Modification of seed exudates by seed-colonizing microbes from vermicompost alters pre-infection behavior of *Pythium aphanidermatum* zoospores

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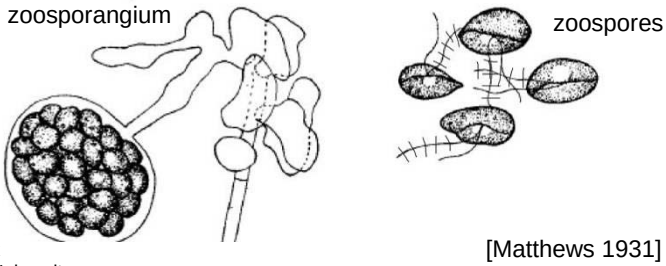
Thank you and good morning. Today I'm pleased to have the opportunity to discuss with you our investigation into the microbial mechanisms responsible for the phenomenon of disease suppressive composts. The goal of this project is to increase our understanding of how compost microbial communities prevent plant pathogens from causing disease. It is our hope that this detailed understanding can increase the efficacy of compost use in horticulture for disease suppression and provide insights into spermosphere ecology.

Pythium aphanidermatum

- Wide host range
- Pre- & post-emergence damping off
- Complex life cycle, including zoospore production



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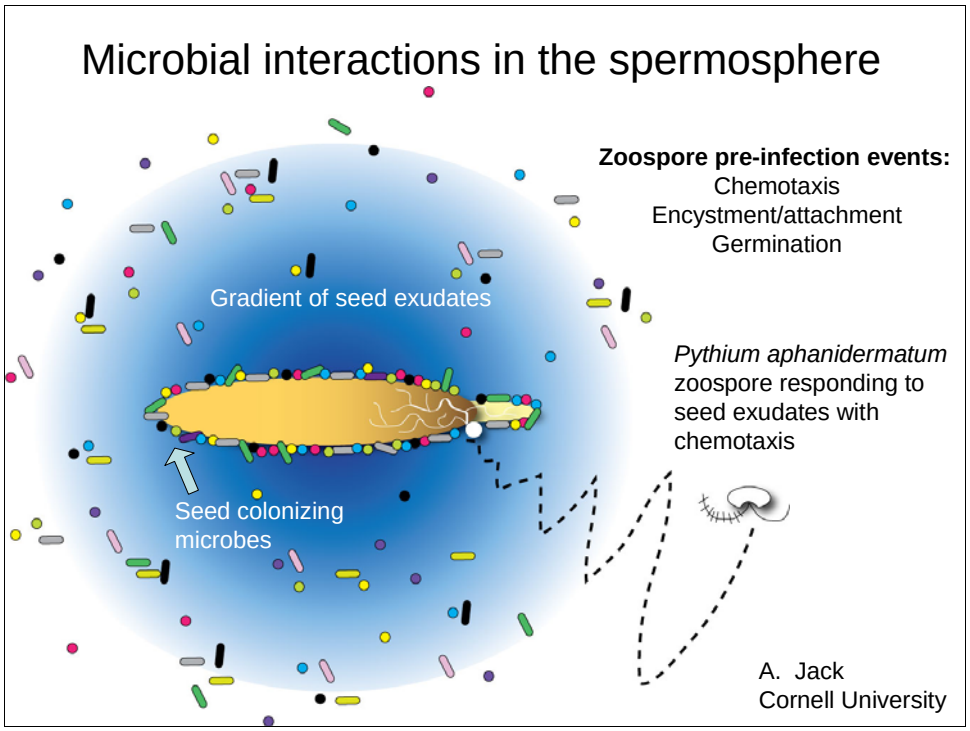
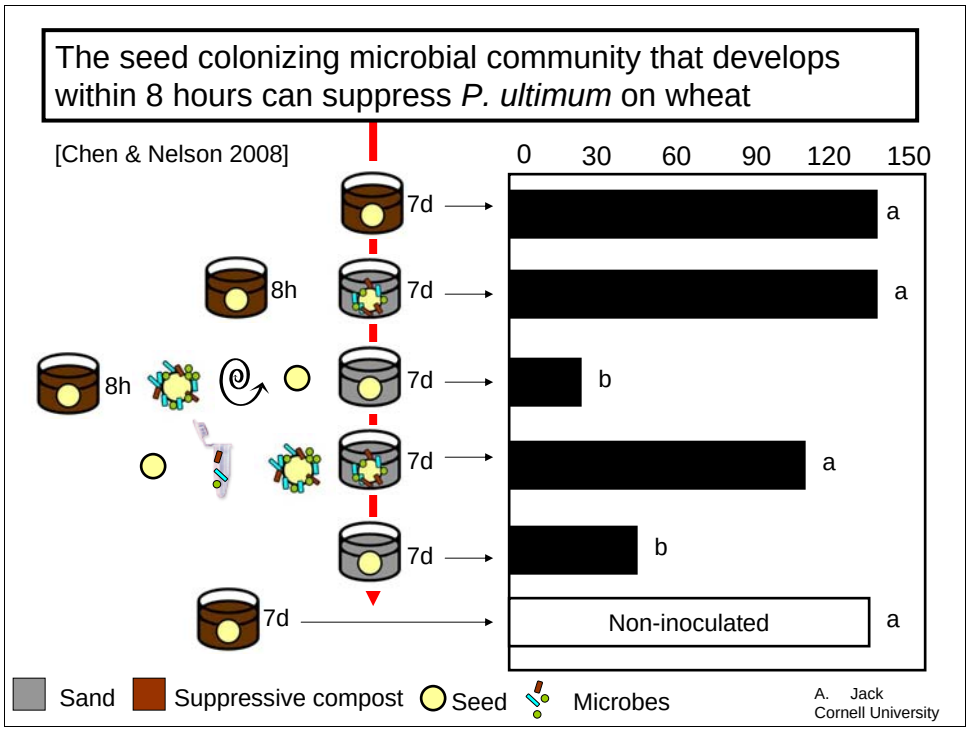
[Matthews 1931]

