Do Microbes Help or Hinder Germination?
A Learning Game Designed by USDA-REEU Undergraduate Students to Explore Seed-Endophyte Interactions

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Introduction

- Today seeds are facing many challenges both in agriculture and in our natural habitats. Disease and environmental stressors (such as drought, heat, abrupt freeze, and high salt content in the soil) reduce germination and seedling growth. Climate change is only going to make these stressors more of a challenge. The beneficial aspects of microbes have largely been unexplored, and they are an untapped resource that has the potential to solve important problems in our world.
- Undergraduate students participated in a USDA-REEU (Research and Extension Experiences for Undergraduates) summer program called Seeds For The Future. We present examples of student research that focused on seed endophytes (microbes within healthy plant tissue) and seed stressors impacting germination and seedling stages.
- During the program, students designed a game to disseminate concepts that were part of their research. The learning game was designed for middle school, high school, and college age students.

Photo 1. Photo of Seeds For The Future USDA-REEU participants and mentors in Summer 2022. Photo by J. Beckstead.

Methods

Students surface sterilized seeds of several species and plated them on four types of agar media to isolate seed endophytes. Plates were examined for presence of bacteria and fungi along with morphological microbial traits.

Students explored different types of stress on seeds by examining cell damage that resulted after the stress events during the germination life stage. Stressors included drought stress (10% PEG), freeze stress (-18C), and heat stress (80C). Sequential stress events were investigated. Cell damage was measured by proportional electrolyte leakage (PEL).

Examples of Student Research Results

--Can microbial seed endophytes help seeds overcome stressors that limit germination?

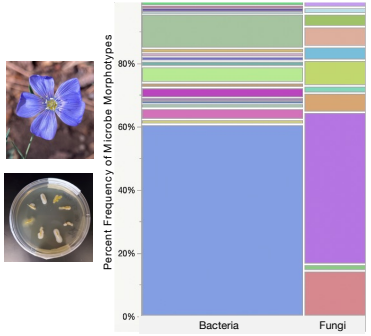


Photo 1 (left upper). Flax (*Linum lewisii*) flower. Photo by J. Beckstead.

Photo 2 (left lower). Seeds on a plate with microbes. Photo by D. Eisenbrandt.

Figure 1 (right). Stacked bar graph with different types of endophytes isolated from flax seeds; morphospecies data shown. Results showed 39% more bacterial endophytes isolated from flax seeds than fungi.

Figure 2. Stressful events resulted in higher electrolyte leakage of germinating forb yarrow (*Achillea millefolium*). Seeds experiencing multiple stress events sustained 3x higher cell damage than controls and nearly 2x higher than seeds experiencing only one stress event compared to controls; significant 1-way ANOVA at p<0.0001. During initial germination, seeds experienced drought stress (10% PEG) for 24 hrs, then imbibed seeds starting to germinate experienced freeze stress for 1-hr at -18C..

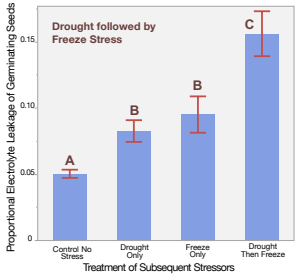
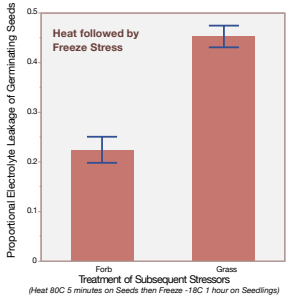


Figure 3. Grass seeds experienced a 50% higher cell damage than forb seeds when seeds experienced hot temperatures simulating a grass wildfire followed by an abrupt freeze event as could occur in the Fall; significant t-test at p<0.0001. Grass species = cheatgrass; *Bromus tectorum*. Forb species = yarrow; *A. millefolium*.

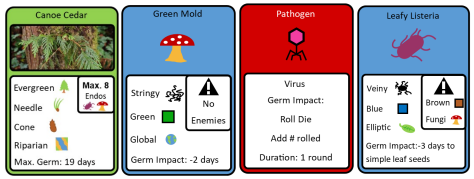


Game Design

Glorious Germination

Goal: *Germinate your seed before the other players by adding endophytes to reduce your days for germination.*

Plant and Endophyte Traits:
- Players select from multiple plant options. Each plant has different traits, such as growth form, type of leaves, and habitat.
- Many endophytes, both bacterial and fungal species, are part of the game. Each microbe has specific morphological and habitat traits, such as growth form, color, and habitat.



Basic Components of Game

Germination Days for Seed Species:
- Maximum germination days listed at bottom of Seed Card.
- Each round you earn days towards germination. Endophytes impact germination by reducing days to germinate.

Endophytes Attached to Seed Species:
- Maximum endophyte number inside small box. Example card, this seed can have a maximum of 8 endophytes attached to it.
- Keep endophytes for duration of game.

Pathogen Cards:
- Pathogen cards must be kept.
- Pathogen impacts to germination are tallied at each round.

Enemy Interactions with other Endophytes:
1. No enemies. Card will not attack other endophytes.
2. Enemies of fungi. Discard endophyte cards with traits listed.
3. Enemies of bacteria. Discard endophyte cards with traits.

Game Student Outcomes

- While playing this game, students will learn about endophytes, microbial interactions, and impacts of microbes to seeds. In addition, this plant-microbe game covers ecological species-interactions, solutions to help plants during climate change, plant survival, and some aspects of plant life cycles.
- This fun learning experience provides students with an opportunity to discover a world they have never considered before.
- Plant restoration and agriculture in the future will depend on future biologists that can tap into unexplored tools to help plants establish under stressful conditions.

Get Glorious Germination Game!

- ***Link to copy of the Game:*** http://www.gonzaga.edu/SeedsforTeachers
- ***YouTube Video – How to play:*** https://www.youtube.com/watch?v=gUXCDYw8F4E

Acknowledgements

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