Analyzing Plant Growth After a Flight to an Altitude of 30,000 Meters via a CubeSat Satellite Carried by a High-altitude Balloon

Jake Pearman², Alysa Suissa¹, Marcos Klingler¹, Andre Fernandes², Dr. Oscar Curet² (Faculty Advisor), Dr. Xing-Hai Zhang¹ (Faculty Advisor)

¹ Charles E. Schmidt College of Science, Department of Biological Sciences, Florida Atlantic University
² College of Engineering and Computer Science, Department of Ocean and Mechanical Engineering, Florida Atlantic University

Abstract

Knowledge of the effects of space travel on plants’ growth and development has tremendous importance for the future of human exploration and colonization of the Moon and Mars, and in discovering methods for growing food in outer space. Environmental conditions at high altitudes are known to be hostile to living beings, including plants, due to low temperature, low pressure, and high levels of cosmic radiation. To explore the feasibility of growing plants during space flight, a miniature satellite known as CubeSat was developed by a team of undergraduate students. This prototype CubeSat contained an automatic sensors-equipped Environmental Control System (ECS) to house a plant growth chamber. The CubeSat carrying several alfalfa and tobacco seedlings flew to an altitude of 30,000 meters above sea-level by a helium-filled balloon. After a 27-minute flight, the CubeSat safely landed, and the plants were retrieved. The sensors worked as designed and the data during the flight were recorded and saved. The plants were recovered from the extreme conditions and grown normally in the lab. This experiment has demonstrated that the prototype CubeSat/ECS worked as expected and could serve as a cost-effective platform for future study of plant growth during high altitude or space travel. The results of this endeavor demonstrated a successful interdisciplinary collaboration and should be of significant interest to NASA, as well as other international space agencies to pursue a future of growing plant food in space.
INTRODUCTION

Understanding plant growth and development in out-of-Earth environments is vitally important not only for astronaut health, but for the success of future long-term off-Earth habitation (Wamelink et al., 2014). The increasing interest in space exploration, demonstrated by initiatives like NASA’s Artemis and SpaceX’s Starship, have raised crucial questions about cultivating food in space and on other astronomical objects. Particularly, little is known about the resilience of plants exposed to elevated levels of radiation in places other than Earth and the feasibility of plant growth in Lunar and Martian regolith (NASA Science Editorial Team, 2022). The risk of space radiation to both humans and plants is a great concern. To assess and understand the risks of cosmic radiation, NASA in collaboration with the U.S. Department of Energy has established the NASA Space Radiation Laboratory at Brookhaven National Laboratory (La Tessa et al., 2016). A high-power ion beam is used to simulate cosmic radiation and evaluate the effects on cells and different equipment. Even though experiments with the NASA Space Radiation Laboratory have been highly valuable to understand the radiation in plants (Zhang et al., 2022), experiments using actual space radiation for an extended period will be critical to examine the long-term effects on the growth, yield and development of plants. Space radiation is generated by various forms of high energy particles (e.g. protons, ions) emitted from the Sun or from outside the Solar System moving at a speed close to the speed of light. On Earth, the magnetic field and atmosphere provide natural protection from this radiation. Plants grown in the space environment would not have the protection that is naturally occurring on Earth.

The establishment of Lunar outposts, such as the Artemis Program’s Gateway, underlies the urgency for low-cost investigations on the effects of chronic cosmic radiation and different soil media on various plant species (Connell, 2023). Despite the advances and numerous experiments of growth chambers on the International Space Station, there are still many critical gaps in knowledge for cost-effective, optimal plant production in space environments, including, among others, the effects of gravity, radiation, CO$_2$, pressure, airflow and pollination in space, as well as the development of water delivery/recovery systems, hardware/sensors, and automated processes for optimal yield. Understanding these effects is vital for the design and development of Lunar and Martian plant life,
contributing to the success of future space missions (Davies et al., 2003).

NASA’s efforts to explore radiation effects involve platforms like the Materials International Space Station Experiment-3/4 (MISSE-3/4) and the Space Radiation Laboratory (Reckart, 2021; Brookhaven National Laboratory, n.d.-b). The MISSE program, initiated in 2001, utilized basil seeds to study effects of radiation on plant germination and growth in space, however, the results were not officially published (Reckart, 2021). In 2003, the Space Radiation Laboratory was established to emulate space radiation’s effects on materials, organisms, and spacecraft (Brookhaven National Laboratory, n.d.-a). Nevertheless, challenges persist in replicating the continuous low-dose rates of space radiation found in chronic cycles on Earth (Simonsen et al., 2020).

