Slides and notes from a talk given with Alex Stone from Oregon State University at the Midwest Organic and Sustainable Education Service annual conference in La Crosse, Wisconsin.

Microbial ecology of disease suppression

Disease suppression… What do we know?

- Single organism biological control is well understood in specific cases
- Suppression of disease by a complex community of microbes is much more complicated!

Brief history of disease suppression research

- **Late 1800s**: suppressive soils documented [Huber & Schneider 1982]
- **1930s – 1940s**: Link made between composts and soil health [Howard 1943]
- **1959**: Biological nature of suppression documented [Menzies 1959]
- **1970s - 1980s**: Extensive work done on suppressive composts [Hoitink & Kuter 1986, Weltzein 1989]

Huber, D.M. and Schneider, R.W., 1982. The description and occurrence of suppressive soils. In: Schneider, R.W. (Eds.), Suppressive Soils and Plant Disease. The American Phytopathological Society, St. Paul, MN, pp. 1-9.

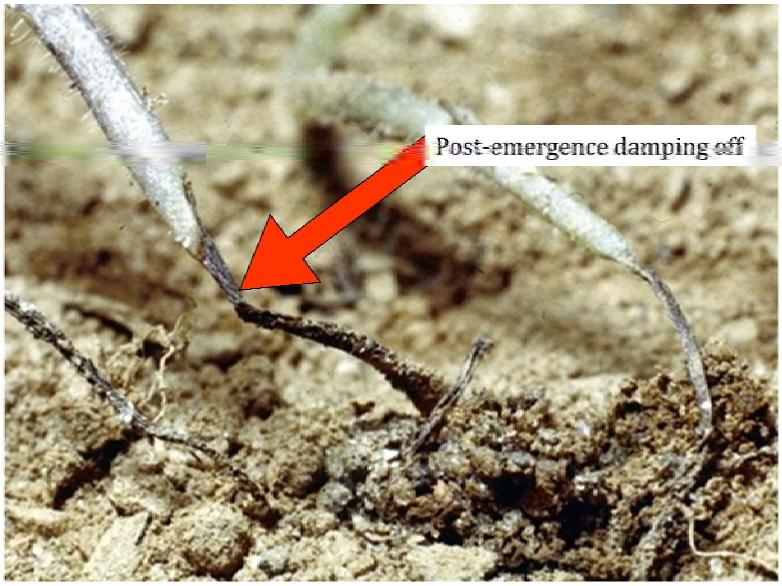
Howard, A. (1943) An Agricultural Testament. New York, Oxford University Press.

Menzies, J. D. (1959) "Occurrence and transfer of a biological factor in soil that suppresses potato scab." Phytopathology 49: 648-652.

Hoitink, H. A. J. and G. A. Kuter (1986) Effects of composts in growth media on soilborne pathogens. Role of Organic Matter in Modern Agriculture. Y. Chen and Y. Avnimelech. Boston, Nijhoff Publishers: 289-306.

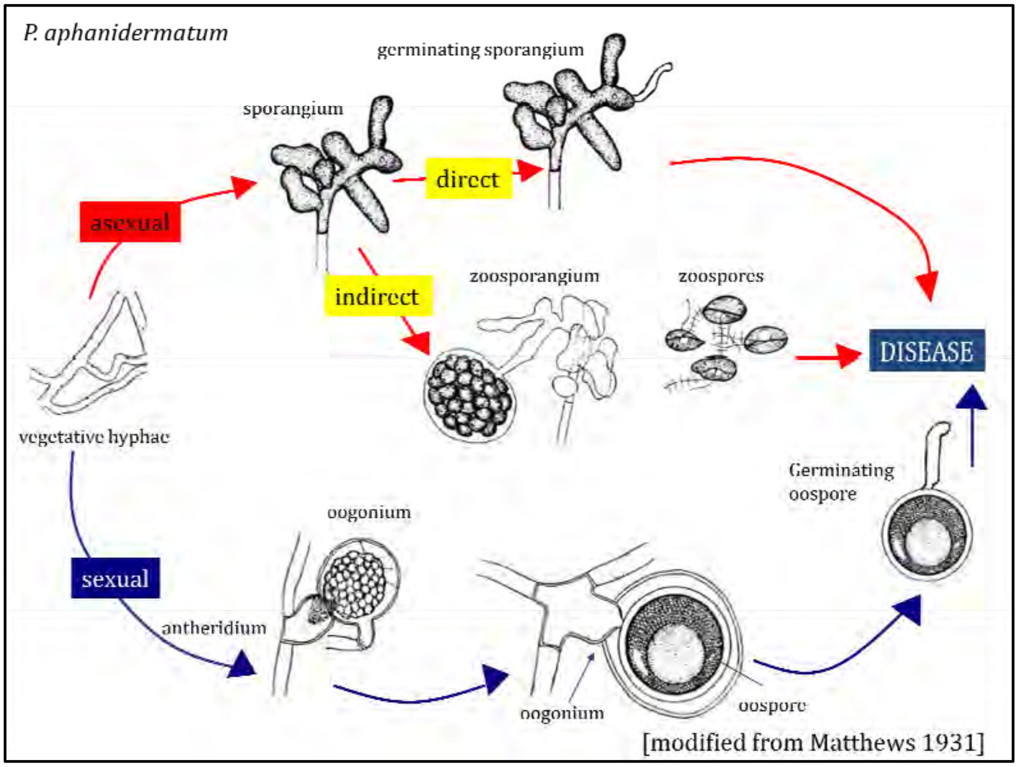
Weltzien, H. C. (1989) "Some effects of composted organic materials on plant health." Agriculture Ecosystems & Environment 27(1-4): 439-446.

Example: *Pythium* spp. (damping off)



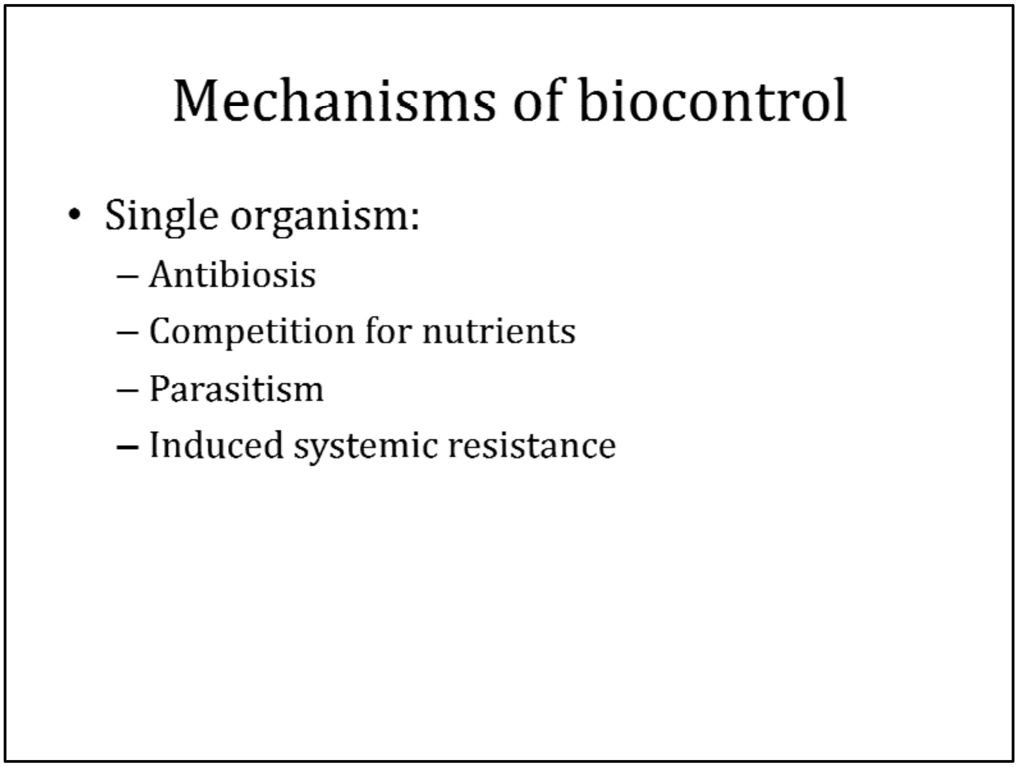
[www.ipmimages.org]

Many species in the oomycete genus *Pythium* can cause seed and root rots. This image shows post emergence damping off of cucumber seedlings. Pythium species have been widely studied in the field of biological control of plant pathogens, and here I will use Pythium to illustrate some important concepts in this field.

