Section I. Proposal Cover Page

Proposal Title: Does microgravity affect the formation of symbiotic relationships between soy and Rhizobium?

Grade Level(s) of Submitting Student Team: Grades 13-16

Submitting School: Collin College

Submitting School District: Collin County Community College District

Submitting Teacher Facilitator:
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Proposal Summary:

The topic of discussion in this proposal is to determine whether microgravity inhibits the formation of symbiotic relationships between soybeans and rhizobium. In the future when astronauts are on missions that take them further out into the solar system, they cannot rely on Earth for food and supplies and will need to find ways to gain a sustainable food source. Soybeans were chosen as the crop in this experiment due to their nutritional value and vitamin content. In addition, they could be used in the creation of bioplastics and other industrial materials, which could be used in manufacturing replacement parts if the need arises while on mission. In a DuPont experiment conducted in 2002, soybeans were proven to be able to sprout in microgravity (SpaceRef, 2003). However, soybeans lack the capability to undergo nitrogen fixation on their own and require Rhizobium to achieve this in a process called inoculation. That process improves the yield of soybeans by 66 percent. This experiment will test to see if the formation of the symbiotic relationship is possible between soy and Rhizobium in microgravity. Inoculation will be determined by measuring if nitrogen fixating nodules are present after germination, compared with soybeans that will be inoculated on Earth. It is expected that no significant differences will be observed.

Section II. Student Team Members and Professional Advisors

Co-Principal Investigators (listed in alphabetical order)

Name: Henry Elmendorf Grade
level: 16

Name: Stefano Sacripanti
Grade level: 14

Professional Advisors

Name: Chase Brooke
Organization: Texas A&M Agrilife Extension
Contribution to Team: Soybean

Name: Cristina Sabilov
Organization: LSU
Contribution to Team: Help explain process of inoculation

Name: Marty Matlock
Organization: University of Arkansas/ U.S. Soybean Export Council Contribution
to Team: Explained soil conditions

Name: Wendy Laffoon
Organization: Visjon Biologics
Contribution to Team: Information on how Inoculants work

Name: Mark Davis
Organization: Agrauxine
Contribution to Team: explained the ratios of inoculants to soybeans

Name: Eduardo Rosetto
Organization: TerraMax
Contribution to Team: How inoculants are used in farming

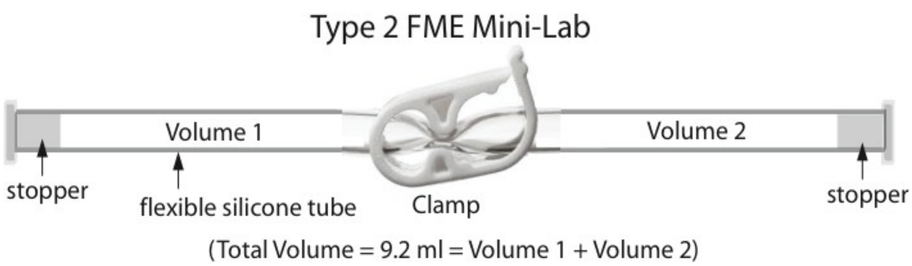
Section III. Experiment Materials and Handling Requirements

1. Fluids Mixing Enclosure (FME) Mini-laboratory Proposed to be Used (check one):

- ☐ Type 1 FME Mini-lab (1 experiment volume: no clamps used)
- ✓ Type 2 FME Mini-lab (2 experiment volumes: one clamp used)
- ☐ Type 3 FME Mini-lab (3 experiment volumes: two clamps used)

2. List of Proposed Experiment Samples (Fluids and Solids to be Used)

If you are using a Type 2 FME Mini-lab



Volume 1
List each fluid/solid to be used and the amount of each sample:

Potting Mix: 1.32g Inoculum:

15µg

Soybeans: 5 soybean seeds

Volume 2
List each fluid/solid to be used and the amount of each sample:

Distilled Water: 3.2 mL

IMPORTANT: Are any of the proposed samples human in origin? (check one):
- ☐ Yes
- ✓ No

3. Special Handling Requirements During Transportation Table

1: Requesting Thermal Control for Your Experiment

		Refrigeration	Ambient Conditions
PRE-FLIGHT	Shipping from your Community to NanoRacks in Houston		**X**
	At NanoRacks until Handover to NASA		**X**
FLIGHT	Handover to NASA Until Arrival at ISS		**X**
	Onboard ISS		**X (required)**
POST-FLIGHT	From ISS until Arrival at NanoRacks		**X (required)**
	At NanoRacks through Return Shipping to Community		**X**