Pythium aphanidermatum is an oomycete plant pathogen with a wide host range of over 50 species including field crops, vegetables and ornamentals. It is capable of causing both pre and post emergence damping off of seedlings as well as a variety of root rots in mature plants. Pythium aphanidermatum has a complex life cycle which includes the formation of zoospores that act as motile infective propagules in wet soils. Because the life cycle is so complex, with multiple stages capable of infecting hosts, we've chosen to focus exclusively on the zoospores and their interaction with microbes in suppressive compost.

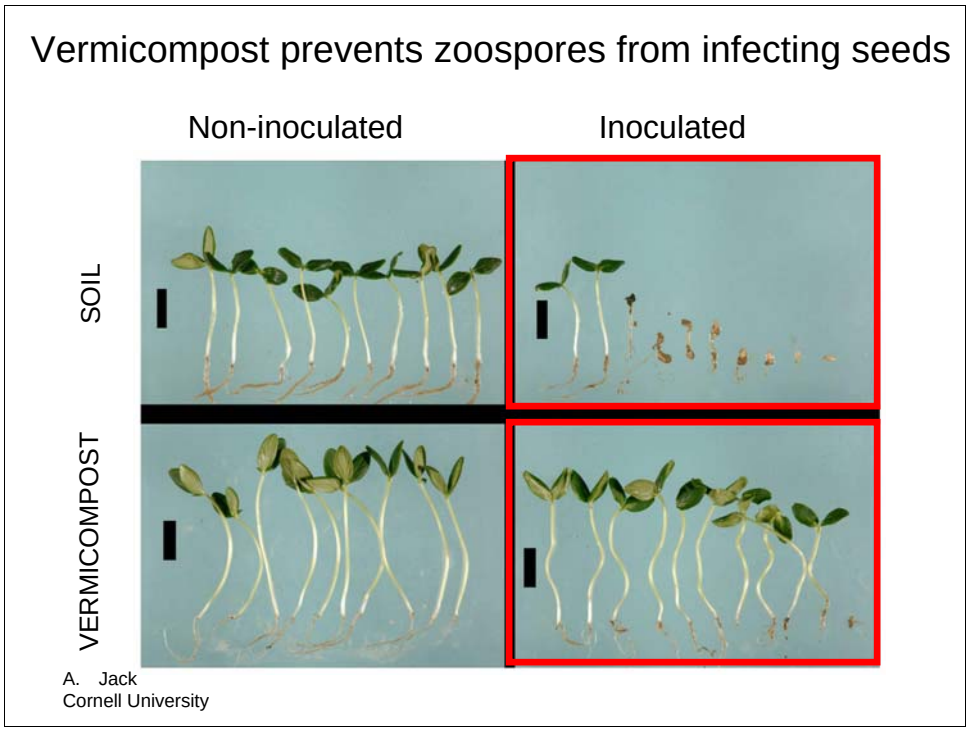


The spermosphere is the primary infection court of P. aphanidermatum. As seeds imbibe water and germinate, they passively release cell contents due to the build of up a high level of turgor pressure. These exudates consist primarily of organic acids, amino acids, and sugars which form a chemical gradient that extends into the surrounding soil. Motile zoospores exhibit chemotaxis in response to these chemical signals which results in an overall directional movement towards the host. Once they reach the surface of the seed, zoospores shed their flagella, encyst and secrete adhesive compounds to secure their position. Zoospore cysts then germinate, initiating the infection of host tissue. However, Pythium is not the only organism present in the infection court. A subset of the total microbial community also respond to exudates and colonize the surface of the seed. Interactions among seed colonizing microbes and Pythium zoospores can determine whether or not disease occurs. We focus on the spermosphere to ask the question: What role does the seed colonizing microbial community play in preventing zoospore infection of seeds?