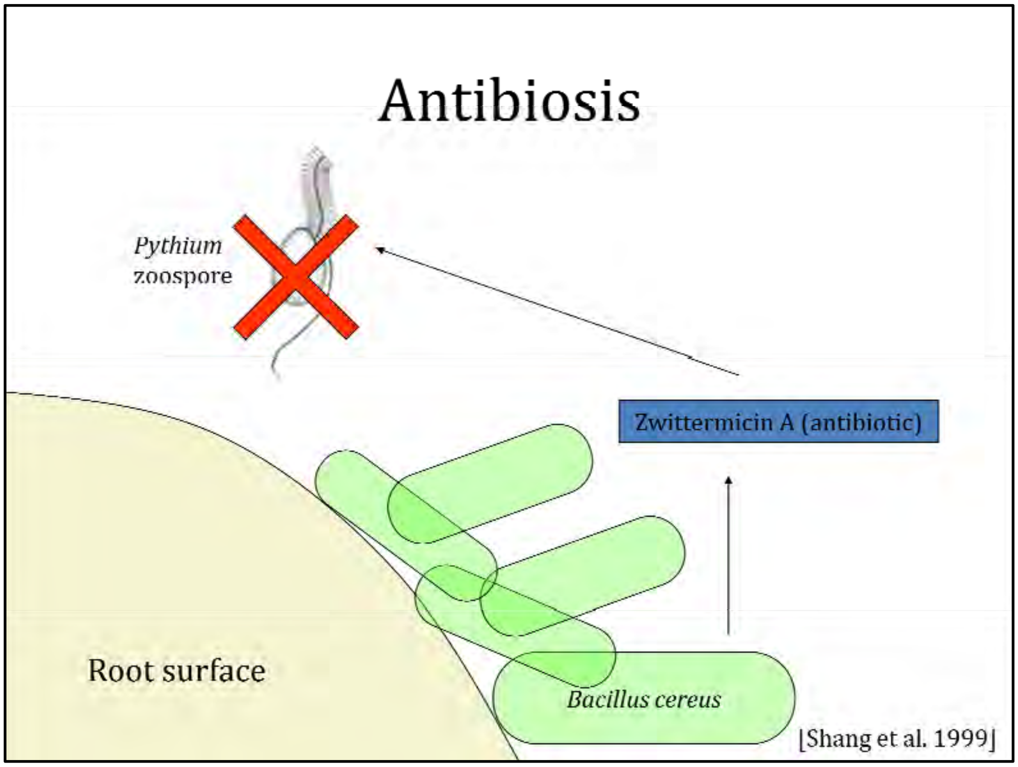


Pythium aphanidermatum infects over 50 crop species and has a complex life cycle with multiple stages capable of infecting plants. During asexual development, sporangia are produced which can germinate directly (by extending germ tubes) or indirectly via the formation of motile zoospores.

Matthews, V.D., *Studies on the Genus Pythium.* 1931, Chapel Hill, NC: University of North Carolina Press. 101.



The following are examples of each of the documented mechanisms of biocontrol with a single organism.

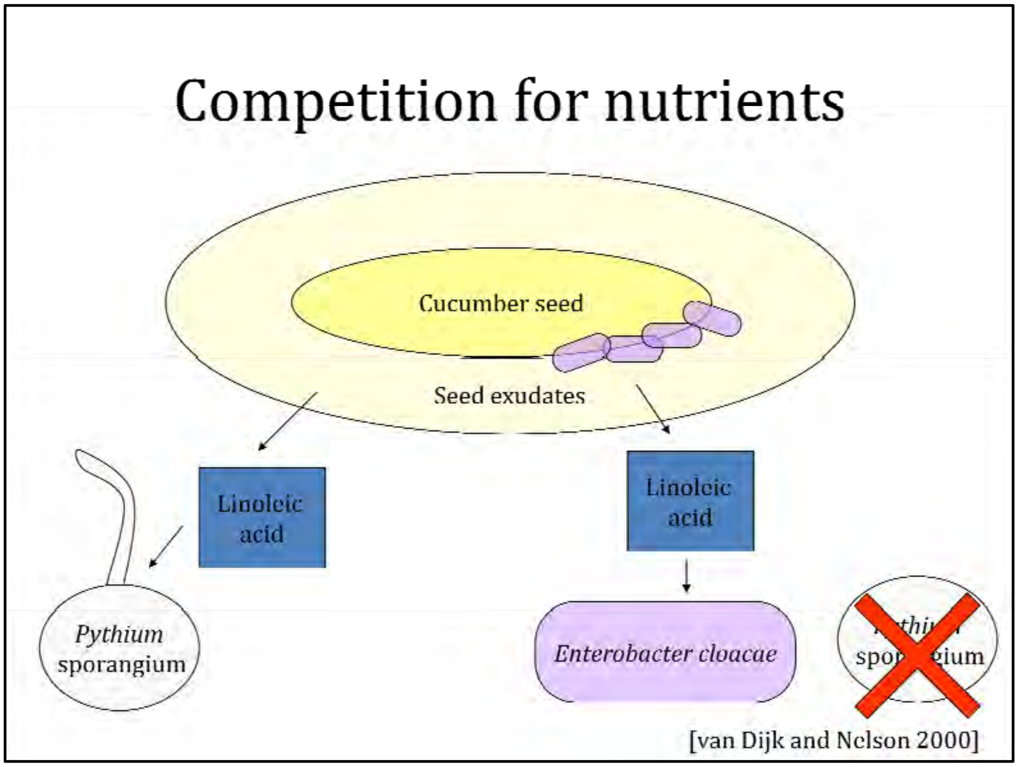


Antibiosis refers to the production of antibiotics. Pythium zoospores attack plant roots. However, when roots are treated with a biocontrol bacterium, *Bacillus cereus*, disease is prevented. The bacteria produce an antibiotic zwittermicin A that prevents the germination of zoospore cysts which contributes to the protection from disease.

Shang, H., J. Chen, J. Handelsman, and R.M. Goodman, *Behavior of Pythium torulosum zoospores during their interaction with tobacco roots and Bacillus cereus.* Current Microbiology, 1999. 38(4): p. 199-204.

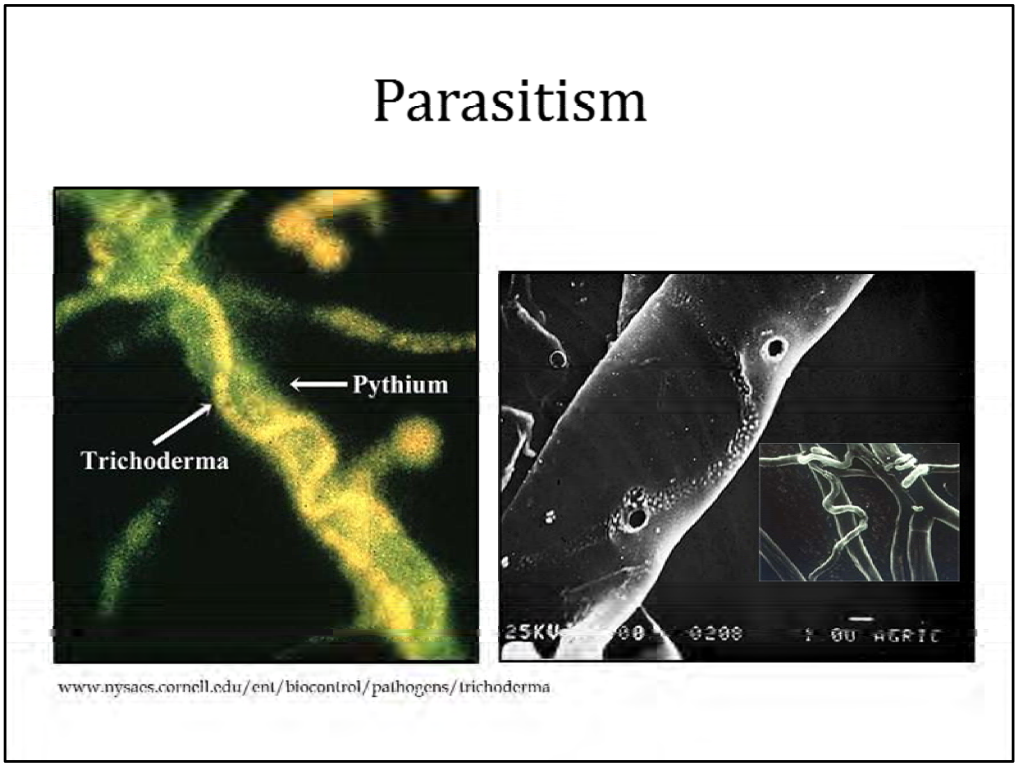
In vitro vs. *In vivo*

Here you can see the zone of inhibition around a colony of *Bacillus* spp. bacteria growing on a Petri dish. Antibiotics produced by the bacterium diffuse into the agar growing medium. The plant pathogenic fungus, *Botrytis* spp. is not able to grow in the presence of the antibiotics. It's important to note that although antibiosis can easily be studied *in vitro*, i.e. "in glass", what happens on a Petri dish does not always reflect what happens in the presence of the plant host in the soil environment *in vivo, i.e. "in life"*.