Regarding regolith studies, NASA stationed the Veggie vegetable production system (VEG-01) and Advanced Plant Habitat (APH) on the International Space Station (ISS). The Veggie vegetable production system (VEG-01) is a small plant growth chamber to grow vegetables in space (Massa et al., 2017). VEG-01, initiated in 2014, relies on astronaut integration and lacks independent functionality. The APH, developed in 2017, is an autonomous system capable of having minimal astronaut interaction (Sempsrott, 2023). Despite its success in producing natural and nutritious crops, the APH is bulkier and requires a higher power output compared to Veggie (Costa, 2021), which would be cost prohibitive for growing plant food on the Moon or Mars.

As part of an undergraduate research project, Florida Atlantic University engineering students attempted to develop and optimize a self-contained robotic system to grow plants during space travel. This project addresses gaps in knowledge and the need for low-cost, high-volume data analysis via investigation of the potentiality of CubeSat for autonomous plant environments in space. Successful completion of this project required hands-on skills and problem-solving ability in the domains of engineering, such as systematic integration of various self-controlling and automatic data recording components for many parameters, and the technical procedures for launch of the CubeSat to space. It also required working knowledge and technical know-hows in the research areas of plant biology. Therefore, this project is a collaborative effect in the interdisciplinary investigation of astrobiology, a venture that bridges the realms between engineering and biology.
The CubeSat designed by us, featuring autonomous and integrative systems for CO$_2$-releasing, watering, lighting, atmospheric regulation, and sensor data collection, strives to serve as a research platform for beyond-Earth farming techniques. The CubeSat containing a plant growth chamber was launched on a high-altitude balloon to simulate an Earth orbit. It monitored plants during the flight, with automatic inputs and storage of environment parameters and ECS state data. Our CubeSat adhered to NASA’s CubeSat structural, vibration, and thermal launch requirements, with the High-Altitude Balloon following FAA restrictions.

Plants play a significant role in the field of biological sciences, both directly and in aiding space exploration endeavors. Plants offer invaluable insights into gravity, radiation, and other biological phenomena. They contribute significantly to advancing our understanding of how living things adapt to the unique environments encountered in space exploration (Paul et al., 2022).

As a first step towards understanding plant growth response in space, we chose to test alfalfa and tobacco plants. Alfalfa is a fast-growing, hardy, and nutritious legume plant, which may serve as a viable candidate for food supply for long-term space travel (The Editors of Encyclopaedia Britannica, 2023). Tobacco is a genetic model organism widely used in genetic and biotechnology studies. We decided to take advantage of the numerous genetic varieties created in our lab that were particularly suitable for studies of environmental stresses such as cold and radiation (Hill et al., 2016; Murashige & Skoog, 1962). Via a high-altitude balloon carrying the CubeSat with a self-controlled and self-contained Environment Control System (ECS) where a plant growth chamber was housed (Zabel et al., 2016), we attempted to record the growth of alfalfa and tobacco seedlings during the short trip to the Earth’s stratosphere. Therefore, this endeavor allowed us to test the reliability of the CubeSat system and to observe plants’ responses to the high-altitude environment. This project established the foundation for future improvement of launch vessels and greater investigation of biological processes of plants in non-Earth environments.

In this report, we present the efforts in the design, development and launch of the CubeSat research platform (illustrated in Figure 1) and the assessment of the growth of plants that had experienced an altitude of 30,000 meters above sea level for a brief period of time.
Figure 1. Journey of the balloon carrying a plant growth chamber in CubeSat.