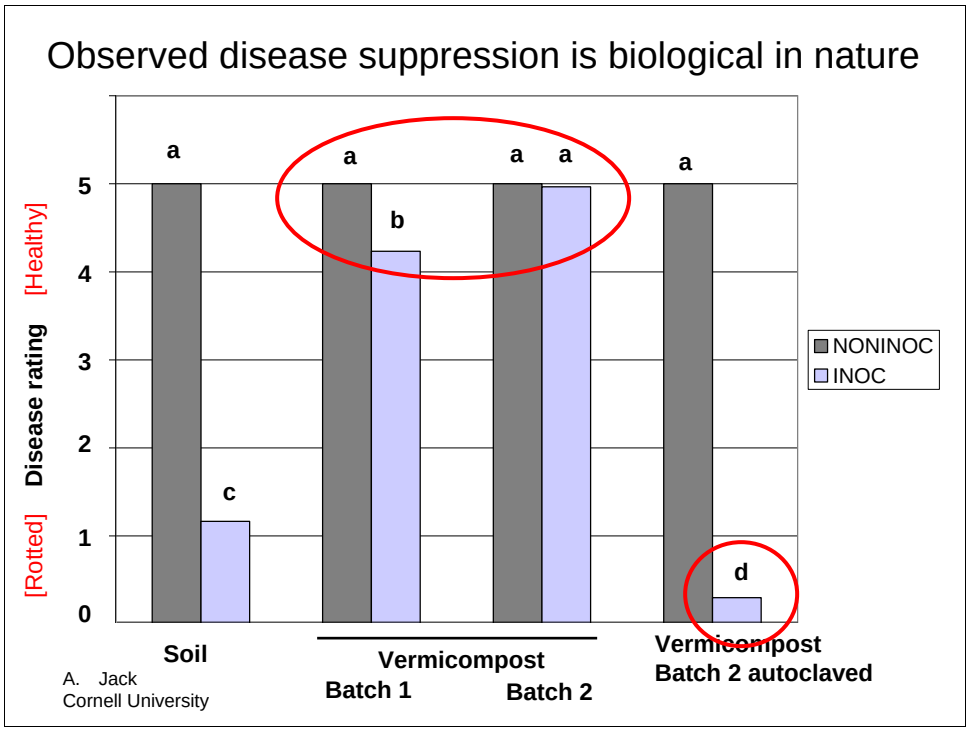


Recent work in our lab has shown the importance of seed colonizing microbial communities in preventing disease caused by Pythium ultimum in wheat. The brown cells represent suppressive compost, and the grey cells represent sterile sand. Yellow discs are seeds and the tiny colored shapes represent seed colonizing microbes. We use shoot height as a metric for disease level. When wheat seeds are sown in suppressive compost and inoculated with P. ultimum sporangia, the resulting seedlings are no different than non-inoculated controls. A variety of time points were tested to determine how long it takes for a suppressive community to develop on the seed surface. By 8 hours, if a seed is removed from the suppressive compost, transplanted into sterile sand, and inoculated, the 7 day old seedlings are no different than the non-inoculated controls. This is evidence of a high level of disease suppression. However, when seeds are started in suppressive compost, and vortexed to remove the seed colonizing microbes before being transplanted, suppression is lost and the 7 day old seedlings are no different from those sown in sterile sand. When the seed colonizing microbes removed from a seed sown in suppressive compost are used as a seed treatment for a sterile seed, suppression is restored.

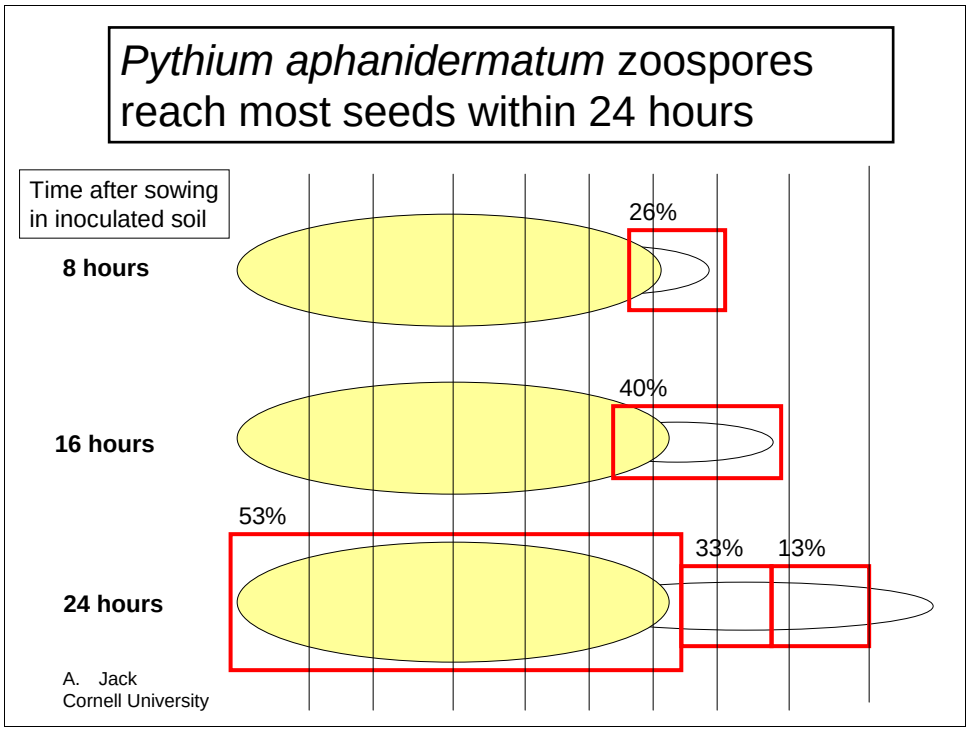
This finding allows us to narrow our search for the key microbial species directly involved in suppression. Instead of considering the entire microbial community present in the suppressive compost, we can focus only on those microbes capable of colonizing the seed within critical timepoints.