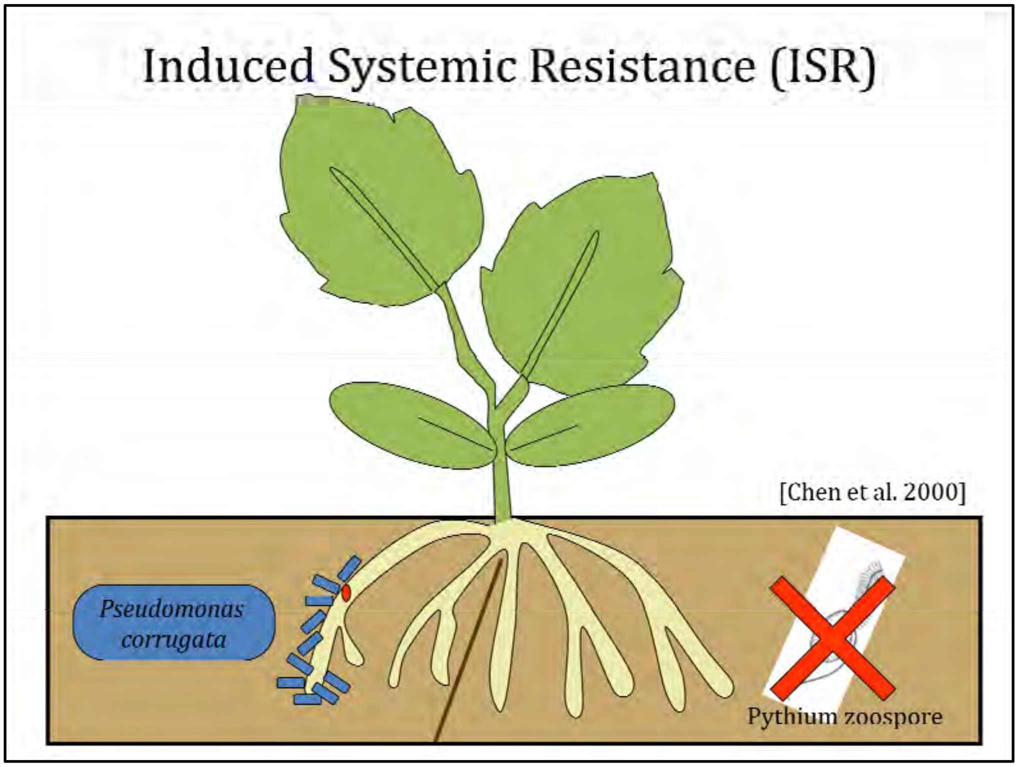
Some biocontrol agents consume chemicals that the pathogen needs as a cue to germinate or a vital nutrient for growth. Sporangia of *Pythium ultimum* lie dormant in the soil until they are exposed to linoleic acid, a fatty acid released by germinating seeds. Linoleic acid acts as a germination cue which initiates infection. The bacterium *Enterobacter cloacae* metabolizes linoleic acid thus removing the cue to germinate. Although the *Pythium* sporangium is still viable, it does not germinate and no infection occurs.

van Dijk, K. and E.B. Nelson, *Fatty acid competition as a mechanism by which Enterobacter cloacae suppresses Pythium ultimum sporangium germination and damping-off.* Applied and Environmental Microbiology, 2000. 66(12): p. 5340-5347.



Some biocontrol agents carry out direct parasitism of the pathogen. For example, fungi of the genus*Trichoderma* encircle *Pythium* hyphae, puncture the cell wall and drain cellular contents. Several commercially available biocontrol formulations include *Trichoderma* species.

Harman, G. "*Trichoderma spp*., including *T. harzianum, T. viride, T. koningii, T. hamatum* and other spp. Deuteromycetes, Moniliales (asexual classification system)" in Weeden, C.R., A. M. Shelton, and M. P. Hoffman. *Biological Control: A Guide to Natural Enemies in North America*. http://www.nysaes.cornell.edu/ent/biocontrol/pathogens/trichoderma.html [accessed on 8-16-2010]



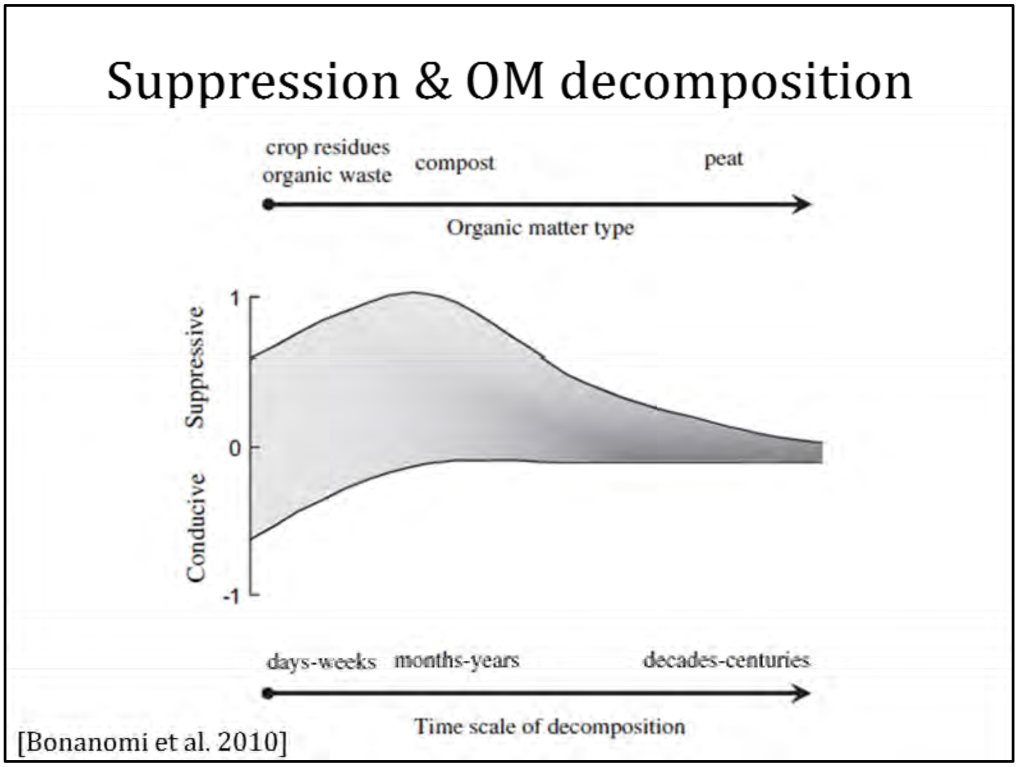
Plants have a system of protection against pathogens that is somewhat analogous to mammalian immune systems. One component of this is Induced Systemic Resistance or ISR. Split pot experiments can demonstrate how microbes can indirectly affect the interaction between plants and pathogens. In split pot experiments with cucumber plants, one part of the root system was exposed to the bacterium *Pseudomonas corrugata*. A separate part of the root system was exposed to *Pythium aphanidermatum* zoospores, but in plants treated with the bacteria no disease occurred. In this case the biocontrol bacterium had no direct contact with the plant pathogen. Instead, the bacterium initiated the plant's defense response and a phloem mobile signal carried this information to the other parts of the plant. With the arrival of this chemical signal, the plant starts to make protective compounds like antioxidants, nitrous oxide, and hydrogen peroxide that can lessen the tissue damage caused by pathogens.

Chen, C.Q., R.R. Belanger, N. Benhamou, and T.C. Paulitz, *Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and Pythium aphanidermatum.* Physiological and Molecular Plant Pathology, 2000. 56(1): p. 13-23.

Multiple organism biocontrol

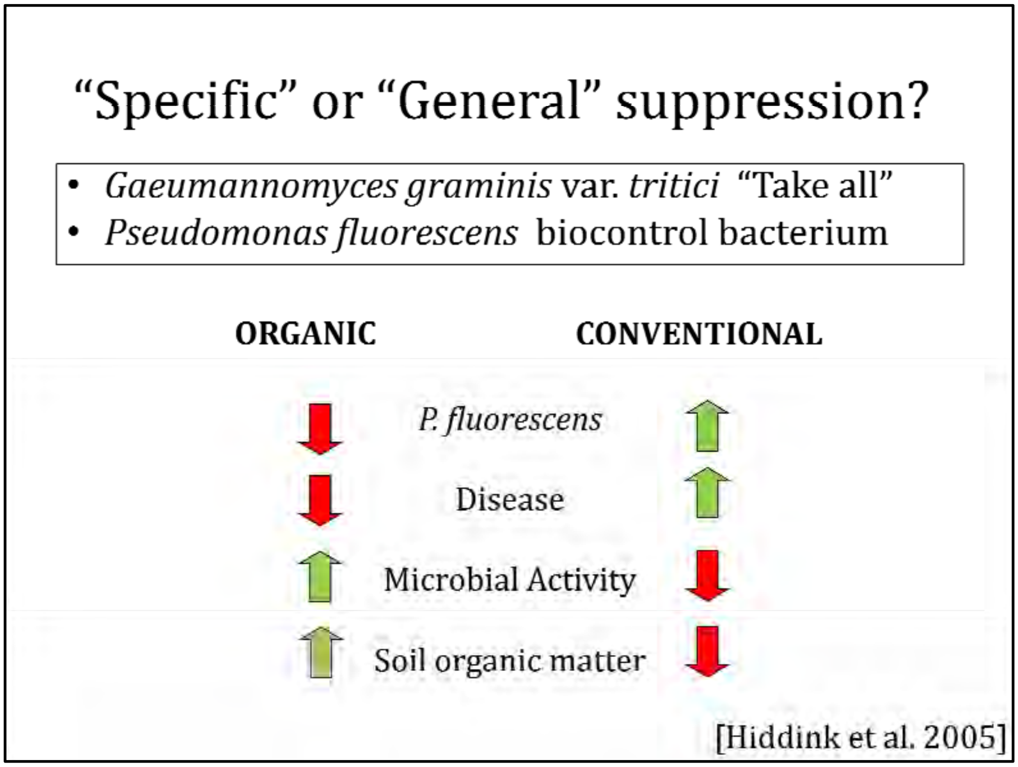
- Often associated with high microbial biomass and activity, but not always
- Unclear which organisms are involved and how they interact with each other, the plant and the pathogen
- Much more complicated to study

While scientists have learned a substantial amount about how individual species of microbes can prevent plant disease, understanding how the complex communities of microorganisms present in soil, compost and decomposing cover crops suppress disease has proven much more difficult. Although some commercial compost testing facilities claim to be able to predict which compost will be suppressive with certain biological measurements, there is no scientific basis for this claim. The reality of the situation is much more complicated as I will attempt to explain here.



