FINAL SCIENCE REPORT– CRESS

December 9, 2016

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Student Demographics

Name	Gender	Ethnicity	Race	Student Status	Disability
R. Austin Schmitz	Male	non-Hispanic	White	Undergraduate	No
Ana M. Ward	Female	non-Hispanic	Asian	Undergraduate	No
Natasha Sng	Female	non-Hispanic	Asian	Ph.D. Candidate	No
Collin LeFrois	Male	non-Hispanic	White	Ph.D. Candidate	No

Abstract

The CRESS (Cosmic Radiation Exposure System for Seeds) project exposed wild-type *Arabidopsis thaliana* (Arabidopsis) 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 protons, 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. The biological targets, 250,000 Arabidopsis seeds, flew dormant and without need for extensive life support during the flight. Though dormant, the seeds are capable of capturing the biological effects of any ionizing radiation that was encountered during the flight, and those effects will be revealed by post flight analysis of those seeds.

Mission

There are two interconnected goals for the Cosmic Radiation Exposure System for Seed (CRESS) Project. The first aim was to develop the flight hardware necessary to present Arabidopsis seeds to the radiation environment of the stratosphere by integrating the payload into a high altitude balloon platform. These goals were realized at integration and testing in Palestine, Texas and flight operations in Fort Sumner, New Mexico this past August and September, respectively. The second aim of this project is to conduct a proof of concept experiment that combines passive radiation detection techniques with biological assays to evaluate the effect of stratospheric radiation on Arabidopsis seeds.

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Hardware Development

Hardware design for the CRESS payload progressed considerably during the six-month span between concept and flight. Design changes were influenced by a continuously evolving understanding of various elements of the payload including 3D-printing materials, the exposure limits of Arabidopsis seeds, and

Initial Requirement

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

Initial Desired Features

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

1. Thermal control
2. 1 atm. sealed container
3. Film or electronic radiation detection system, ideally with some physical relationship to the seed cassette to enable physical mapping of the impacts.
4. Record the internal and external temperature and pressure environment (e.g. HOBO data logger).

Final Requirement

- Payload must fly **250,000** seed to the Earth's upper atmosphere.

Final Desired Features

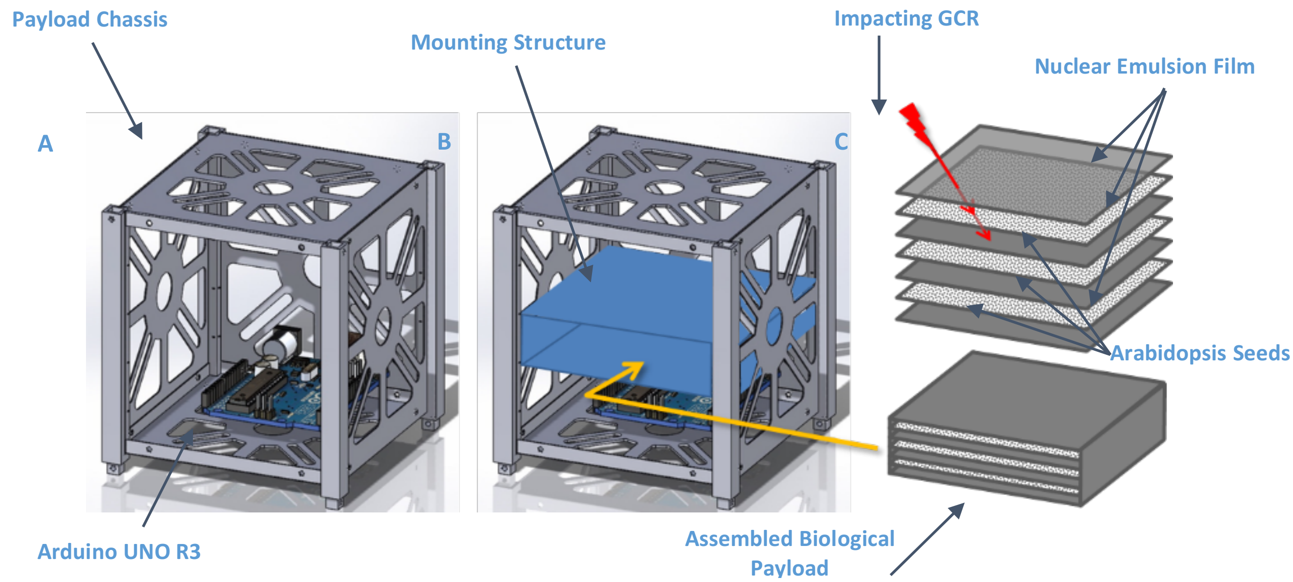
These elements are preferred but not required for a successful flight.

1. A containment vessel capable of maintaining approximately 1-atmosphere of internal* pressure
2. Internal temperature and pressure data for the duration of the flight processes, including pre and post-flight procedures.
3. CR-39 Solid State Nuclear Track Detector (SSNTD) sheets for the recording of galactic cosmic ray impacts.
4. A well-organized seed container allowing for highly controlled post-flight processing and analysis.