MATERIALS AND METHODS
1. Design of Environment Control System for plant growth in a CubeSat

The Environment Control System (ECS) was designed to house a growth chamber that was able to hold multiple small plants in the 6-unit CubeSat, equipped with cameras and auto-controlled sensors. This CubeSat could monitor and record pressure, temperature, gas composition, humidity, and radiation. The CubeSat also contained a water delivery system for the plants. The ECS plays a vital role in maintaining optimal conditions for plants throughout the launch process. Its frame is built using A1-6061 T6, ensuring NASA material properties regarding launch are maintained, but also providing lightweight, soft metal for ease of assembly. The ECS components include the Grove SCD30, which monitors CO$_2$ levels, temperature, humidity, and a BarO2 for temperature and pressure backup data. To protect against extreme cold temperatures (~65°C) experienced during the High-Altitude Balloon mission and while in orbit, the ECS is equipped with a multi-layer insulation (MLI), an industry-standard thermal control method. Concurrently, the 5V heating elements, coupled with the heat sinks on the GrowLED PCB, ensured a
consistent temperature of 25°C during the day and 20°C at night; creating a stable environment for the plants. The GrowLED PCB also supplied a diverse color spectrum for optimal plant growth. Other components of the ECS include RaspberryPi camera boards, Pocket Geiger radiation detector, ExHale CO₂ bag, two plant pillows, and a WetLink penetrator. Overall, the ECS components, including sensors, insulation, and heating elements, work together under the control of a custom-designed PCB motherboard to safeguard plant health during launch and the mission. The ECS/CubeSat is shown in Figure 2.

![Environment Control System (ECS) and ECS & CubeSat](image)

**Figure 2. Environment Control System (ECS)/CubeSat module.** Top-left: Growth chamber. Bottom-left: fabrication of the growth chamber. Right: Growth chamber in a CubeSat setup.

2. **High-altitude balloon**

A high-altitude balloon was purchased from Kaymont Consolidated. Before the launch, helium was pumped into the balloon to achieve an expected ascent rate of 5 m/s ± 2 m/s to an altitude of 30 km ± 3 km, maintain altitude for 60-90 minutes, and descend at a rate of 3 m/s ± 2 m/s.
3. Plants as payload for high-altitude balloon

Three types of plants were used in this experiment: (1) alfalfa (*Medicago sativa*), (2) wild-type tobacco (*Nicotiana tabacum*), and (3) genetically modified tobacco named DsupR that expresses a protein called Damage Suppressor Protein (Dsup) from the tardigrade *Ramazzotius varieornatus* (Kirke et al., 2020). The Dsup gene produces a DNA-associating protein which lowers the rate of DNA breaking due to radiation, stabilizing the DNA. The transgenic plant allows for greater examination of possible cellular or genomic damages occurred during the up and down flight, exposed to high levels of radiation. In theory, the DsupR seedling should be more resistant to radiation exposure and other stresses than the wild-type plants.

The seeds were planted in the round cheese-cloth pats (also called pillows) (4-cm diameter and 3.5-cm height) filled with potting mix soil (Sta-Green) and grown in the growth room at 25°C under 8 hours of darkness and 16 hours of cool white light of ~200 μmol photons m⁻² s⁻¹. The day before launch, two pats with a mixture of two-week-old alfalfa and tobacco seedlings were placed in the growth chamber in the CubeSat. The day after the CubeSat landed and returned to the lab, the plants were removed from the soil pats, transplanted in 6-foot soil pots and grown in the growth room to maturity.

**Results**

1. Launch of a high-altitude balloon

On March 28, 2023, the high-altitude balloon was launched from Duette, Florida, carrying a 6-unit CubeSat with ECS where growing alfalfa and tobacco plants were securely placed, traveling to an altitude of 30,000 meters and 125 kilometers from west to east, and safely landed in South Florida (Figure 3). The entire flight lasted approximately 27 minutes.
2. Plant growth-related environmental conditions during the balloon flight

During the balloon flight, atmospheric pressure outside the CubeSat dropped as the balloon ascended, to -15 Hpa at the altitude of 30,000 meters (green line in Figure 4a). After the balloon ruptured and the CubeSat descended, the outside atmospheric pressure rapidly rose (grey line in Figure 4a). In contrast, the pressure inside the ECS remained steady at around 1,000 Hpa (red line in Figure 4a), which is close to the pressure at the sea level on the ground, suggesting the ECS maintained a suitable condition of pressure for plant growth.

Likewise, as shown in Figure 4b, the outside temperature decreased as the altitude increased, dropping to as low as -55°C at an altitude of 30,000 meters, which would instantly kill plants. Yet, inside the ECS maintained a plant-friendly temperature of 22 to 26°C throughout the flight (Figure 4b).

Figure 3. A short journey to “space” by the plants living in CubeSat.