A recent metanalysis of over 400 scientific studies on disease suppressive amendments has emphasized some important trends. In this graph different types of amendments are listed in order of their decomposition status. On the low end of the decomposition spectrum, cover crop residues and raw organic wastes are either highly suppressive (prevent disease) or highly conducive (allow disease). More highly decomposed materials such as compost are more likely to be disease suppressive, although not all batches are. On the high end of the decomposition spectrum, peats are most likely to be neutral, i.e. mildly suppressive or conducive. Although this general trend is informative, looking at specific examples illustrates the variability and complexity of these systems.

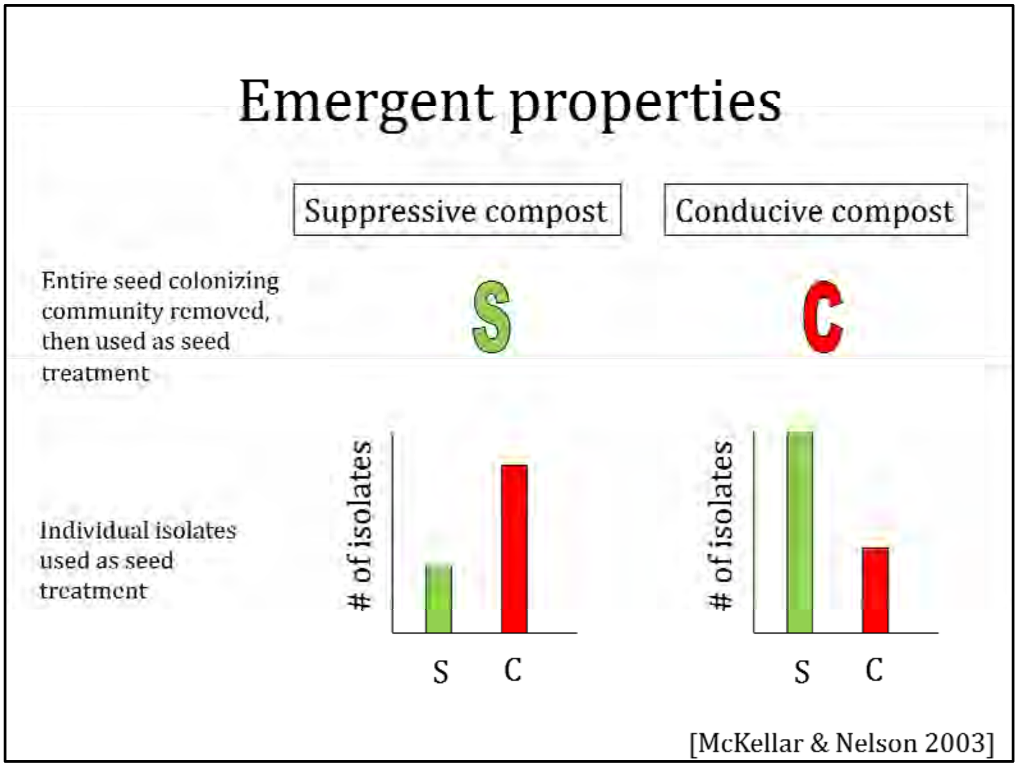
Bonanomi, G., V. Antignani, M. Capodilupo, and F. Scala, *Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases.* Soil Biology and Biochemistry, 2010. 42(2): p. 136-144.



Suppression of plant pathogens is sometimes classified as either specific, caused by a single microbial species or general, associated with multiple chemical, physical, and biological factors. Take all decline of wheat is often mentioned as a classic example of “specific” disease suppression where the biocontrol bacterium *Pseudomonas fluorescens* produces antibiotics toxic to the fungal pathogen. But the distinction between specific and general suppression can fall apart when you look at the interaction in an ecological context.

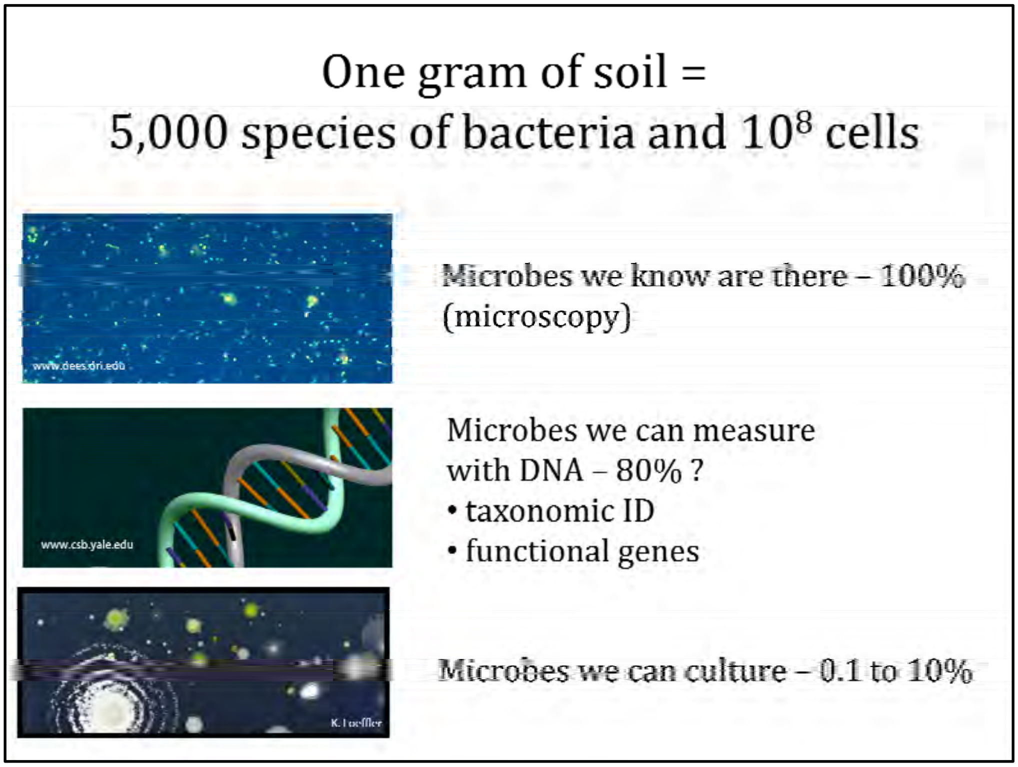
In one case, soil organic matter management had an unexpected effect on take all suppression. Organically managed soils had lower levels of antibiotic producing *Pseudomonas fluorescens*, but still had low levels of disease even with the presence of the pathogen. Conventionally managed soils had high levels of antibiotic producing *P. fluorescens*, but also had high disease. The reality of suppressive soils is much more complex than it is usually presented when you take into account soil organic matter characteristics and the overall activity of the soil microbiota. So even in cases with well studied and extensively documented biocontrol organisms, there are aspects of the system that we are just beginning to understand.

Hiddink, G.A., A.H.C. van Bruggen, A.J. Termorshuizen, J.M. Raaijmakers, and A.V. Semenov, *Effect of organic management of soils on suppressiveness to Gaeumannomyces graminis var. tritici and its antagonist, Pseudomonas fluorescens.* European Journal of Plant Pathology, 2005. 113(4): p. 417-435.



While the previous example emphasized the importance of total microbial activity in soils, we'll now take a closer look at complex microbial communities and how they contribute to disease suppression. This study compared two types of compost, one that suppresses *Pythium ultimum* on cotton and one that does not. Seeds were sown in both composts for 4 hours, removed and the microbes colonizing the seed surface were collected. When this group of microbes was used as a seed treatment, the microbes from the suppressive compost offered protection from disease while those from the conducive compost did not. Individual microbes were isolated from the seed colonizing microbial communities and tested one by one as seed treatments. One might assume that the group of microbes that effectively suppressed disease would contain a high number of individual isolates that were effective biocontrol agents. Instead the authors found the opposite, that only a few of the microbial isolates from the suppressive compost were able to prevent disease on their own, while the conducive compost contained a high number of suppressive isolates. This finding is an example of the concept of emergent properties where "the whole is greater than the sum of its parts". Even when we have characterized the suppressiveness of individual microbial isolates, we still cannot predict how those same microbes will interact with the plant host and the pathogen when applied as a group.

McKellar, M.E. and E.B. Nelson, *Compost-induced suppression of Pythium damping-off is mediated by fatty-acid-metabolizing seed-colonizing microbial communities.* Applied and Environmental Microbiology, 2003. 69(1): p. 452-460.

