# Effect of Gravitational Changes on Susceptibility of Cotton Seedlings to Reniform Nematode Infection.

## Introduction

Plants have been 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 *Rotylenchulus reniformis (*Reniform nematode*),* a nematode responsible for significant root damage. Students will need access to cotton seed, nematodes, and guidance on extraction and 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
Engineering Design ETS1.A, ETS1.B, ETS1.C

**Objectives:**

- To investigate whether changes in microgravity causes changes in susceptibility of cotton seedlings to Reniform nematode
- 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:**

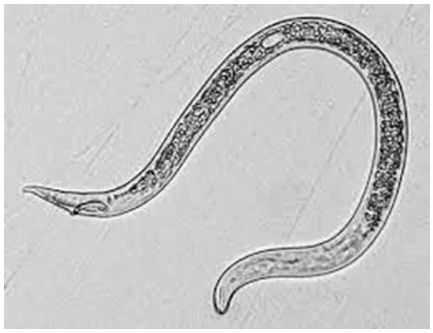
This project investigates whether changes in gravity could result in a plant stress response causing changes in susceptibility to disease causing organisms, such as bacteria and nematodes.

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. Neither are human pathogens.

**Reniform nematodes** are a root pathogen in cotton. Nematodes juveniles (larvae) penetrate cotton roots to establish a feeding site. Females lay 60 to 200 eggs, with eggs hatching one to two weeks later.. Reniform nematodes can survive without a host in dry soils for two years.

Reniform nematodes can cause plant stunting and grainy, small roots. This is a result of sand sticking to the egg masses.

Yield loss attributed to reniform nematode pressure in untreated fields can be up to 59%.



Reniform nematode and its damage to cotton roots. Infected roots are on the left.

**Purpose: Will a cotton seedling exposed to changes in gravity have a change in susceptibility to Reniform Nematode?**

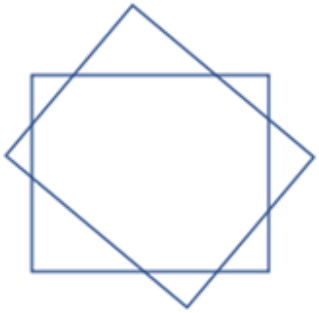
**Hypothesis**:*Do some research! What do you think, and why?*

**Procedures:**

1. Germinate cotton seedlings: Layer approximately 400 seed in damp paper towels in Ziploc bags 3 days prior to Zero-G flight.
2. 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.
3. Upon returning to classroom, plant seedlings 2 per pot and place in a humidity chamber for 2 days.
4. Extract nematodes from roots for inoculation 2 weeks after planting (see extraction instructions)
5. Inoculate half of each set of plants (0-G and 1-G), leaving half not-inoculated as a control for both sets. (see inoculation instructions)
6. LABEL TRAYS VERY CAREFULLY: (0-G Inoculated), (0-G Non-Inoculated), (1-G Inoc), (1-G Non-Inoc)
7. Grow plants in identical environments for 2-3 weeks after inoculation
8. Measure plant growth
9. Carefully remove plants from soil and rinse roots for root analysis.

**Nematode Extraction and Inoculation of cotton seedlings with Reniform Nematodes**

1. Place mesh screen inside of glass pie pan
2. Lay two Kleenex down on the mesh. Kleenex should be offset, as in diagram below
3. Place soil with nematode infested cotton roots on top of the Kleenex.
4. Lift a corner of the Kleenex and add 200 mL water to the pie-pan (be careful not to dislodge the soil or roots from the Kleenex into the water
5. Wrap the wet Kleenex around the soil.
6. Let it sit for 1-2 days at room temperature
7. Lift the mesh + Kleenex off the pie-pan. Soil / Kleenex can be thrown away.
8. Pour the water into a beaker. The water contains the nematodes which will be used to inoculate the seedling roots.
9. Identify the presence of nematodes using a microscope.
10. Combine water with nematodes, and bring all water to a volume of 5-10 mL water/nematode mixture per plant.
11. Occasionally stirring the water, pipette 5-10 mL of nematode solution into the soil next to the stem.
12. The nematodes should be able to infect the plants within a few days and complete their life cycle