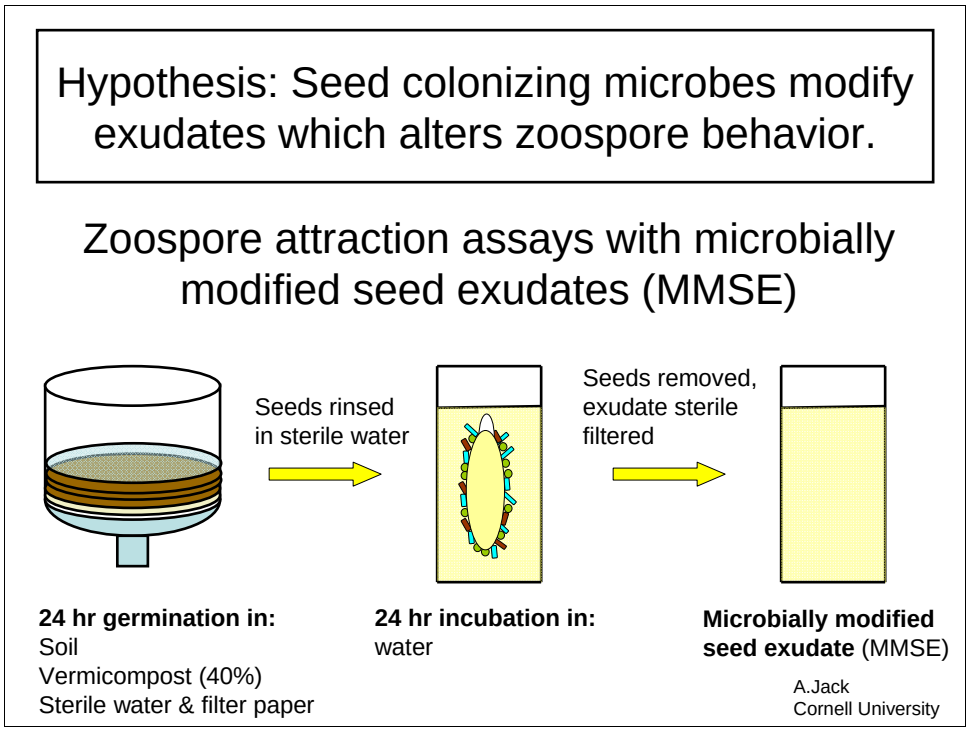
We are currently working with vermicomposted dairy manure from a large scale facility in Western NY that suppresses P. aphanidermatum on cucumber. In the left column non-inoculated seeds were sown in soil and soil amended with 40% vermicompost. In the right column seeds were inoculated with a controlled amount of P. aphanidermatum zoospores. These assays were carried out in fritted glass buchner funnels in a water column of a set length to control for matric potential in the growing media, which we've found can greatly influence zoospore behavior. You can see that amendment with vermicompost provides a high level of suppression. Because the vermicompost also has a significant growth promotion effect, we have had to use statistical models that can separate growth promotion from disease suppression.