4. Proposed Timeline of Crew Interactions – Your Proposed Crew Interaction Days and Crew Interactions Aboard ISS

Table 2: Proposed Timeline of Crew Interactions

Allowed Crew Interaction Day	Requested Interaction
A=0	
A+2	
U-14	
U-5	
U-2	Unclamp A, shake gently for 10 seconds

Section IV. The Question to be Addressed by the Experiment

Plants have long been studied in microgravity experiments onboard Russia's Mir, NASA's Space Shuttle program, and the International Space Station. Soybean specific experiments have ranged from simple proof that they can grow in the given environments to seed-to- seed experiments (NASA, 2002). Currently, NASA is slated to experiment with soybeans on the International Space Station once again by returning seeds produced by plants grown in microgravity to Earth, processing the seeds, and sending them back to the ISS to study whether there are any advantages to those space-grown seeds (NASA, 2022). On Earth, soybeans yield better results when the seeds are inoculated with nitrogen-fixing bacteria, like rhizobium (Brooke, 2022), forming a symbiotic relationship where the soybeans get the nitrogen they need to grow, and the bacteria are fed with carbohydrates from the soybean plant. (Mueller, 2015) It would be beneficial to be able to inoculate seeds during the duration of missions to produce the highest yields and highest nutrient-rich crops as possible.

Image 1: Soybean plants grown onboard the Space Shuttle, July 2002
Photo Credit: Wisconsin Center of Space Automation and Robotics, 2002

Image 2: "Astronaut Peggy Whitson with the ADVASC soybean plant growth experiment during Expedition 5."
Photo Credit: NASA, 2020

In our gravity environment on Earth, there are two main ways to mix the inoculant before planting the seeds: seed-applied and soil-applied (Thelen, 2018). The soil-applied method requires the application of beneficial bacteria directly to the soil to increase the health of the soil and increase the quality before planting begins. This method of inoculation is typically more expensive and much more time consuming since entire fields need to be treated with nitrogen-fixing bacteria inoculate many times before planting to improve soil health enough to support the crop. With the seed-applied method inoculant is mixed with water, coating the seed with the combined mixture, and planted quickly. However, in a microgravity environment, liquids do not behave in the same way that they do on Earth. In space, liquids conform to the shape of the container and run in stream form- they float in spheres. Could this difference in the property of water make inoculation harder? Could it be done via a combination of the two methods, combining water, inoculant, and seeds together at once in a smaller test environment? We decided to test the combined methods not only because of the limitations of the FME mini-lab (volume and clamps available), but because you don't want to mix your inoculant solution far in advance of when you use it. In this case, it would be several weeks from assembling the mini-lab to the clamp being opened in microgravity, which is a greater length of time than necessary. Due to the constraints, it was decided it may be more advantageous to mix the powdered inoculant with the dry media and dry seeds, then add the water when the time is appropriate to initiate the process.

The successful growth of healthy, high-producing, and high-nutrient soybeans would be beneficial to space missions in several ways. On Earth, soybeans are used in countless food and nutrition applications, providing a source of proteins and other nutrients. Soy is commonly found in dairy-alternative milks, vegetarian and vegan “meats”, protein bars and drinks, and often labeled as vegetable oil on supermarket shelves. The adaptability of soy in food and nutrition makes it a useful fresh food option on long missions.

Soy can also be used in the production of biofuels, which is essential to long-duration missions and non-Earth based habitats. The ability to produce fuels on-station is critical, since it is inefficient, and perhaps impossible, to bring along as much fuel as needed for these journeys and long-term habitation.

Soy is even capable of being used as a 3D printing material. The applications of 3D printing in space is vast and could be vitally important. As of 2019, 7,000 pounds of spare parts were sent to the ISS every year, with 29,000 pounds of spare hardware stored onboard, and 39,000 pounds stored on Earth awaiting its need (NASA, 2019). With the use of 3D printing those numbers could be significantly reduced onboard ISS, but for longer missions to the Moon and Mars, its use is critical. There is no quick way to get a spare part to Mars if something breaks, and it’s not practical to bring spares of everything along for the journey, so having a way to create new parts in right there and then is the only solution for long- term mission success.

