk for significant thermal expansion and contraction for certain materials. The extreme cold of high altitude nighttime flight also poses a risk for electronics and may reduce the structural integrity of many types of materials. Extensive thermal testing will be conducted in the months leading up to HASP integration. Seeds are robust and can survive the extreme environments of the HASP flight even without any kind of thermal regulation. In the case of a complete failure of the thermal regulation system the biological payload will still hold valuable information.

3.2 Vacuum Testing

At Altitude the HASP payload can experience pressures as low as .001mbars. This low pressure could pose a threat to the sealed 1 atm. payload. Rapid depressurization is by far the greatest risk to this payload. Extensive testing will be conducted in local Vacuum chambers and climate controlled vacuum chambers. These tests will be conducted on payload mock-ups that have been through thermal cycling treatments to ensure that even the toughest conditions will not cause a structural failure.

3.3 Systems Testing

Full software and electrical system tests will be conducted to ensure full integration of the Arduino Uno R3, temperature, pressure, and thermal regulation system. Once basic tests are passed HASP high altitude simulation tests will be conducted to certify that all systems will operate normally under the extreme temperature and pressure environments of the upper atmosphere.

3.4 Risk Analysis

The greatest risk facing the CRESS payload, which would lead to the failure of the scientific mission, is the loss or destruction of the biological payload containing the 100,000 Arabidopsis seeds. The risk is considered low as the biological payload will be protected by the payload chassis, the seeds within the biological payload are capable of withstanding the environmental profile of HASP flights, and the biological payload is designed in such a way that the final *g* forces experienced during touchdown will not damage the unit. Other risks include the loss of pressure and/or temperature regulation during the flight and the failure of electronic components (Arduino, sensors, fan). These risks to the electronic components are mitigated in part because of their standing reputation on high altitude flights. Extensive testing will still be done to ensure this is true.

3.5 HASP Integration

The CRESS payload will be designed to integrate seamlessly with the footprint and power systems of the HASP system. Testing will be completed using the same volt and amp profile as the HASP system. The CRESS payload has no preference for placement on the high altitude balloon craft.

4.0 Flight Procedures

4.1 Pre Flight Procedures

Arabidopsis thaliana seeds will be cultivated in laboratory growth chambers to a level that will yield a harvest of about 500,000 seeds. Of those seeds, 100,000 will be loaded into the containment cassette and then positioned in the CRESS payload, and 100,000 will be used in the comparable Ground Control. Final systems test will be performed to ensure everything is working as designed. From here the seeds do not require any delicate handling and can easily be shipped to the CSBF facility in a passively cooled container.

4.2 In Flight Procedures

The CRESS payload will not require any in-flight manipulation.

4.3 Post Flight Procedures

The Cress payload will not require any delicate handling during the recovery, packing, and return shipment. Refrigeration is preferred but not necessary.

5.0 Data Analysis

Upon reception of the payload in Florida the seed-containing cassette will be removed from the CRESS payload. The nuclear emulsion film surrounding the seeds will be removed, stored in a protective casing, and shipped for development. Developed nuclear film will then be manually or automatically scanned for impacts. If impacts are found the seeds corresponding to that area of the biological payload will be removed and allowed to germinate on nutrient containing sterile agar plates. If the nuclear film does not prove to be a reasonable means of scanning then *all* seeds will be screened for germination and altered phenotypes. The nuclear film could provide the information necessary to make specific selections of seed on the cassette which would greatly decrease screening time. Visual scans for obvious mutations will be performed. Any plants with atypical phenotypes will be transplanted to sterile soil and monitored carefully. DNA from mutant sectors will be extracted for full genome analysis. The mutant plants will be grown to maturity and the resulting second generation seeds will be collected and planted. The number of non-germinating seeds will be recorded and compared to values seen in the ground control. This system will be repeated for all seeds in the payload.

6.0 Project Management

The project will be led by Austin Schmitz as a major part of his undergraduate research program in the UF SpacePlants lab, which is overseen by Drs. Ferl and Paul. Austin will be responsible for project tracking and development and reporting. Graduate students Eric Schultz and Natasha Sng will advise and participate in the hardware design (Eric) and the postflight plant analyses (Natasha). Drs. Ferl and Paul will provide project oversight, funding for those activities needing support, and will also be responsible for integrating the CRESS project into the broader context of the space biology experiments. The work proposed here would be taking place in the same calendar year as two spaceflight experiments to the ISS and will provide a tremendous perspective both scientifically and operationally. The UF SpacePlants lab conducts weekly lab meetings and Austin, Eric and Natasha will present weekly updates and discuss project questions and solutions during those lab meetings.

7.0 Notional Timeline

- Fall, Winter 2015
  - Science concept design
  - Initial SolidWorks mock-ups
  - 3D-print 1U cube-satellite
  - 3D-print seed carrying wafer
- January 2016
  - Begin bulking wild-type Arabidopsis seeds
  - Purchase Arduino
  - Purchase Temperature/Pressure Sensors
  - Purchase thermal regulation system
  - Purchase atmospheric containment system
  - Seed freezing and hypobaria tests for risk reduction
- February 2016
  - Software system design and testing
  - Atmospheric containment design and testing
  - Clean/prepare first seed bulk set
- March 2016
  - Continue software system design and testing
  - Continue atmospheric containment design and testing
- April 2016
  - Finalize software system design
  - Finalize thermal and atmospheric containment system design
  - Compile preliminary PSIP document
- May 2016
  - Begin thermal and atmospheric containment system testing
  - HASP power integration testing
- June 2016
  - Send in final PSIP document

    - Continue thermal and atmospheric containment system testing
- July 2016
    - Final FLOP document due
    - Final thermal and atmospheric containment system testing before payload integration
- August 2016
    - Payload integration in Palestine, Texas
    - Final flight preparations
- September 2016
    - Flight
    - Recovery and payload return
    - Begin screening seeds
- December
    - Continue screening seeds
    - Final science report due

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