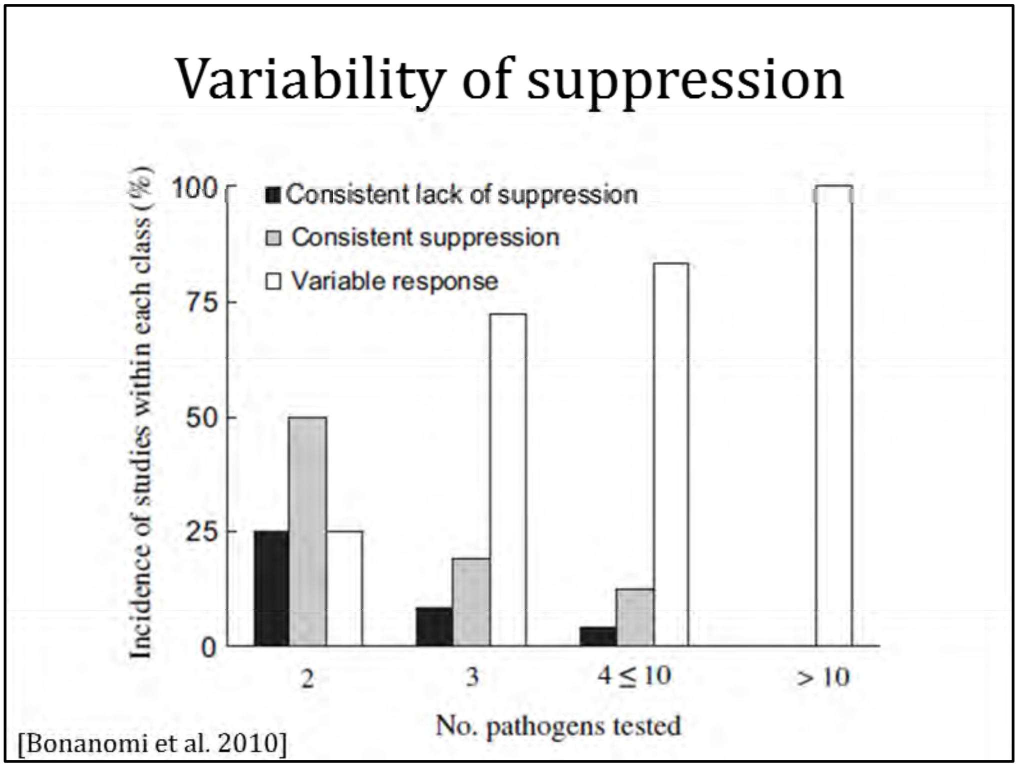


Our ability to study soil microbial communities is complicated by the incredible complexity of this environment. Basic microscopy allows us to see and count exactly how many microbes are present in soil, but doesn’t help identify the species of microbe or its role in the soil environment. Traditional plating methods allow for intensive physiological study of individual species, but only offer access to 0.1 to 10% of the total microbial community. This phenomenon is often referred to as “the plate count anomaly”. In the past few decades molecular techniques using DNA extracted from soil have allowed scientists to access much more of the bacterial community. These techniques rely on a national DNA sequence database for identification of many of the microbes. The identification of individual microbes and sequence fragments is commonly outpaced by the rapid generation of sequence data. Global measures of microbial communities are helpful in some situations. But its important to keep in mind that counting soil microbes with a microscope is like counting the total number of living creatures in the rainforest while not knowing if they are ants or jaguars.

Suppression: "it depends"

- Suppressive composts are well documented in the scientific literature
- But, suppression depends on:
  - Amendment rate
  - Type of feedstock
  - Temperature
  - Presence of synthetic fertilizers
  - Potting media substrate
  - Type of pathogen

Variability of suppression with composted materials is extremely high. Scientists don't understand enough at this point to predict if a batch of compost will suppress a certain disease on a certain crop which is a major obstacle to using these materials effectively in farming. Many of the studies that test multiple batches of compost in a variety of pathosystems come up with the somewhat unsatisfying conclusion that "sometimes it works, sometimes it doesn't and we're not totally sure why".



Compiling the results of over 400 scientific papers shows that the variability of suppression increases with the number of pathogens tested in the study. So that even if a particular batch of compost is consistently suppressive towards one plant pathogen, it is likely to have a range of effects on other pathogens. This variability is of great relevance to growers because very rarely is there only one pathogen causing a problem in a specific production system.

Real world complications

- **Suppression:** 50% control of powdery mildew (*Uncinula necator*)
- **Some suppression:** gray mold (*Botrytis cinerea*)
- **No effect:**
  - Black rot (*Guignardia bidwelli*)
  - Phomopsis (*Phomopsis viticola*)
- **Increased:** downy mildew (*Plasmopara viticola*)

[M. Ryan Rodale Institute unpubl. Data, synopsis in New Farm online magazine]

Testing compost-based materials in the field can be complicated by the fact that multiple pathogens are naturally present. In this on-farm trial of aerated liquid compost extracts conducted by the Rodale Institute, the authors found that treatment with the extracts significantly controlled some of the pathogens while actually exacerbating the symptoms caused by other pathogens.

Sayre, L. (2003) *Compost tea research enters its second year: Study aims to shed light on current debates over the safety and efficacy of compost tea as an organic material.* New Farm. http://www.newfarm.org/depts/NFfield_trials/0404/tea.shtml

Dangerous Assumption

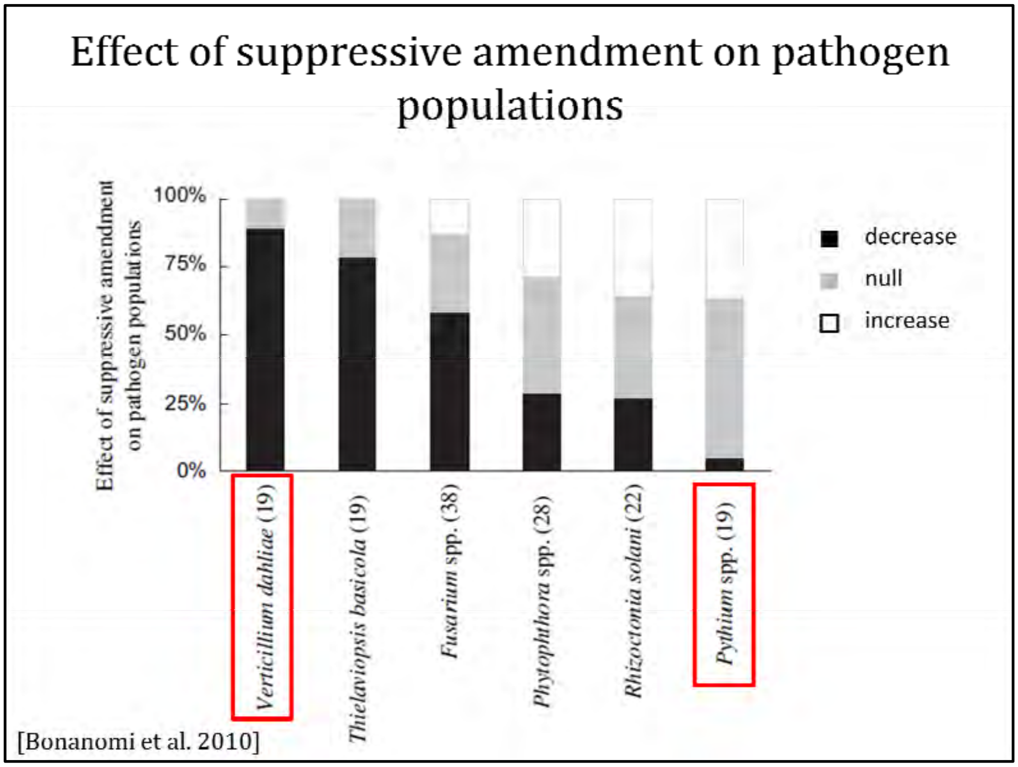
- All composts and cover crops that suppress disease do so by reducing the amount of pathogen present in the soil
- It's much more complicated than this!

One reason for the variability of suppression seen with composts among a range of pathogens is that different mechanisms are involved in the suppression of different types of pathogens.

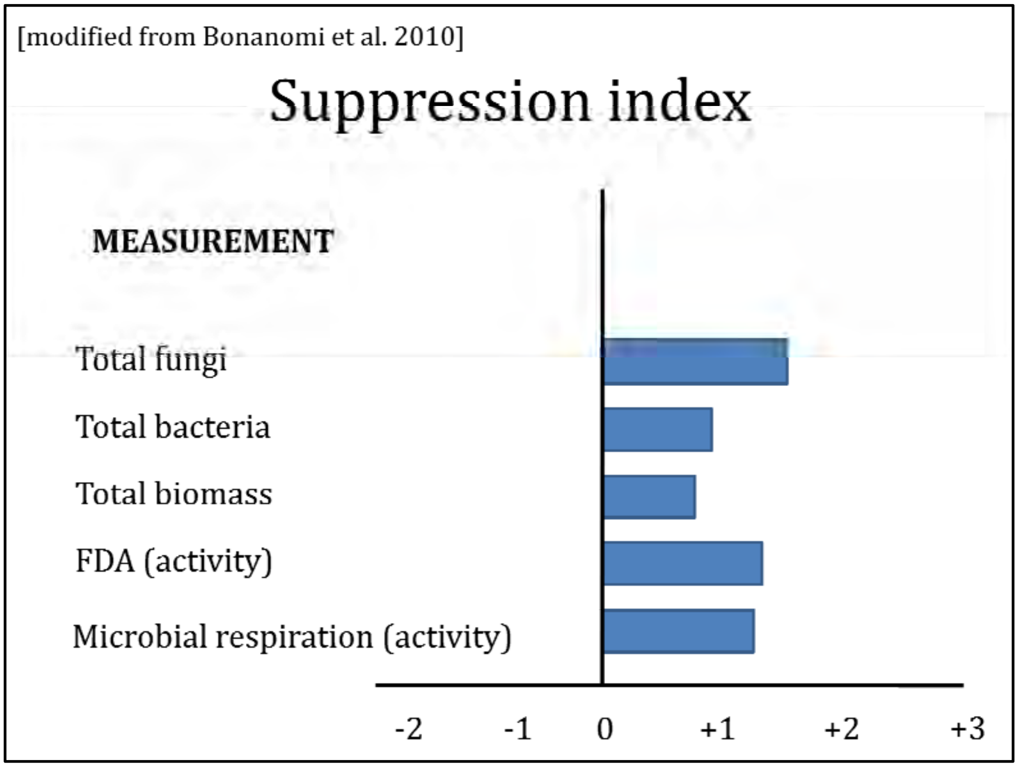
Ecology of plant pathogens

- Verticillium spp. (wilts)
  - Survival structures in soil - microsclerotia
  - Weak saprotroph
  - Microsclerotia are killed by chemicals released from decaying organic matter
- Pythium spp. (seed, root and fruit rots)
  - Survival structures in soil – oospores
  - Strong saprotroph
  - Mechanism of suppression is less clear