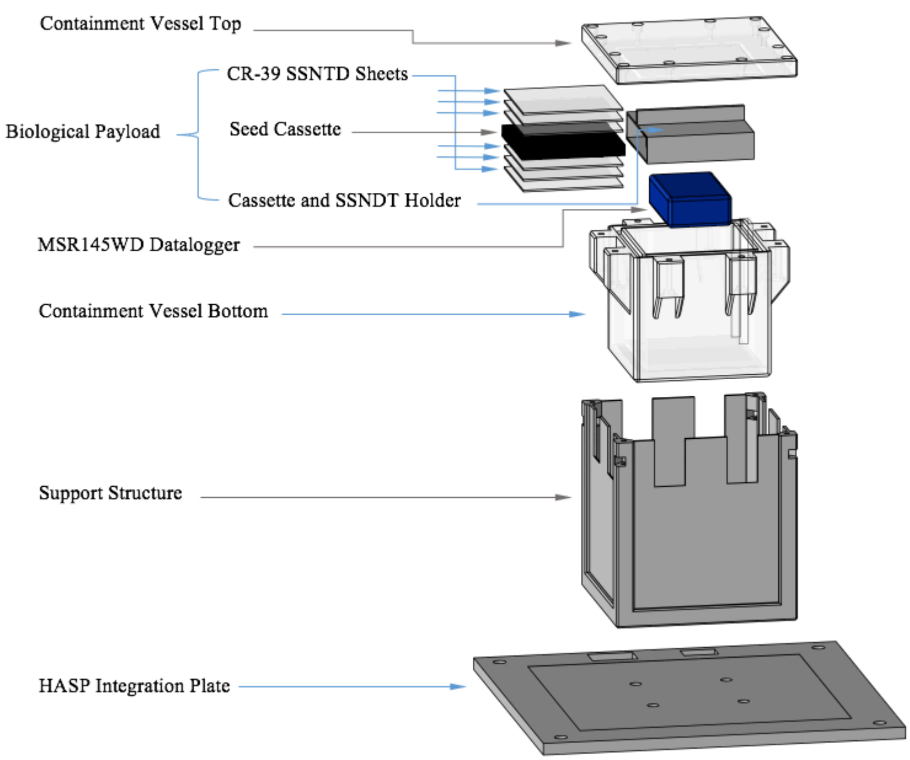
* The sealed volume within the containment vessel, containing the seeds and Solid State Nuclear Track Detector Sheets.

In order for a successful mission, the CRESS payload needed to bring seeds to the Earth's upper-atmosphere. The noticeable change in the quantity of seeds required, 100,000 initially to 250,000 for the final design, is simply the result of the method by which the seeds were organized. During development, it was determined that 250,000 seed could be stored. There are several obvious changes in the desired features from initial design to final design. 1. Thermal Control was removed after seed exposure testing proved thermal life support measures were not necessary. 2. (1 atm. sealed container) was retained until the final flight design. See section *Design Evolution* for more information on the containment vessel.

Initial Concept

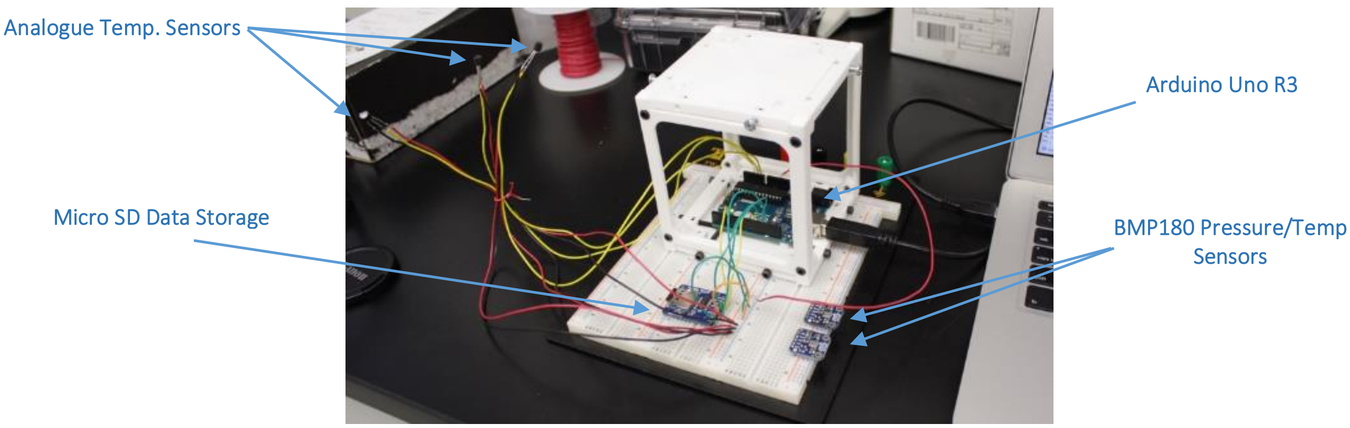


Final Payload Design



Design Evolution

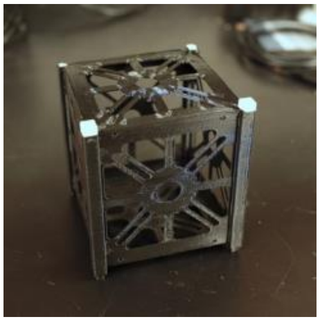
The initial design of the CRESS payload was heavily influenced by the perceived need to protect the biological target (seeds) from the extreme environment of the upper atmosphere. It was believed that the freezing temperatures and low pressure environment may affect the germination rates of the Arabidopsis seeds. In order to combat these extreme conditions, the initial design included microcontroller responsible for the measurement of internal environmental conditions and the counteraction of those conditions by toggling an internal heating element. This system consisted of an Arduino R3 microcontroller, a gamut of several temperature and pressure sensors, a small resistive heating sheet, and appropriate power adapters.