Section V. Experiment Design

The question for this proposal came from the combination of researching past SSEP flight-chosen and honorable mention experiments and looking at NASA experiments. A common SSEP proposal theme is "Will ___ seed germinate in microgravity?" but that question has been sufficiently evaluated over the past 15 flown missions (SSEP, 2022). NASA has been growing a variety of plants for decades and have tried numerous technologies and methods to best grow their crops. Since basic germination and ability to grow have been thoroughly proven, the next question to answer is how to get the crops that can grow in microgravity to thrive and produce high yields in microgravity. Crops that provide a wide range of utilizations should be evaluated first. We chose soybeans as the subject for this work because of their versatility and multitude of uses. On Earth, soybeans benefit from being inoculated with Rhizobium to aid in nitrogen fixation, but it is often a process done just before planting that uses a water/ inoculate slurry to coat the seeds. We questioned whether this process could be done successfully in microgravity. Together these pieces created the proposal: Does microgravity affect the formation of symbiotic relationships between soy and Rhizobium?

This experiment will only begin to answer whether inoculation in space is possible. If successful, soybean seeds will sprout into seedlings, which can then grow into mature plants with nodules on the roots showing inoculation success. This experiment will also show if a mixture of traditional on-ground inoculation processes will yield positive results.

Within the mini-lab will be all materials necessary for inoculation and sprouting a soybean, including soybean seeds, media, inoculum, and water. The materials used are all easily obtained and do not require special documentation. Distilled water can be purchased off the shelf from any local grocery store and was chosen for use over tap water to avoid any imperfections within the local tap water, and any differences between samples collected at different times. Soybean seeds may be acquired from the Collin College Agriculture program seed selection if available or purchased online. Media can be purchased off the shelf at any local home improvement store, gardening center, or farm supply, and is sterilized before bagging. An autoclave is also available to sterilize any samples, should the need arise. Rhizobium inoculate may be acquired from the Collin College Agriculture program if available or purchased online.

This experiment is initiated when assembling the FME mini-lab. During this process the clamp needs to be placed and closed in the correct position prior to any other actions, approximately 2/3 of the way down the length of the mini-lab. Next, a selection of 5 appropriately sized soybean seeds are chosen that can easily fit the inner diameter of the tube, though most are a snug fit due to the larger nature of soybean seeds. The seeds are layered inside the larger section of the tubing with ~1.32g of sterilized media with ~15µg of Rhizobium inoculant mixed in, and the stopper placed on that end. Using the opposite open end, ~3.2mL of distilled water is added to the mini-lab, and the final stopper is inserted.

Once onboard the International Space Station, the experiment will remain in waiting until the final action day. At this time the clamp will be opened, the water will be allowed to enter the media/ inoculant/ seed mixer, some gentle shaking will aid in water infiltration, and it will be returned to its storage container to await the return to Earth. The introduction of water not only allows the inoculant to help stick to the seeds, but begins the process of imbibition and seed germination. By waiting until the last action day, the seed will only just begin its growth cycle and be inoculated in microgravity before swiftly returning to be sown and grown back on the ground.