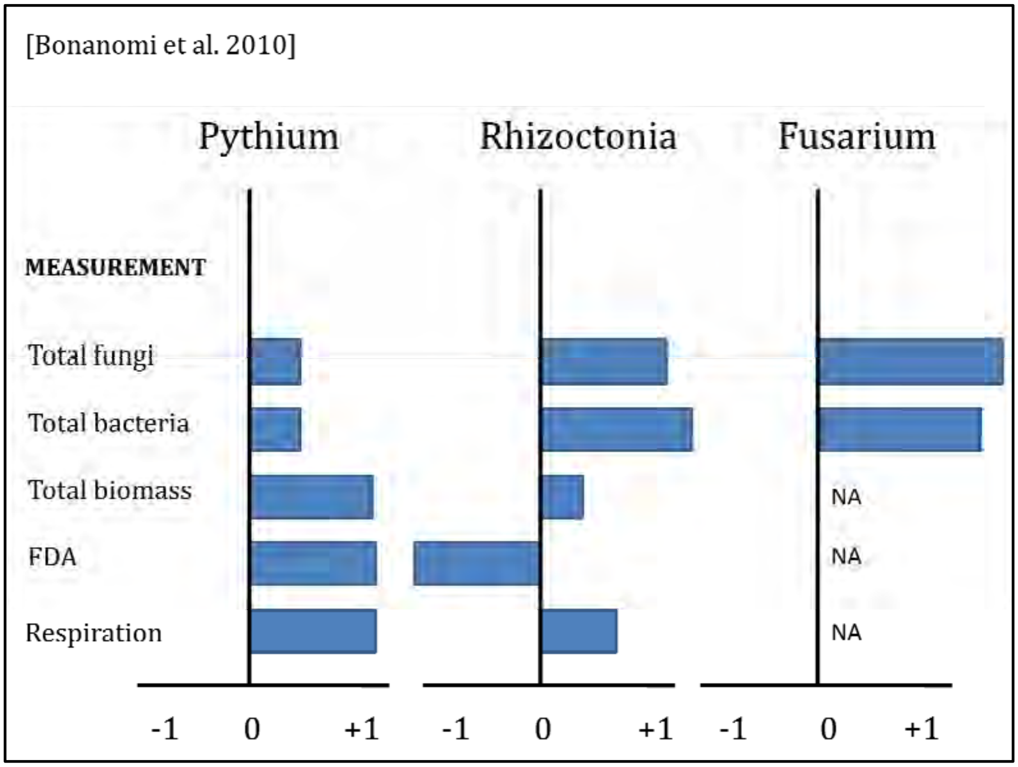
Pathogens in the fungal genus *Verticillium* and the oomycete genus *Pythium* have distinct strategies for existing in the soil environment. *Verticillium* is a weak saprotroph, meaning that it cannot survive well by decomposing dead plant tissue, while *Pythium* is able to switch between being a pathogen and a decomposer.



The soil population of plant pathogens are affected by compost amendments differently depending on the pathogen’s ability to survive without the presence of its plant host. Poor saprophytes such as *Verticillium* spp. are more likely to be killed by suppressive compost amendments, while strong saprophytes, *Pythium* spp., are more likely to remain alive and even flourish, but somehow lose their ability to cause infection.



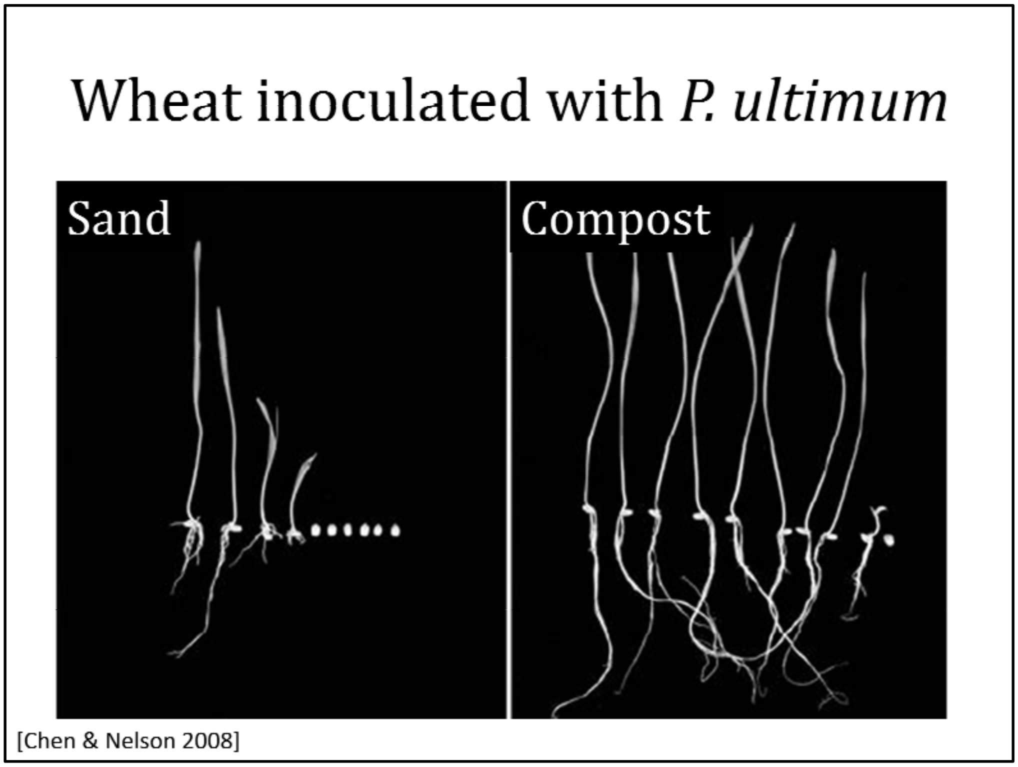
Specific measurements are often associated with suppression as seen in this "suppression index". Positive numbers on the index indicate that a high value of a measurement like microbial activity or total bacterial cell counts positively correlates with suppression and vice versa. When all available scientific data on all pathosystems is combined several indicators stand out as being consistently associated with suppression.



However, when you look at the suppression index for different types of pathogens the variability becomes more apparent. For example high levels of FDA hydrolysis (a measure of microbial activity) is positively correlated with suppression of *Pythium* spp., but negatively correlated with suppression of *Rhizoctonia* spp. This variability of reliable indicators depending on the type of is one of the reasons suppression is so difficult to predict.

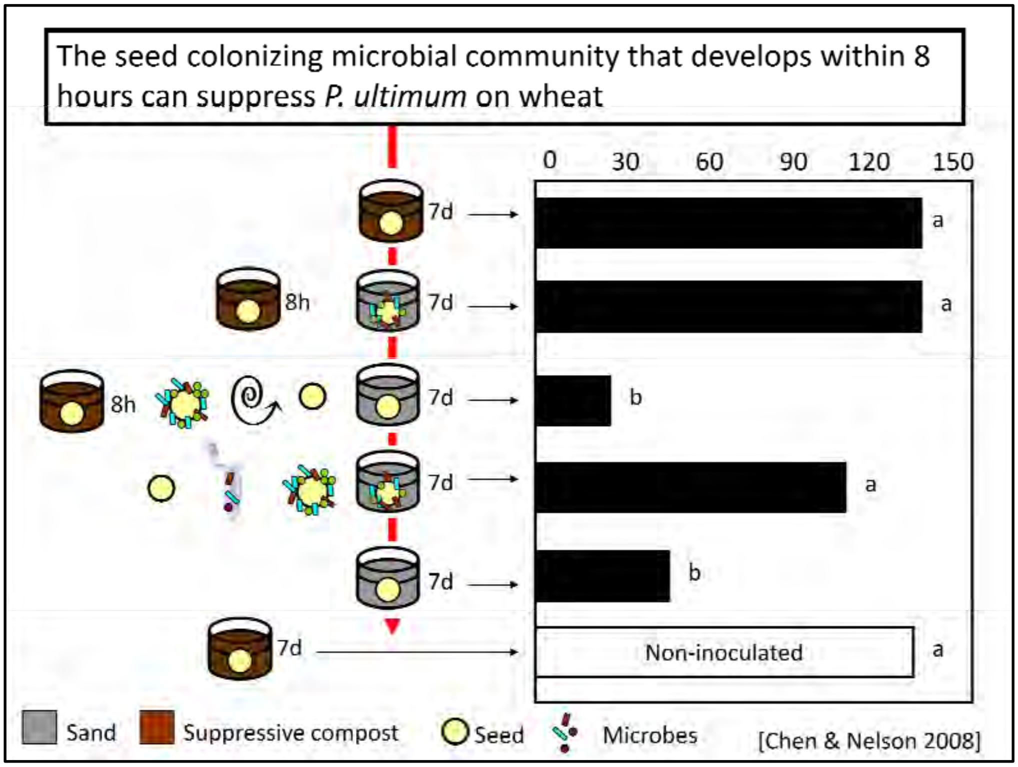
Probing the black box:
What makes composts
suppressive?