The complexity of the above system led to a number of complications inherent in electrical systems operating in low pressure environments. In addition, the viability of Arabidopsis seeds in extreme upper-atmospheric conditions was not well understood. Several tests were performed to define the temperature and pressure conditions required to retain viable seeds. For further details regarding seed environmental testing see *Biological Testing*. It was determined, through several extreme environmental seed tests, there was no need for extensive environmental control. This insight allowed for the removal of the Arduino-controlled thermal management system, greatly simplifying the payload. The design-goal to maintain internal atmospheric pressures at or near 1 atm was retained as a precautionary measure. In addition, the nature of an atmospheric containment vessel has the added benefits of high structural integrity, propensity to attenuate extreme temperature, and ability to reflect damaging solar UV radiation.

Design timeline:

January 2016

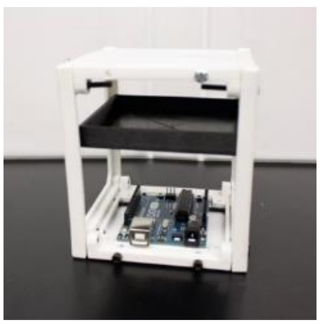


Description: "The Cube Sat" was the initial design for CRESS. The .stl file for this design was found on GrabCad by contributor, Armin Yousefi Kanani.

Advantages: Easy to 3D-print. Proper form-factor of a 1-Unit cube satellite.

Disadvantages: Thin walls. Difficult to access inner components. Incapable of containing 1 atm. of internal pressure.

February 2016

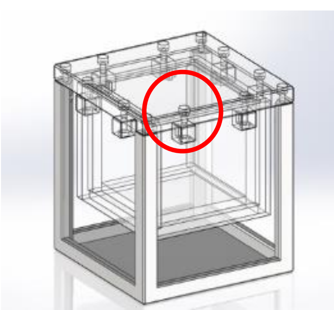
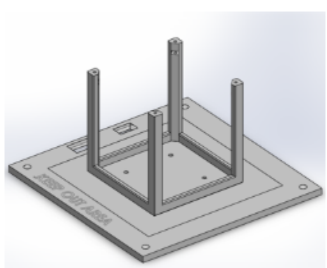


Description: "Ardusat" was the second form-factor for the CRESS payload. This design was created by the company, Ardusat, for specific use with Arduino microcontrollers. At the time, CRESS still implemented the use of and Arduino R3 microcontroller for thermal and data management.

Advantages: Easy to 3D-print. Proper form-factor of a 1-Unit cube satellite. Easy to access inner components. Easy to disassemble.

Disadvantages: Failed drop testing. Incapable of containing atm. of internal pressure.

March 2016

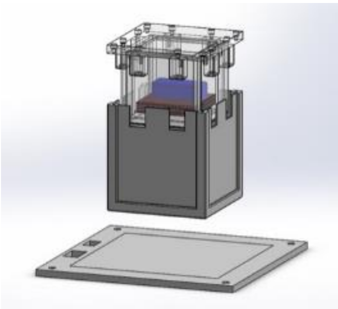




Description: March marked the greatest change in the design of the CRESS payload. The need for a containment vessel and a simple integration system motivated the development of a custom design. In addition, seed exposure testing proved that advanced thermal life support was not necessary, leading to a simpler design that required only a simple, self-contained datalogger. The original design called for a CNC-cut acrylic containment vessel (clear structure) and 3D-printed support structure (Middle: shown attached to the HASP integration Plate). This design eventually evolved to become the final iteration that flew in New Mexico.

Advantages: Easy to 3D-print support. Proper form-factor of a 1-Unit cube satellite. Cheap materials. Large internal volume for seeds and datalogger.

Disadvantages: Difficult to seal CNC-cut acrylic (incapable of containing 1 atm. pressure). Flimsy support structure. Flanged bolt supports (circled in red) of 3D-printed-acrylic-substitute containment vessel failed lid tightening test.

April 2016



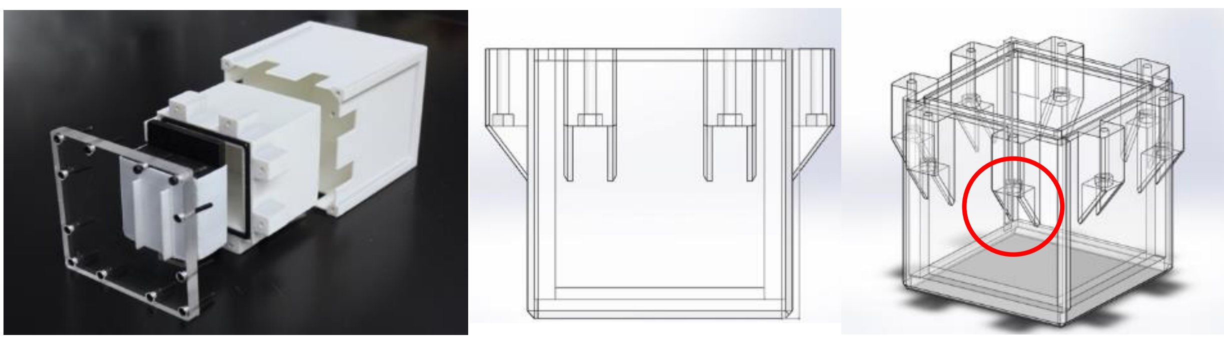
Description: Several important developments were made in April including larger flanged bolt supports and side-walls added to the support structure. The use of acrylic for the containment vessel was abandoned and instead the containment vessel was 3D-printed using Makerbot FDM* technology. However, several vacuum chamber tests proved that the 3D-printed containment vessel was incapable of holding 1 atm.

Advantages: Stronger support. Easy to integrate the containment vessel. Containment vessel is structurally sound

Disadvantages: Still incapable of containing 1 atm. of internal pressure.

* Filament Deposition Modeling. The most common 3D-printing technique. Used by Makerbot and Craftbot.

