Effect of Gravitational Changes on Susceptibility of Cotton Seedlings to *Xanthomonas* Leaf Spot Disease.

Introduction

Plants are found to exhibit molecular stress when flown in reduced and microgravity environments, leading to the question if this stress changes a plant's interaction with disease causing organisms. This experiment will investigate whether brief changes in gravity impact cotton's susceptibility to the disease causing agent, *Xanthomonas*, a bacteria responsible for foliar leaf blight of cotton. Students will need access to cotton seed, bacterial inoculum, and guidance on inoculation techniques.
Students gain valuable experience in basic plant anatomy and physiology, plant-disease interactions, current research trends in space science, gravitational force, engineering, experimental design, and critical thinking, even before the actual flight.

Note that once seed or seedlings are flown in microgravity, any number of experiments can be conducted. Therefore, these projects can be modified for many aspects of plant health. .

Target Grade Level: 6th-12th
Suggested Time Frame: 30 Days (due to plant growth and disease development)

US Next Generation Science Standards (NGSS)

Force and Motion PS2.A, PS2.B
Space Systems ESS1.A, ESS1.B
From Molecules to Organisms: Structures and Processes LS1.1, LS1.2, LS1.3, LS1.4, LS1.5, LS1.6
Ecosystems, Energy, and Dynamics LS2.1, LS2.2, LS2.4, LS2.5
Biological Evolution: Unity and Diversity LS4.4, LS4.5, LS4.6

Objectives:

- To investigate whether changes in microgravity causes changes in susceptibility of cotton seedlings to *Xanthomonas*
- To investigate plant-pathogen interactions
- To gain experience in measurements, data collection, and analysis
- To bring forward and investigate new questions in the field of space science
- To provide an authentic learning experience for students which deepen understanding of force and motion, space exploration, plant biology, microbiology, and engineering design process, and scientific inquiry.

Background:

This is a student driven, authentic research project investigating whether changes in gravity could result in a plant stress response causing changes in susceptibility to disease causing organisms, such as bacteria and nematodes.

This will need to be a collaborative project between the middle or high school and a university. For this experiment, seeds were germinated prior to flight, with half flying on board G-Force One. All seedlings were used to study infection susceptibility by *Xanthomonas* bacteria and Reniform nematode.

Bacterial blight (*Xanthomonas citri subsp. malvacerarum*)

Bacterial blight, or angular leaf spot, is a common disease on cotton, causing spotting , cankers, and defoliation of the plant. The bacteria enters leaf tissue through stomates or wounds, and survives in crop residue left in the field. High humidity and warm temperatures favor disease development. It has been shown by TAMU Department of Plant Pathology that environmental stress may contribute to an increase in susceptibility to secondary pathogens. Students at Salado Middle School would like to know if gravitational changes cause stress to the plant, resulting in changes in susceptibility to disease.

Bacterial leaf blight on mature cotton leaf and on cotton seedling

Purpose:

Do gravitational changes affect susceptibility of cotton seedlings to bacterial leaf blight (*Xanthomas sp*.)?

Hypothesis:

Procedures

1. Germinate cotton seed: Layer approximately 400 seed in damp paper towels in Ziploc bags 3 days prior to Zero-G flight.
2. Label bags carefully
3. All seed should be treated the same (ex: fly all seed to Florida on commercial airliner)
4. Fly half of the seedlings on a 15-parabola flight, consisting of (3) 20-second periods of lunar gravity, (12) 20-second periods of microgravity, and (15) 30-second periods of 1.8 G.
5. Upon returning to classroom, plant seedlings 2 per pot and place in a humidity chamber for 2 days.
6. Once cotyledons are expanded, inoculate the undersides on half of each set of plants (0-G and 1-G). The non-inoculated plants serve as a control for both sets.

***Xanthomonas* inoculations**:

1. Grow the bacteria (*Xanthomonas*) on bacteria media for 3-4 days.
2. Using a toothpick, coat the tip in bacteria and scratch the underside of the cotyledons
3. *LABEL TRAYS VERY CAREFULLY: (0-G Inoculated), (0-G Non-Inoculated), (1-G Inoc), (1-G Non-Inoc)*
4. Return plants to humidity chamber for 2-3 days after inoculation, then remove plants from chamber for an additional 10-12 days..
5. Evaluate leaves for symptoms of bacterial leaf blight.