Image 3: Testing materials

Image 4: Testing different numbers of soybeans within the mini-lab

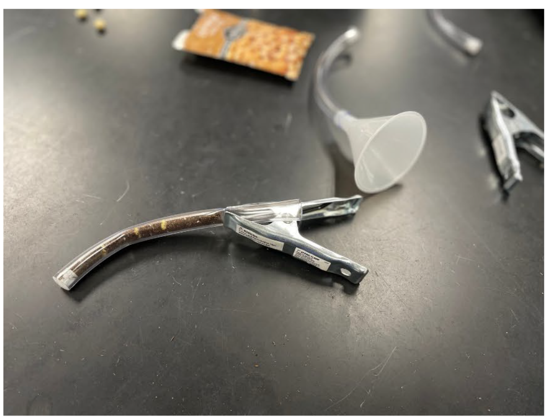


Image 5: Trial mini-lab with a clamp in-place prior to adding water

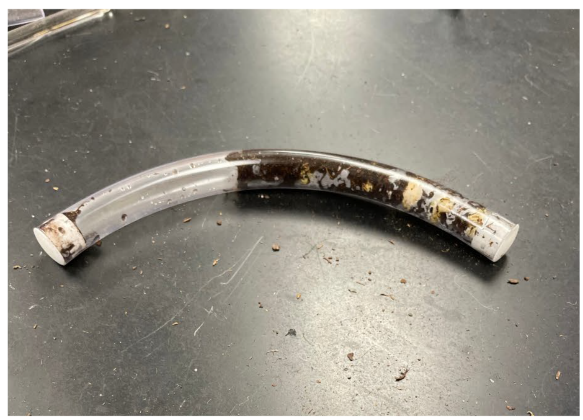


Image 6: Trial after adding materials, releasing the clamp, and shaking

The ground elements necessary for this experiment take place post-flight as a continuation of the started experiment. The goal of the experiment is to inoculate the soybeans in-orbit, but the way to prove success is only by allowing the plant to grow and seeing what happens. Once the mini-lab returns, seeds/ sprouts will be planted into 3-inch seedling containers with sterilized media, transferred after 2-3 weeks to a 1-quart pot as they grow bigger, then approximately 2-3 weeks later transferred into a 1-gallon pot. After the plant has grown large enough, it will be carefully uprooted, and the roots and nodules will be observed. During the growth period the plants will be cared for in the Agriculture program lab under grow lights, receiving water, and being monitored. Typically, a ground-planted seedling takes approximately 2 days to sprout, and may take a week (or more) to break through the soil. From this point it may take 4-5 weeks for the sprout to develop and mature to a size that is mature enough for nodules to be sufficiently formed for observations on inoculation to take place.

Final analysis will take place after several weeks once the plant has grown to a mature enough size for nodules to have formed on the roots. Nodule growth is the key indicator of inoculant success. A further indication of success within the nodules is the observation of pink flesh inside (Voight, 2018). If the nodules are cut open after uprooting and show pink, inoculation has been successful, if the flesh is not pink, it has not been successful.

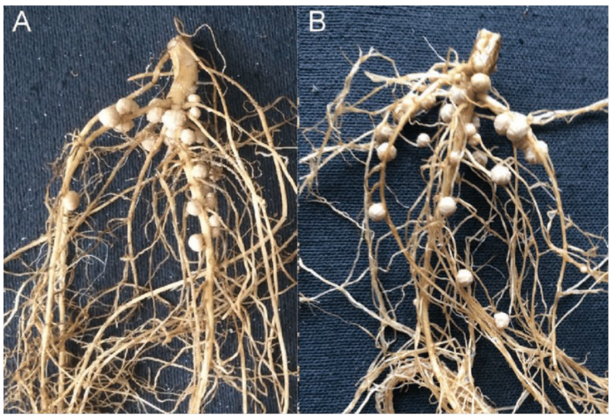


Image 7: Soybean root nodules
Photo Credit: Vorobey, et al., 2022

Image 8: Pink inner flesh indicating Rhizobium inoculation
Photo Credit: Mariangela Hungria, 2018

Regardless of the results, this experiment would only be the first step in a longer process of answering if inoculation can occur in a microgravity environment. Could it work in the gravity environment of the Moon or Mars, which each have a higher gravity than the ISS in orbit, but lower than on Earth? Could inoculation be successful in deep space on long- duration travel missions? This experiment could act as the first step in a proof-of-concept to the larger project of proving inoculation can be successful in space environments, and the best ways to accomplish inoculation. Once the methods have been established, the production of soybean crops can be maximized and utilized in all the previously discussed methods and more.

Section VI. List of Reference Publications

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Xie, D.-M., Zhao, X.-L., Li, Y.-D., Weng, Y., & Zeng, J.-B. (2022, November). *Biobased dynamic polymer networks derived from castor oil and anhydrous piperazine*. Industrial Crops and Products. Retrieved, October 2022, from https://web-s-ebscohost-com.library.collin.edu

Section VII. Letter of Certification by the Teacher Facilitator

November 2, 2022

I certify that the student team designed the experiment described herein and authored this proposal, and not a teacher, parent, or other adult. I recognize that the purpose of this letter is to ensure that there was no adult serving to lead experiment definition and design, or write the proposal, and thereby provide content and/or professional expertise beyond that expected of a student-designed and student-proposed experiment.

I also understand that NCESSE recognizes that facilitation of thinking across the student team through advice and counsel by the team's Teacher Facilitator, other teachers, and local area and national researchers, is not only to be encouraged but is absolutely vital if students are to receive the necessary guidance on the process of scientific inquiry, experimental design, how to do background research in relevant science disciplines, and on writing the proposal. I also understand that it is appropriate for the Teacher Facilitator and other teachers to provide editorial comment to the student team on their proposal drafts before proposal submission.

I also certify that the samples list and the special handling requests listed in this proposal are accurate and conform to the requirements for SSEP Mission 17 to ISS. I confirm that the team, after reviewing their procedure and budget for obtaining the samples for the experiment, is certain that they will be able to obtain the necessary samples for their experiment in time to meet the deadline for shipping the flight-ready FME to NanoRacks. If using human samples, the team is aware that these samples must be tested for prohibited viruses before the experiment can be selected for flight.

Finally, I certify that the student team will have access to the proper facilities and equipment to prepare the FME mini-laboratory for flight and to analyze the samples after the flight.

Tamara S. Basham

Tamara S. Basham
Teacher Facilitator