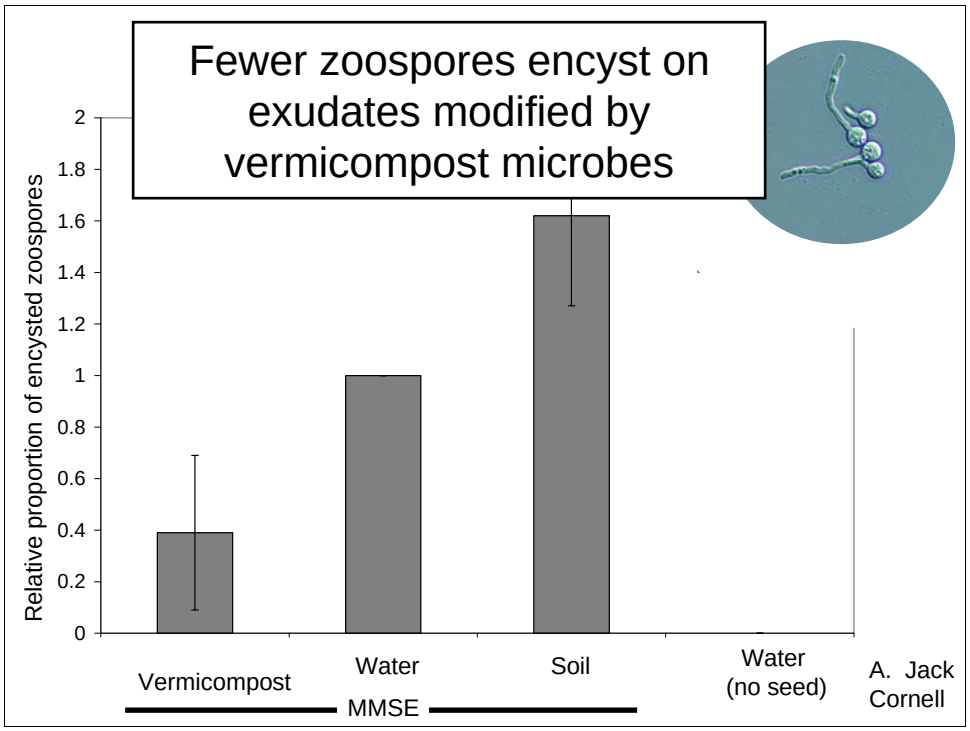
We know that the observed disease suppression is biological in nature, by documenting that heat sterilized vermicompost offers no protection from P. aphanidermatum infection. Disease rating is plotted on the y-axis with 0 representing completely rotted seedlings and 5 representing healthy seedlings. Dark bars represent non-inoculated seeds and light bars represent seeds that have been inoculated. We are finding low levels of batch to batch variability in suppression and are currently assaying additional batches and storage techniques to determine the extent of the variation. Amendment with autoclaved vermicompost resulted in significantly lower seedling quality than the conducive soil, possibly due to the addition of nutrients to the system. After documenting the biological nature of this phenomenon, we moved on to characterize microbial interactions on the seed surface. The first question we asked was, “when does Pa reach the seed?”



To find out when zoospores reach the seed surface and begin to infect the host, we carried out a series of sectioning experiments. Seeds were sown in soil inoculated with Pa zoospores and removed at 8, 16 and 24 hours. Seeds were then cut into 1 mm sections and the tissue was plated on water agar to assay for the presence of Pa hyphae derived from zoospore cysts. A total of 15 seeds were used for each time point. By 8 hours, Pa was present at the tip of the radicle in 26% of the seeds. Within 16 hours, there was an increase in the presence of Pa in the emerging radicle to 40%. By 24 hours a majority of the seeds were colonized by Pa throughout the cotyledon and extending into the radicle. One interesting observation from this set of experiments is that although the tip of the radicle is the first area of the seed to be colonized, by 24 hours only a low percentage of seeds had Pa present in the radicle. No Pa was present at the expanding tip of the radicle, or the first mm here. These experiments identified 24 hours as a critical timepoint because any interactions leading to disease suppression must occur before the seeds are colonized by zoospores.