May 2016

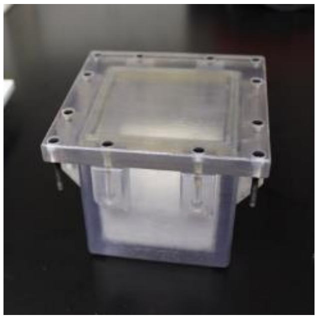
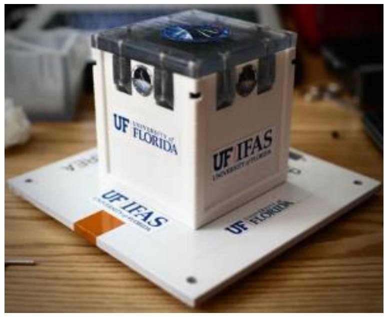


Description: In May of 2016 the containment vessel (CV) final design was completed. The walls were thinned and additional supports were added to the flanged bolt supports (circled in red). The CV successfully contained 1 atm. of internal pressure through multiple exposures to low pressure. Minor edits to the support structure enabled quicker integration of the CV and seamless integration to the HASP integration plate.

Advantages: Objet Eden Veroclear model capable of containing 1 atm. of internal pressure. Quick and efficient integration and reintegration. Robust CV and support.

Disadvantages: FDM CV still incapable of containing 1 atm. internal pressure. Difficult to access biological payload and MSR within CV after lid is tightened down. Veroclear print somewhat sensitive to high heat exposure (see section *Thermal Testing).*

June 2016

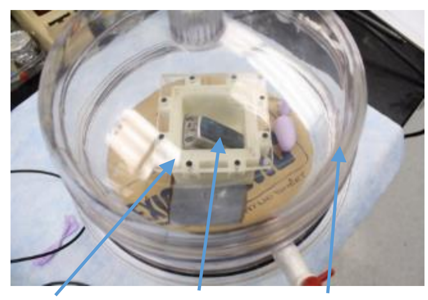
Description: Left: Final print of the containment vessel to be used for flight in New Mexico. This model was used during Integration and Thermal-Vacuum testing in Palestine, Texas proving that the payload was capable of fulfilling all desired features. Slight warping was observed after extended time bolted shut. However, there were no issues with the CV's ability to contain 1 atm. of internal pressure.

Advantages: Performed nominally through several environmental exposure tests. The clear material allows for easy assessment of internal data logger status.

Disadvantages: Difficult to access biological payload and internal datalogger after CV is bolted shut. Slight warping in high heat.

Vacuum Chamber Testing

Initial pressure testing was completed using a dome vacuum chamber. The containment vessel designs were bolted shut and exposed to the low pressure environment of the vacuum chamber for approximately 30 minutes. Absolute pressure and temperature inside the containment vessel was recorded using an MSR datalogger. This particular vacuum chamber is capable of creating a reduced pressure environment near 250mbars. Although this pressure is much higher than that experienced during a high altitude flight, these tests proved useful for the initial verification of the containment vessel seal.

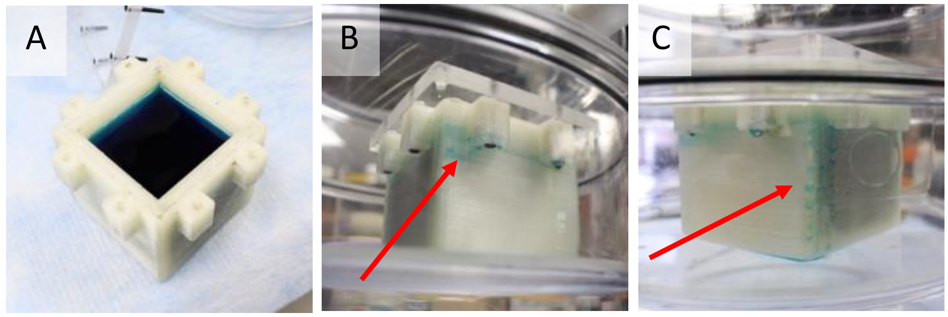


Containment Vessel MSR Vacuum Chamber

3D-Printed Containment Vessel

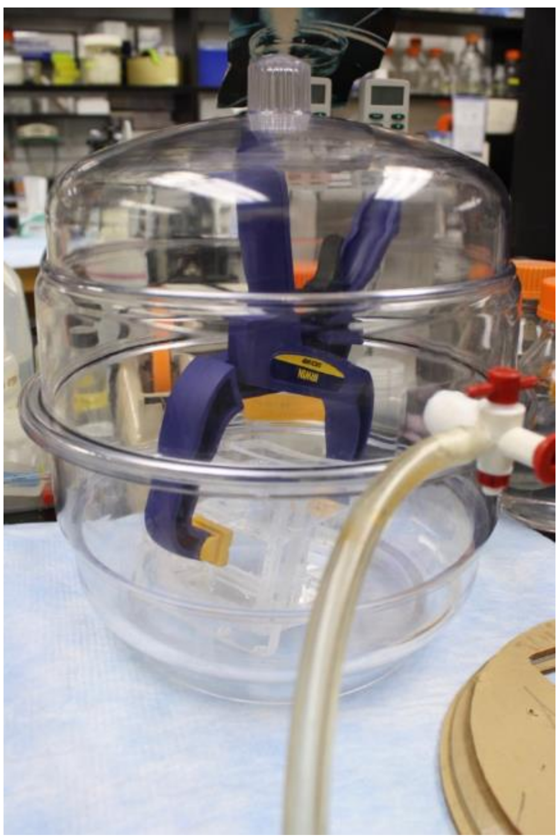
It was observed that the above containment vessel configuration (Acrylic top, 3D-Printed base) cannot maintain normal atmospheric pressure upon pulling a vacuum. The seal between the acrylic top and the 3D-printed base was first assumed to be the point of failure. However, it was discovered using a simple test that the 3D-Print is actually the point of failure. Filament Deposition Modeling, a technique Craftbot and Makerbot 3D-printers utilized to produce prints, often leaves tiny spaces between layers of filament. It is through these tiny holes that allows passage of gas and fluids.