Results: Bacterial Blight (*Xanthomonas*) SMALL GROUP RESULTS

	Not Inoculated (no flight)	Not Inoculated (0-G)	Inoculated (no flight) *Xanthomonas*	Inoculated (0-G) *Xanthomonas*
Plant 1 Bacterial Infection 0= no infection 1= infection				
Plant 2 Bacterial Infection 0= no infection 1= infection				
Plant 3 Bacterial Infection 0= no infection 1= infection				
Plant 4 Bacterial Infection 0= no infection 1= infection				
Plant 5 Bacterial Infection 0= no infection 1= infection				
AVERAGE (% infection)				

	Not Inoculated (no flight)	Not Inoculated (0-G)	Inoculated (no flight) *Xanthomonas*	Inoculated (0-G) *Xanthomonas*
Plant 1 Health (0-5 scale)				
Plant 2 Health (0-5 scale)				
Plant 3 Health (0-5 scale)				
Plant 4 Health (0-5 scale)				
Plant 5 Health (0-5 scale)				
AVERAGE (0-5 Scale)				

Bacterial Blight of Cotton- SMALL GROUP RESULTS (AVERAGE)

	Not-Inoculated (1-g)	Not-Inoculated (0-g)	Xanthomonas Inoculated (1-g)	Xanthomonas Inoculated (0-g)
Average TOTAL seed germinated				
Average Diseased Plants				
% DISEASE				
Average Dead Plants				
Average Diseased Plants				
% DEAD (SEVERITY)				

Percent Disease- CLASS RESULTS

	Not-Inoculated (1-g)	Not-Inoculated (0-g)	Xanthomonas Inoculated (1-g)	Xanthomonas Inoculated (0-g)
Group 1				
Group 2				
Group 3				
Group 4				

Percent Dead (Severity)- CLASS RESULTS

	Not-Inoculated (1-g)	Not-Inoculated (0-g)	Xanthomonas Inoculated (1-g)	Xanthomonas Inoculated (0-g)
Group 1				
Group 2				
Group 3				
Group 4				

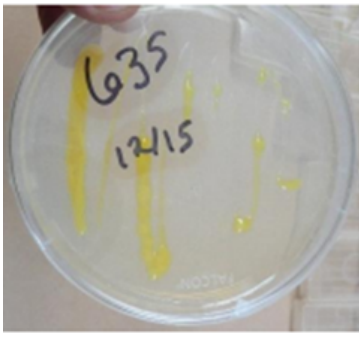
Analyze data as a class.

Assessment

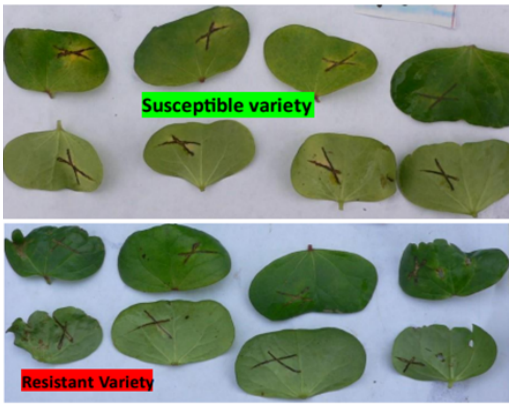
1. Is there a difference between inoculated and non-inoculated control? (supporting data)
2. Obvious difference between the number of infected plants that were exposed to changes in gravity and those not flown on Zero-G plane? (supporting data)
3. Obvious differences in overall health in plants exposed to changes in gravity and those not flown on Zero-G plane? (supporting data)
4. What problems were encountered in this experiment?
5. What changes should be made to this experiment?
6. Are there other questions that should be addressed?
7. Why is this important to both earth and space science?

<u>Additional Information and photos</u>

Seedlings planted into pots and labeled



Xanthemonas culture



After one week, half of cotton seedlings inoculated with *Xanthomonas*
Seedlings examined daily for symptoms of bacterial infection

Additional Resources:

- Plant Pathology for Kids!

  https://kids.britannica.com/kids/article/Plant-Disease/601361

- Plant Pathology Short Course:

  https://www.apsnet.org/edcenter/foreducators/TeachingNotes/remotelearning/Pages/default.aspx

- Cotton Diseases:

  http://cotton.tamu.edu/NematodesAndDisease.html

  http://cotton.tamu.edu/Nematodes/16_FS_FC010_Cot_Bact_Bl.pdf

- NASA Technical Reports (search by topic)
  https://ntrs.nasa.gov/