I'd like to shift gears and talk about some of the work going on at Cornell in Eric Nelson's lab on disease suppressive composts. Many of our research projects aim to increase our understanding of the microbial mechanisms of suppression in specific pathosystems.



Bioassay results showed that a biosolids – leaf compost made in NY state consistently suppressed *Pythium ultimum* infection of wheat.

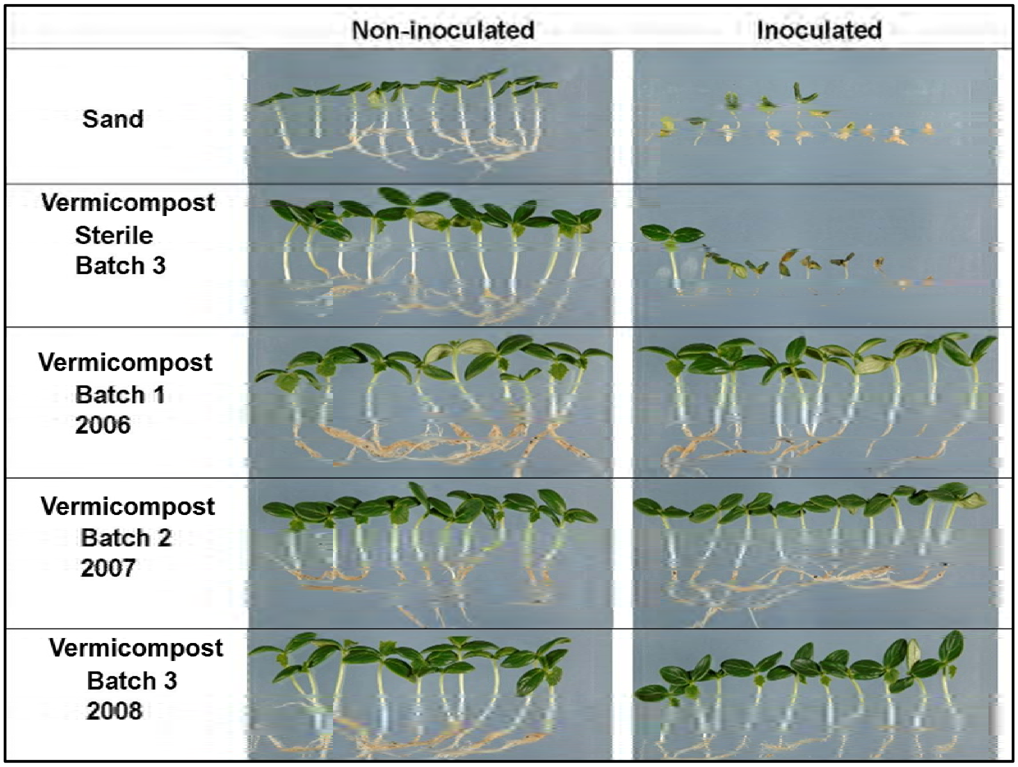
Chen, M.-H. and E.B. Nelson, *Seed-colonizing microbes from municipal biosolids compost suppress Pythium ultimum damping-off on different plant species. Phytopathology, 2008. 98(9): p. 1012-1018.*



Recent work in our lab has shown the importance of seed colonizing microbial communities in disease suppression. 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.

Mention that sporangia were still viable, just didn't germinate, or germinated a little then retracted their germ tubes.



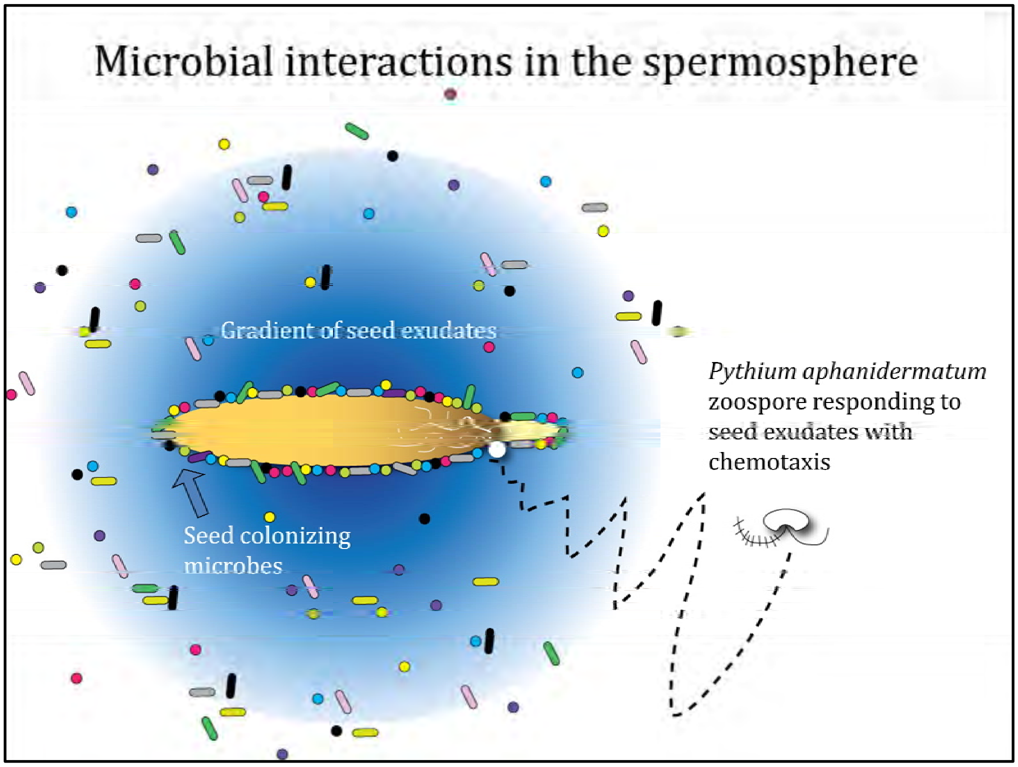
I'm currently investigating the role of the seed colonizing microbial community in the suppression of *Pythium aphanidermatum* in cucumber by vermicompost. 7 day old seedlings were grown in sand or sand amended with 40% v:v vermicompost. Photos show representative seedlings in non-inoculated and inoculated treatments. All inoculated seedlings received a set number of zoospores and amendment with vermicompost consistently suppressed infection. Heat sterilized vermicompost offered no protection from infection.

Zoospore pre-infection events

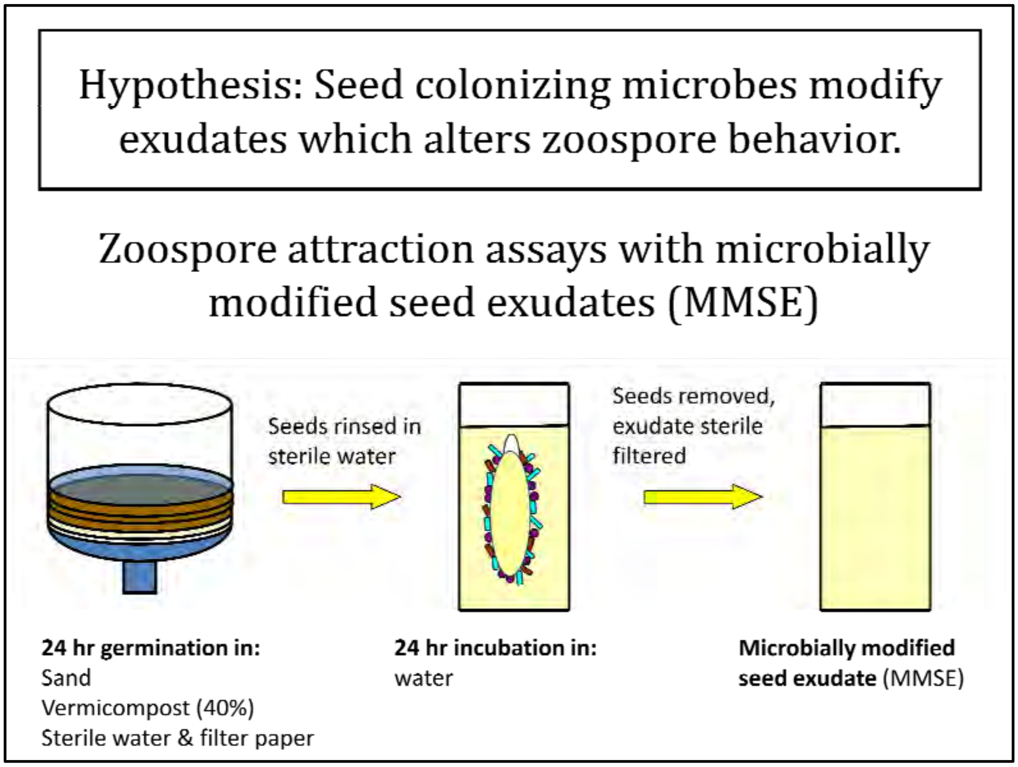
Please see separate video file: “zoospore behavior”.