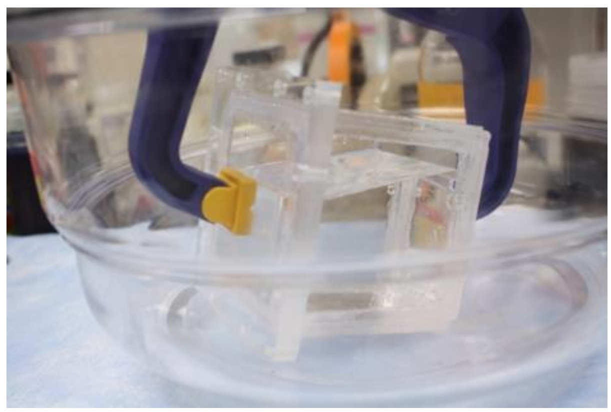
Water colored blue using food dye was used to pinpoint the location air leaks. Soon after lowering the pressure within the chamber, blue dye was seen permeating the walls of the 3D-printed base as seen in photos B and C to the right.

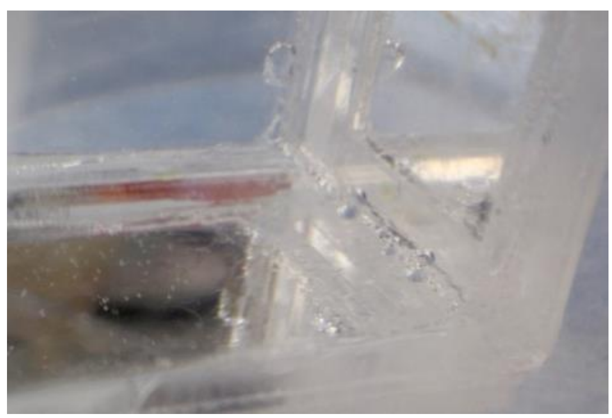


Acrylic Containment Vessel

An acrylic containment vessel was constructed in parallel to a 3D-printed containment vessel. The acrylic was milled using a –axis CNC mill. The individual acrylic cuts were cemented together using 16 Fast Set acrylic cement from SCIGRIP™. Upon pulling a vacuum on the water-filled acrylic vessel bubbles began to form on the inside of the acrylic. There are clearly small holes along the cement points. These inner edges were then sealed, but did not hold pressure under vacuum.



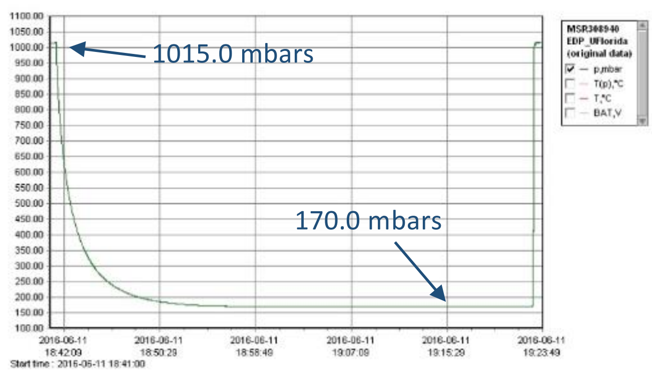




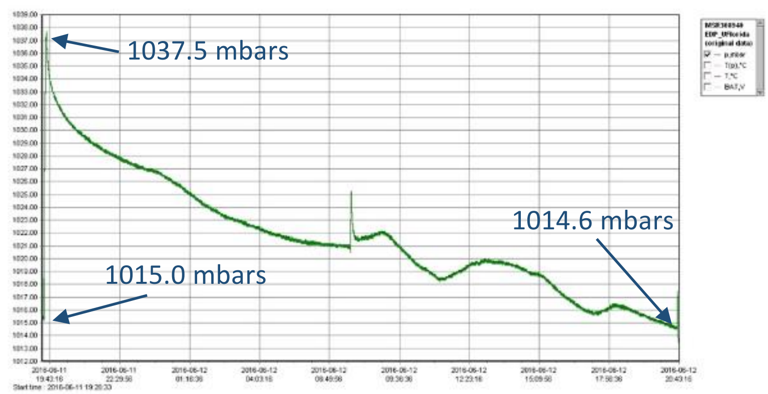
The final design in VeroClear resin, printed a containment vessel that performed well during a 25 hour low pressure test @ ≈ 170 mbars. An MSR datalogger was used to record the pressure within the vacuum chamber and within the containment vessel. Ambient atmospheric pressure hovered around 1015 mbar. Upon attaching and tightening the top of the containment vessel to the bottom of the containment vessel the internal pressure jumped to 1037.5 mbars. Over the course of the 25 hour test the pressure within the containment vessel fell from 1037.5 mbars to 1014.6 mbars. This equates to a loss of 0.9 mbars of internal air pressure per hour the containment vessel is exposed to the low pressure environment.