- a. The balloon carrying the CubeSat in ascendance.
- b. The moments before the balloon ruptured at 30,000 meters.
- c. The CubeSat payload (yellow arrow) and parachute (red arrow) post-landing.
- d. HAB launch trajectory
Plants need a constant supply of carbon dioxide (CO₂) to survive (Wheeler et al., 2001). Inside the ECS, CO₂ was released from two CO₂-emitting bags (ExHale CO₂) installed on the walls of the ECS, so that the chamber maintained a CO₂ level of around 780 ppm (Figure 4c) to support plant growth.

**Figure 4. High altitude balloon (HAB) flight conditions.**

- **a.** Changes in atmosphere pressure (green line) or inside (red line) of the Environment Control System (ECS) and altitude (blue line) during the flight. X-axis shows flight time in seconds and Y-axis shows altitude in meter (left) and pressure in Hpa (right).
- **b.** Changes in temperature (°C) outside (blue line) or inside (green line) of ECS over flight time.
- **c.** Changes in carbon dioxide levels (ppm, green line) inside the ECS during the initial test.

The plants experienced a total ionizing dose of 0.243 μSieverts. The CubeSat was exposed to an average of 0.6 μSieverts/hour for 27 minutes, which was an equivalent value of 12.7 μSieverts/day. This radiation level is approximately 2.7 times higher than the average daily dose humans are exposed to on Earth.
The gravity at the altitude of 30,000 meters is about 99.1% of that at sea level. Therefore, gravity changes during the balloon flight should have negligible effect on plant growth.

3. Plant growth after the flight

After traveling to ~30,000 meters above sea level, the CubeSat landed by a parachute and was transported to the FAU Boca campus. The plants were retrieved from the ECS (Figure 5a and 5b), immediately transported to garden soil-filled pots, and grown in the growth chamber. Those plants grew normally, and no obvious phenotypic abnormality was observed as compared to non-flown plants. Figure 5c and Figure 5d show, respectively, the representative plants of alfalfa and tobacco 28 days and 42 days after the CubeSat landed. To verify the genotypes of the tobacco plants, leaf disks from these plants were stained for beta-glucuronidase activity (GUS staining) (Bottino, 2020). Upon completion of the GUS staining test, plants with a positive result displayed a blue color whereas negative results remained green. The DsupR plants that possess the GUS gene showed blue color, whereas the wild type plants showed green (Figure 5e).

**Discussion**

This research demonstrates the successful design of the Environmental Control System (ECS) and CubeSat, to withstand launch and return via a high-altitude balloon. The automated sensors integrated within the system have efficiently recorded and saved data on pressure, temperature, and carbon dioxide. More importantly, our research has demonstrated that plants can remain alive inside the ECS even when conditions outside become rapidly harsh and non-survivable for plants. The plants landed with no obvious damage and grew normally in the lab to maturity. We have not yet attempted analysis of any possible genetic mutations for these plants.

For future investigations, several considerations should be noted. Due to short flight time, we were not able to collect sufficient data of radiation levels at high altitudes, which is important to analyze cellular and genomic changes in plants’ response to radiation. A longer stay at the high altitude, e.g. hours instead of minutes, would provide more informative data. Additionally, the high-altitude balloon did not reach the
Figure 5. Growth of plants before and after high altitude flight.

a. Plants one day before the balloon launch.
b. Plants one day after the balloon launch and retrieved from the ECS.
c. Plants 28 days after retrieved from the ECS after balloon landed.
d. Plants 42 days after retrieved from the ECS after balloon landed.
e. Leaf-disk GUS-staining of transgenic tobacco plant expressing tardigrade Dsup gene and wild-type (Wt) tobacco plants.

necessary altitude to experience zero gravity, preventing analysis of its effects on plants. The preliminary test served to assess the feasibility of the system. For future missions, we plan to extend the duration of the plants at higher altitudes as the ECS has displayed successful results in those conditions thus far. Our long-term target is to launch this CubeSat into low-Earth orbit for extended and realistic testing. This research holds significant promise in furthering advancements in development of crops for future space expeditions, and eventual establishment of low-cost greenhouses for plant growth in Lunar and Martian soil (Wheeler et al., 2001). The findings reported here would aid in future development of innovative technologies supporting human exploration and potential habitation on other space bodies.
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**References**