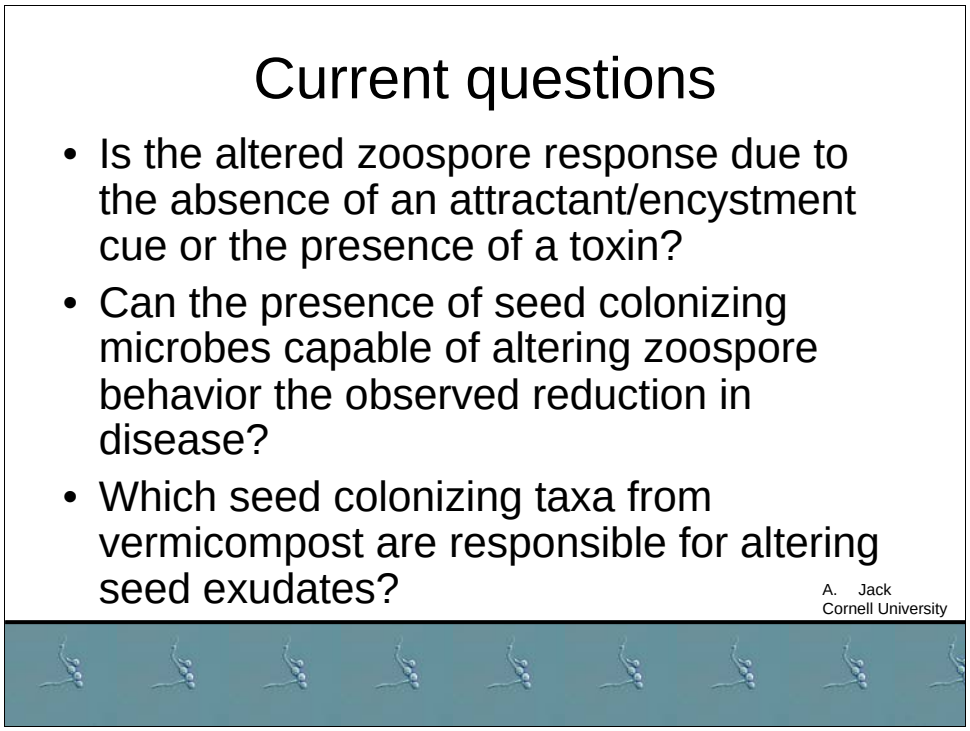


We used this 24 time period to set up our next experiments to test the hypothesis that seed colonizing microbes modify exudates which alters zoospore pre-infection behavior. We tested this hypothesis by carrying out zoospore attraction assays using microbially modified seed exudates or MMSE. MMSE were prepared by sowing seeds in soil, suppressive vermicompost, and sterile water in our fritted glass buchner funnels. After 24 hours, seeds were rinsed, and incubated in sterile water for an additional 24 hours. During this period seeds continued to germinate and release exudates while seed colonizing microbes had a chance to metabolize these exudates. The resulting modified exudates were then sterile filtered and used in zoospore attraction assays.



For the zoospore attraction assays, we infused agar discs with microbially modified seed exudates and submerged them in a zoospore suspension for 30 minutes. We then counted germinating zoospore cysts, which look like this and have rapidly extending germ tubes. We found that less zoospores encysted on discs infused with microbially modified exudate from seeds sown in suppressive vermicompost. By watching in real time under the microscope, we noticed that fewer zoospores appear to be attracted to the vermicompost MMSE, but this has been more difficult to quantify.

Using exudate from seeds sown in sterile moist filter paper as a positive control, we were able to average the results of 9 zoospore attraction assays and express the number of encysted zoospores relative to the control for each treatment. Almost no zoospores were encysted on agar discs infused with sterile water, while a high number encysted on discs infused with MMSE from soil. MMSE from vermicompost showed only 40% of encysted zoospores relative to the control. This result is evidence that seed colonizing microbes from suppressive vermicompost are somehow modifying seed exudates and disrupting the zoospore homing and/or encystment response.



There are a few possible explanations for the changes in zoospore behavior that we have observed. 1) A chemical that serves as a zoospore attractant and/or encystment cue is missing in the modified exudate or 2) the modified exudate contains a toxin that repels zoospores. We're designing experiments based on previous work by Heungens and Parke who answered this same question for pea exudates modified by B. cepacea and P. aphanidermatum zoospores. We're currently conducting transplant experiments like the ones described in this talk to determine if the presence of seed colonizing microbes from vermicompost can fully explain the reduced disease that we've documented. And farther in the future, we will determine which seed colonizing taxa are responsible for altering seed exudates. Our long term goal is to use this information to develop tools for predicting the suppressiveness of composts to increase the efficacy of their use as alternative disease control strategies.

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