FIGURE

GRAPH A: Pressure within Vacuum Chamber



GRAPH B: Pressure within Containment Vessel



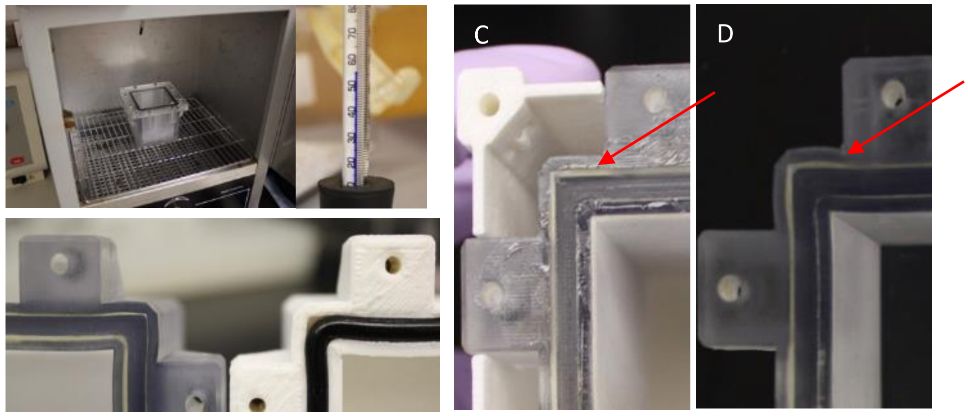
Graph A stands as the control for Graph B. Graph B illustrates the pressure environment within the containment vessel as it is subjected to the 170 mbar environment within the vacuum chamber. The noticeable jump in pressure half-way through the test was due to a temporary loss of vacuum caused by a vacuum pump malfunction.

A follow up test was performed at NASA KSC which subjected the containment vessel to a 0.045 mbar environment. The containment vessel behaved comparable to the initial test. The internal pressure environment of the containment vessel was recorded for the 8 hours leading up to and the 8 hours following low pressure testing at KSC. It has been determined that external temperature has a far greater impact on the internal pressure compared to the external pressure. When placed within a refrigerator for 5 hours the vessel experienced a drop of 50 mbars as compared to a drop of 20 mbars when subjected to a 0.045 mbar external pressure environment. These drops in pressure, whether from external temperature or external pressure, are insignificant and their effects lie well within the pressure bounds for the success of the primary mission goal.

Thermal Testing

The FDM model and the VeroClear resin model were both subjected to temperatures of 50˚-55˚C for 12 hours. The containment vessels are under considerable stress when the bolts are tightened to ensure a proper seal between the top and bottom. In the figure below, it is obvious that this stress caused a deformation in the structure's shape compared to the shape before thermal testing. This slight deformation of the material however, did not affect the containment vessel's ability to hold pressure and did not cause any harm to the HASP gondola or other payloads.

A: The oven used to test both the FDM and Resin model. B: Both the Resin and FDM model show comparable deformation after exposure to 55˚C for 12 hours. This was expected as both material have similar HDT ˚C @ .45Mpa values. C and D: indicated by the red arrows is the location that showed the most significant deformation. This deformation did not significantly effect the internal dimensions of the containment vessel thereby allowing easy integration and removal of the biological payload and MSR datalogger.



Biological Payload

Biological Testing

At the time of initial payload design, the survivability of Arabidopsis seeds in the upper atmosphere was not well understood. In order to facilitate the development of the CRESS payload, environmental testing was performed. The results of these tests dictated the technical development of the CRESS payload.

Temperature Seed Testing (RT, +40, -80)

Seeds were exposed to Room Temperature, 40˚C and -80˚C for 5 days. The seeds were then sterilized and allowed to germinate on sterile nutrient agarose gel plates.

Condition Tested	# of Seeds Planted	# of seeds Germinated	% Germination
Room Temperature	141	139	98.6
+ 40˚ Celsius	191	186	97.4
- 80˚ Celsius	217	214	98.6

For the control, room temperature seeds (24˚-27˚C), the germination rate was 98.6%. Germination was slightly lower for the +40˚C and the same for the -80˚C seeds. These tests suggest that seeds can survive and germinate well in conditions more extreme than those recorded during previous HASP flights.

Temperature/Pressure Seed Testing (RT, +40, -20 ˚C @ 1 atm. and 8mbars)

Columbia-0 seeds were exposed to conditions similar to those present during a high-altitude flight. Despite extremes of both temperature and pressure high germination rates were observed. The following study was supported through the guidance and hardware of Dr. Andrew Schuerger.

Temperature (˚C)	Pressure (mbars)	Germination Rate (%)
-20	1020	230/234 98.2
-20	8	231/234 98.7
24	1020	231/232 99.6
24	8	208/208 100
40	1020	191/193 99
40	8	202/207 97

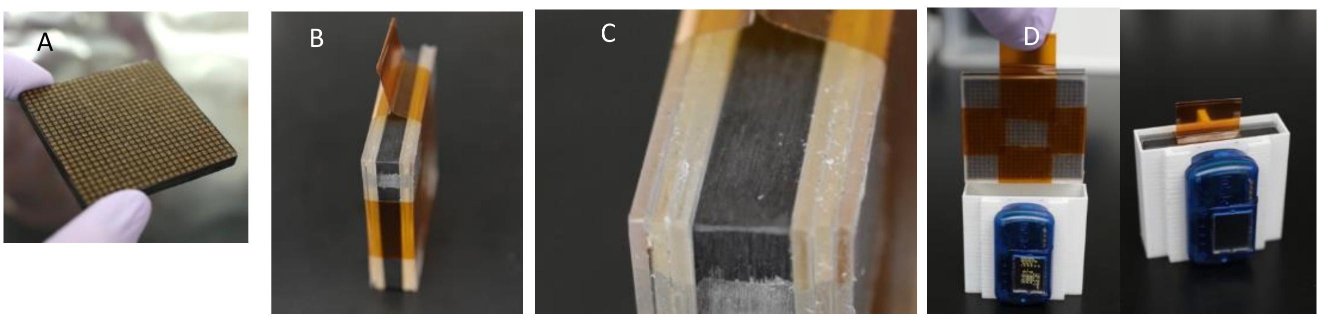
Note: this document is intended for the fulfillment of agency reporting requirements and not intended for release to the public.

