The Effects of Dark Septate Endophyte Symbiosis on Constitutive and Induced Defenses of Plants against Insect Herbivory

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Introduction

Pesticides are widely utilized in modern agriculture despite evidence that they cause extensive damage to both the environment[1] and human health[2] [3]. It is imperative to explore other, more sustainable methods of agricultural pest control. I propose exploring the natural ability of plants to produce defensive chemicals[4] in order to prevent herbivore damage as a potential solution.

- Some symbioses between **fungi** and plants have been observed to **alter plant defensive responses to pathogen damage and herbivory[5].**
- Dark Septate Endophytes, abbreviated DSE, are a poorly studied group of fungi[6] that are **thought to support plant growth in harsh environmental conditions[6]**.
- DSEs have been shown to stay in a mutualistic relationship with plants even when nutrients are abundant, leading to the hypothesis that **they may provide more than nutrients to the plant[7].**

Questions addressed:

1. Does the presence of DSEs alter plant germination rates, morphology, or survival during severe drought?
2. Are changes observed in the constitutive defensive response or in the induced response to herbivory, and what does this reveal about the relationship between plant and DSE?
3. Will the effects of DSE colonization be enhanced or lowered in plants that are drought-stressed? This knowledge may inform an entirely new approach to pest management in agriculture.

Preliminary Results

This experiment is being performed as an Honors in the Major project and is currently in progress, to be completed during the Fall 2022 semester. To address part of the first experimental question, below are my preliminary results for seedling survival rate during the first week of growth. I have also included an overview of my training experience and takeaways from my research over the Summer semester, both funded by the IDEA grant.

Figure 1:Seedling Percent Survival

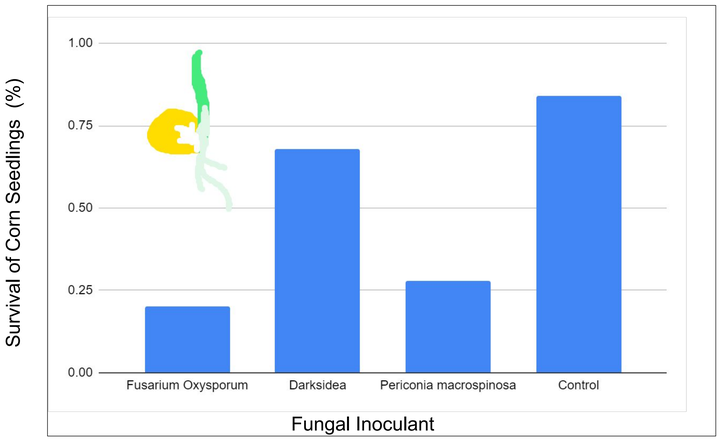
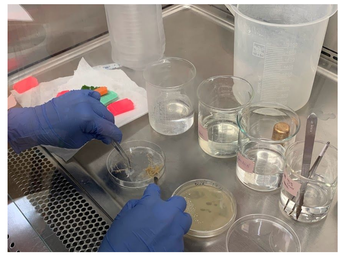


Figure 1: In the *Fusarium oxysporum* group., I found that survival was reduced to 20%. *Periconia macrospinosa* survival was found to be 28%, *Darksidea* survival percentage was 68%, while the control group had a percentage of 84% survival. The *F. oxysporum* group and the *P. macrospinosa* group both had statistically significant differences in survival counts from the control group according to the Fisher's exact test (p-value = 1.155e-05 and p-value = 0.0001, respectively). The *Darksidea* group did not have significantly different survival from the control group (p-value = 0.3209).

A Learning Experience



- I traveled to the Rudgers Lab at the University of New Mexico in May to be trained on dark septate endophyte culturing techniques.
- I successfully inoculated PDA-filled petri dishes with each of three species of fungi. There was minimal contamination.
- I began fertilization of all plants after two months of growth out of concern that the change would influence the growth of the fungi in the colonization stage. In the next round I plan on starting fertilization as soon as corn is planted.
- I determined that the optimal drought period to maintain drought stress is ten days.
- Spider mites were present on almost every corn plant in great numbers. In the next round, I plan on using ladybugs as a natural pest control for the plants.

Methods

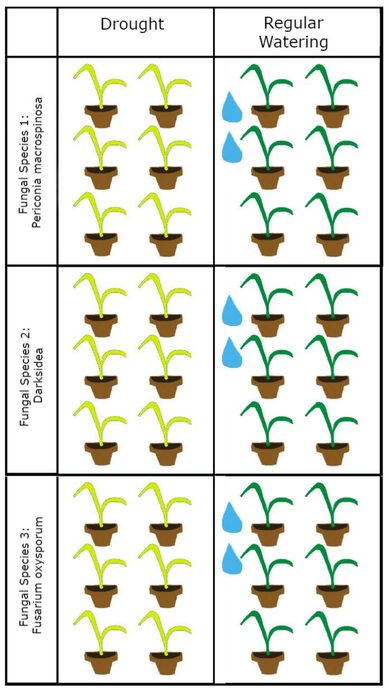
Darksidea, Periconia macrospinosa, and ***Fusarium oxysporum*** were culturted from *Bouteloa gracilis* on potato dextrose agar (PDA).
Zea mays, or corn kernels were surface sterilized and inoculated with each fungal species. Fungi and corn were planted in sterilized soil together as a core of PDA media after one week of growth.

- Half of the corn plants were subjected to drought conditions and half were watered regularly.
- At maturity, corn plants will be harvested and their leaves cored for bioassays to determine larval growth rate.
- Corn roots will be analyzed using a light microscope to determine percent colonization per plant.
- Data will be analyzed using an ANOVA (analysis of variance) statistical test in R to determine differences in larval growth rate. Percent survival of plants will also be calculated and the number of surviving plants from each group will be compared to those of the control via a Fisher's exact test in R.

Spodoptera exigua

Zea mays

P. macrospinosa & Z. mays



Conclusions

This experiment is in its first stage, but predictions can be made based on preliminary data.

- The **mutualism-parasitism continuum** for DSEs may revolve around water availability. In well-watered young plants, DSE were detrimental to seedling survival past the first week. However, this may not be the case in older or drought-stressed plants. In the second round I will monitor plant survival over two months of growth as well as other health indicators including stalk height, number of fruit, and presence or absence of leaf curling (a sign of drought stress).
- While *Fusarium oxysporum* was particularly detrimental to seedling survival, **one *F. oxysporum*-inoculated plant in drought conditions did flower,** which was uncommon for drought plants in any group. This may have also been a result of extreme stress on the corn (the drought coupled with stress from *F. oxysporum*), leading it to flower before dying. While sample size was too small to make statistically significant conclusions, I intend to re-examine the effects of *F. oxysporum* on flowering in drought-stressed plants.

Works Cited

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