**Assembling the pie pans with soil sample.**

**SMALL GROUP RESULTS: Do changes in gravity make cotton seedlings more likely to be infected by Reniform Nematodes?**

| Date_____ | Not Inoculated (no flight) | Not Inoculated (0-G) | Inoculated (no flight) | Inoculated (0-G) |
|---|---|---|---|---|
| Plant 1<br>Root Length (cm) | | | | |
| Plant 2<br>Root Length (cm) | | | | |
| Plant 3<br>Root Length (cm) | | | | |
| Plant 4<br>Root Length (cm) | | | | |
| Plant 5<br>Root Length (cm) | | | | |
| Plant 6<br>Root Length (cm) | | | | |
| Plant 7<br>Root Length (cm) | | | | |
| Plant 8<br>Root Length (cm) | | | | |
| AVERAGE | | | | |

| Date_____ | Not Inoculated (no flight) | Not Inoculated (0-G) | Inoculated (no flight) | Inoculated (0-G) |
|---|---|---|---|---|
| Plant 1<br>Overall Health (0-5) | | | | |
| Plant 2<br>Overall Health (0-5) | | | | |
| Plant 3<br>Overall Health (0-5) | | | | |
| Plant 4<br>Overall Health (0-5) | | | | |
| Plant 5<br>Overall Health (0-5) | | | | |
| Plant 6<br>Overall Health (0-5) | | | | |
| Plant 7<br>Overall Health (0-5) | | | | |
| Plant 8<br>Overall Health (0-5) | | | | |
| AVERAGE | | | | |

**CLASS RESULTS: Do changes in gravity make cotton seedlings more likely to be infected by Reniform Nematodes?**

| Date_____ | Not Inoculated (no flight) | Not Inoculated (0-G) | Inoculated (no flight) | Inoculated (0-G) |
|---|---|---|---|---|
| Group1: Average Root Length (cm) | | | | |
| Group2: Average Root Length (cm) | | | | |
| Group3: Average Root Length (cm) | | | | |
| Group4: Average Root Length (cm) | | | | |
| Group5: Average Root Length (cm) | | | | |
| Group6: Average Root Length (cm) | | | | |
| Group7: Average Root Length (cm) | | | | |
| Group8: Average Root Length (cm) | | | | |

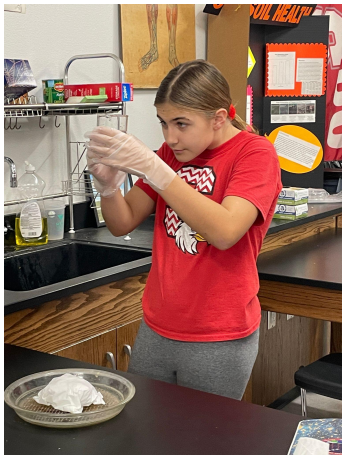
| Date_____ | Not Inoculated (no flight) | Not Inoculated (0-G) | Inoculated (no flight) | Inoculated (0-G) |
|---|---|---|---|---|
| Group1: Health (0-5) | | | | |
| Group2: Health (0-5) | | | | |
| Group3: Health (0-5) | | | | |
| Group4: Health (0-5) | | | | |
| Group5: Health (0-5) | | | | |
| Group6: Health (0-5) | | | | |
| Group7: Health (0-5) | | | | |
| Group8: Health (0-5) | | | | |

Analyze data as a class.

**Conclusion** (essay format, discuss the following)

1. Is there a difference between inoculated and non-inoculated control? (supporting data)
2. Obvious difference between root length in plants exposed to changes in gravity and those not flown on Zero-G plane? (supporting data)
3. Obvious differences in overall root 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?


Soil and root samples from Lubbock, TX for nematode extraction



Extraction of nematodes from water, inoculation at base of each plant.

**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/