These result indicate that the combination of extreme temperature and pressure environment observed during flight will not significantly affect the germination rates of plant seeds.

It was obvious from these tests that biological life support was not necessary. These result greatly simplified the CRESS payload by reducing payload weight and cost and increasing the design efficacy of our system.

Design

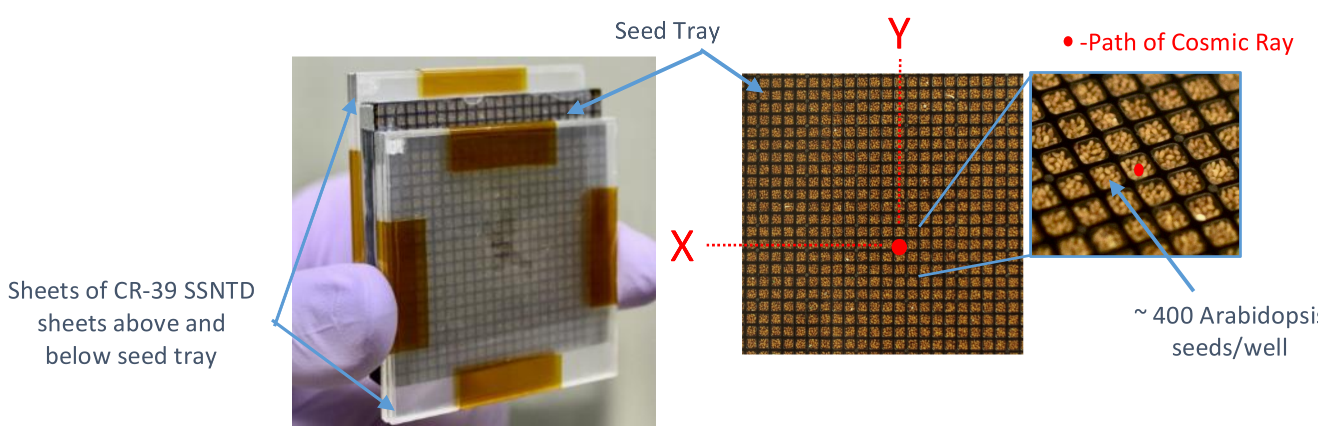
The biological targets of this study, 250,000 *Arabidopsis thaliana* seeds, were organized in a modified 1500 well plate (seed cassette) and stored within the containment vessel. To determine the location of potential Galactic Cosmic Ray hits, CR-39 sheets (supplied by Track Analysis Systems Ltd.) were organized around the seed cassette. The orientation and order of the CR-39 sheets were well documented and this position was used to pinpoint hit locations, and trace the path of events through the seed cassette post-flight.



A: Image of the loaded seed array containing approximately 250,000 Arabidopsis seeds. B: Assembled seed array and CR-39 SSNTD film insert. C: Close-up image of the insert detailing the three CR-39 sheets (clear/white) placed above and below the seed array (black) for a total of 6 CR-39 sheets. D: Image of seed array and CR-39 sheets assembly being inserted within the holder (with MSR datalogger attached).

Determining Hit Location

Seeds and SSNTD sheet are de-integrated post-flight. SSNTD sheets are processed and analyzed for impacts by GCR's. X, Y, Z Coordinates of possibly impacted seeds are determined. Seed are located, removed from the seed tray, and prepared for screening.



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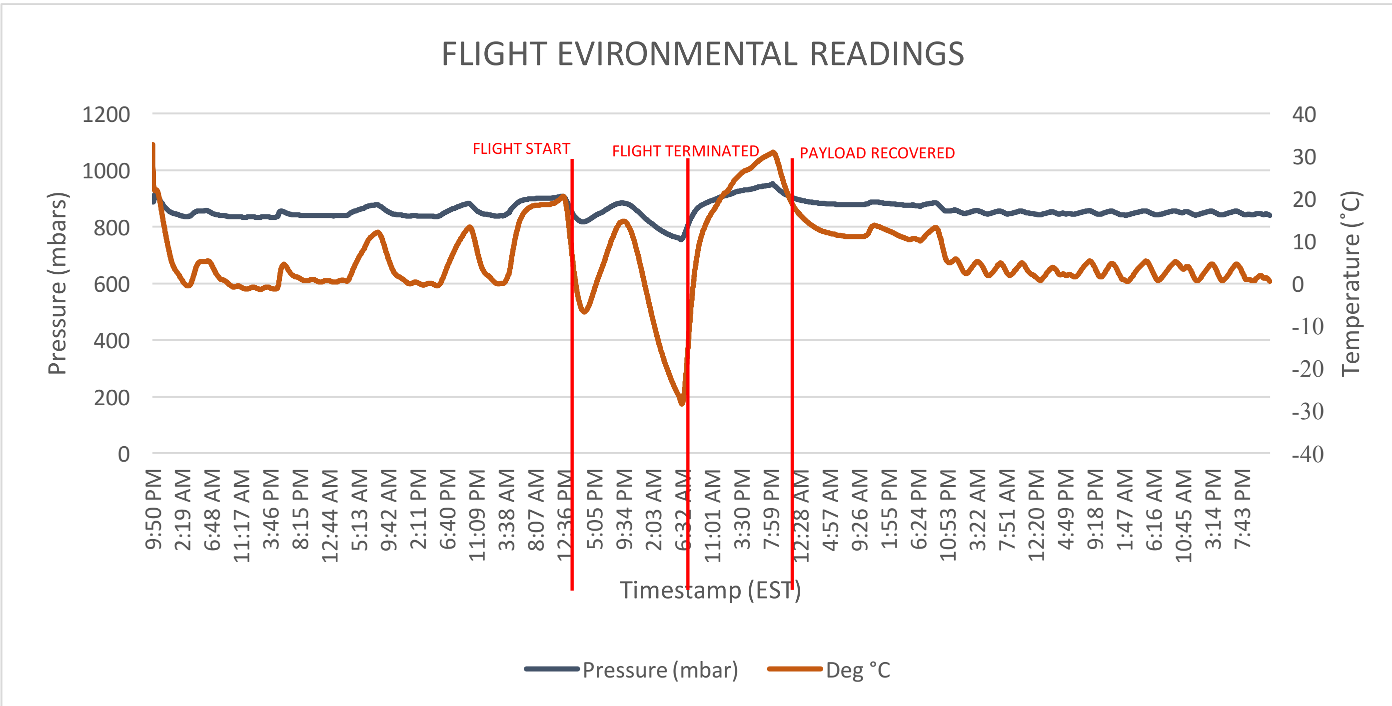
Flight Operations – Fort Sumner, New Mexico

Flight and payload recovery was an overall success. The CRESS containment vessel and support structure withstood the unique temperature, pressure, and *g* environment experienced during the high-altitude balloon launch, 15 hour 8 minute flight, and final impact.

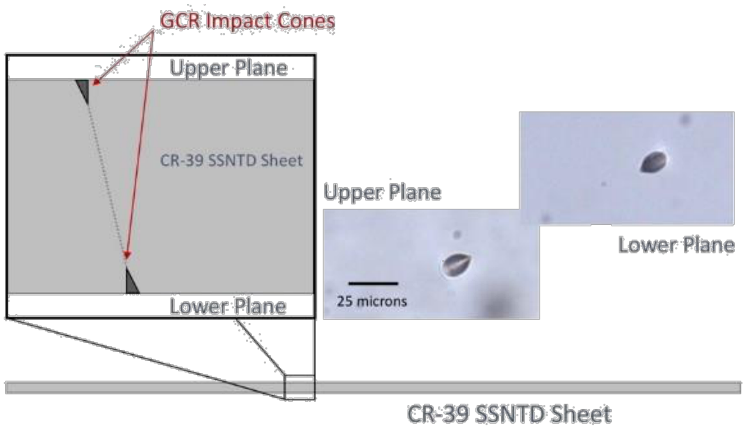


Internal temperature and pressure environmental readings from the MSR datalogger show that the containment vessel performed just as expected and are consistent with pre-flight environmental testing. The payload was recovered and stored on-ice for approximately 12 hours after touchdown. CRESS's integration location within the HASP payload attenuated the harsh desert sunlight and in effect the internal temperature of CRESS only temporarily rose above 30˚C; well within the viability limits of the biology.

Post-Flight Processing

CR-39 Processing

In order to expedite the screening process, etching of the CR-39 sheets has begun. Initial analysis shows possible GCR impact sites. The impact location results from CR-39 etching will dictate the selection of seeds for growth and subsequent screening on sterile growth plates.



Etched CR-39 SSNTD sheets are viewed under a compound microscope to assess impact locations. Impacts form conical holes on both the upper and lower plane of the SSNTD sheets. Pictured right is a possible GCR impact on CR-39 sheets flown on the 2016 HASP flight.

The biology and solid state nuclear track detector (ssntd) sheets are de-integrated and currently stored in a 4˚C refrigerator and -20˚C freezer, respectively. Testing and initial processing of both the biology and the ssntd sheets is underway. Early screening of a flight CR-39 ssntd sheet suggest putative cosmic ray impacts but further analysis is necessary.

Seed Screening

In order to determine the presence of galactic cosmic ray induced genetic mutations that result in a phenotypic change, flown seeds must be screened in such a way that both roots and shoots are examinable. The high quantity of flown seeds also poses a number of logistical challenges. We must balance the use high throughput screening techniques without sacrificing the fidelity of the screening process. In our initial assays, the micro-bin of seeds indicated by the CR-39 trace paths were separated from the remaining seeds in the tray and then planted on solid nutrient plates for growth and evaluated for deviant phenotypes.

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Poster Presentations

A. M. Ward[1], R. A. Schmitz[1], N.J. Sng[2], R.J. Ferl[1-3], and A-L. Paul[1-2]. *Screening for Galactic Cosmic Radiation Induced Mutations in Arabidopsis Seeds*

- UF Genetics Symposium – Gainesville, Florida – November 2016

R. Austin. Schmitz, Ana M. Ward, E. R. Schultz, N. J. Sng, A-L Paul, R. J. Ferl *A Technological Development Study of the Effects of Cosmic Radiation Exposure on Seeds* – Poster Presentation

- American Society for Gravitational and Space Research – Cleveland, Ohio – October 2016

Awards

1st Place – Student Undergraduate Poster Competition, American Society for Gravitational and Space Research Conference, Cleveland Ohio

Funding Awarded

$6000 – NASA Florida Space Grant Consortium: Unsolicited Proposal for the Cosmic Radiation Exposure System for Seeds (CRESS) Project

$2259 – University Scholars Program: Undergraduate student research support

$500 – University Scholars Program Travel Stipend: For travel related expenses to present the CRESS project at the 2016 ASGSR Annual Meeting