This time lapse shows cytoplasm (cellular contents) streaming from sporangia into vesicles. Once in the vesicles, cytoplasm cleaves into individual zoospores which are eventually released into the environment. Zoospores then swim using chemical cues to find germinating seeds. A five hour time lapse shows encysted zoospores on a root border cell germinating and extending their germ tubes to initiate infection.

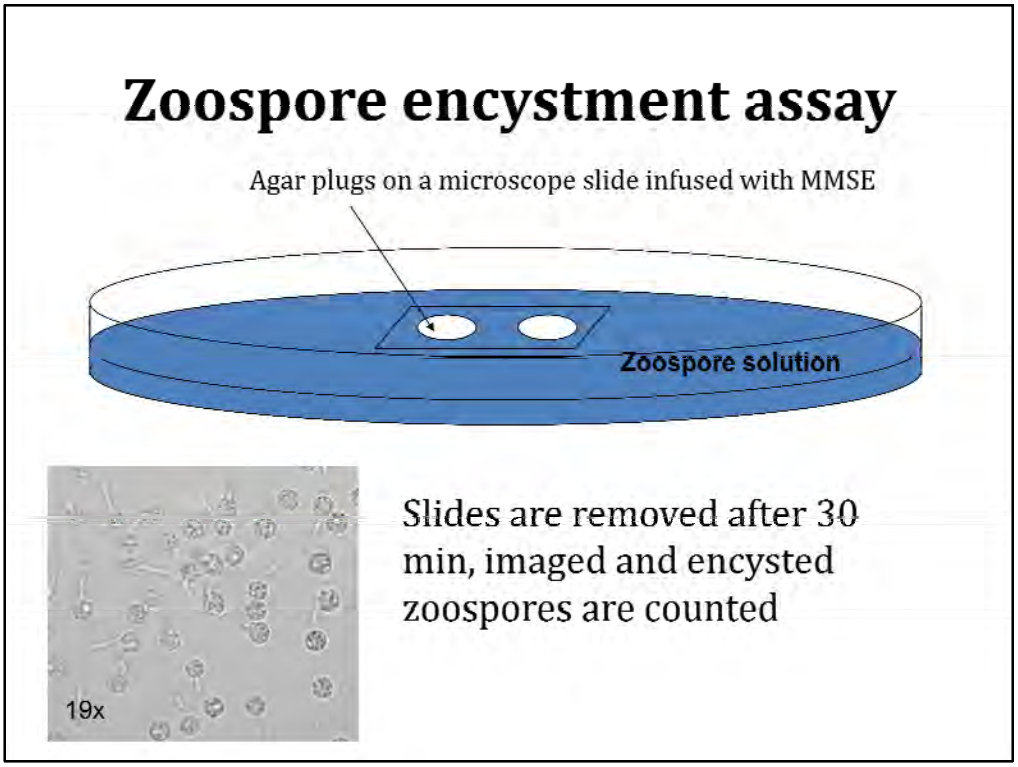
Video credit: E. Carr & A. Jack



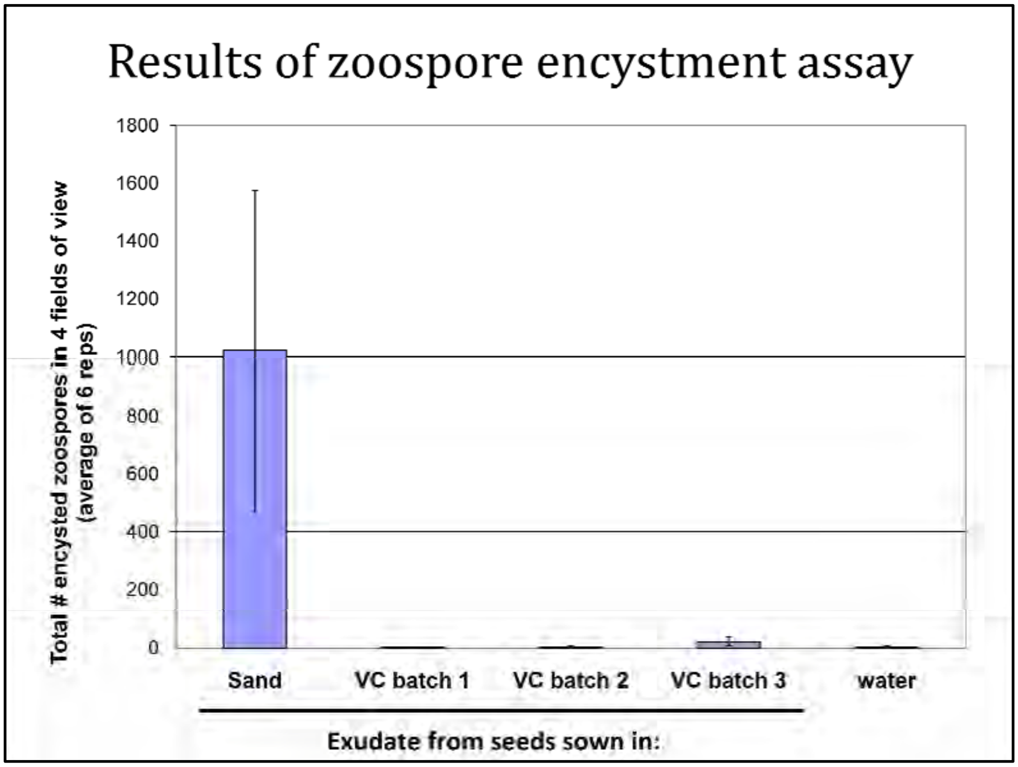
The spermosphere is the primary infection court of *P. aphanidermatum*. As seeds imbibe water and germinate, they passively release cell contents due to the build of up a high level of turgor pressure. These exudates consist primarily of organic acids, amino acids, and sugars which form a chemical gradient 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?



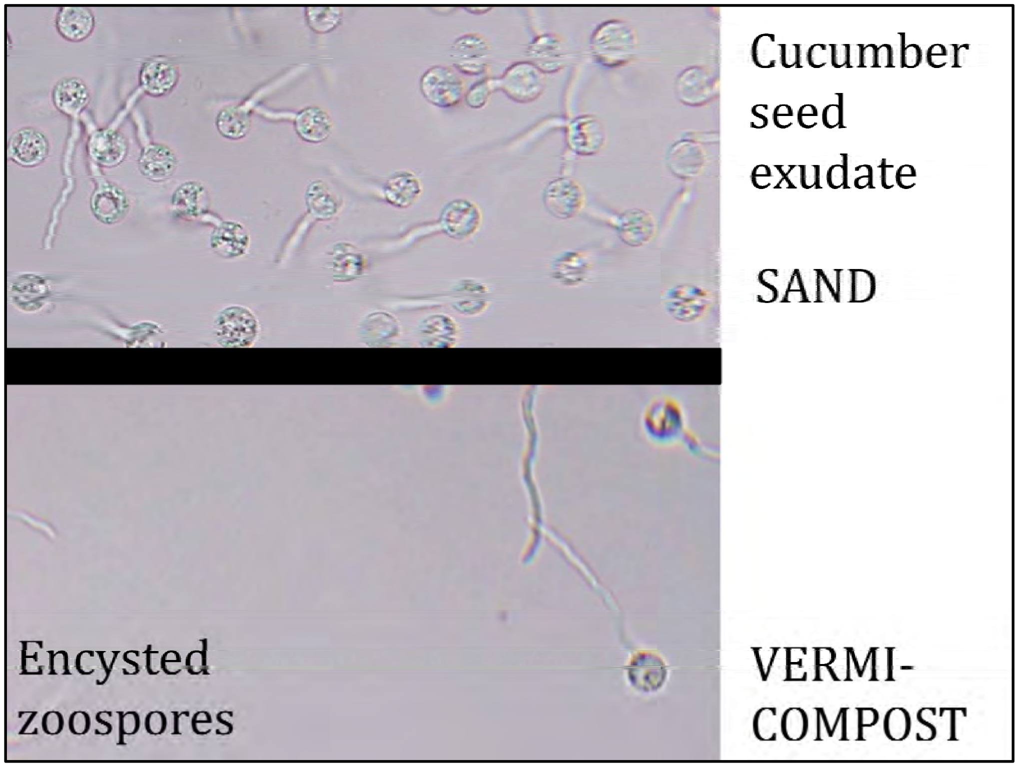
We tested the hypothesis that seed colonizing microbes modify exudates which alters zoospore pre-infection behavior by carrying out zoospore encystment assays using microbially modified seed exudates or MMSE. MMSE were prepared by sowing seeds in sand or suppressive vermicompost. After 24 hours, seeds 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 to remove all microbial cells and used in zoospore encystment assays.

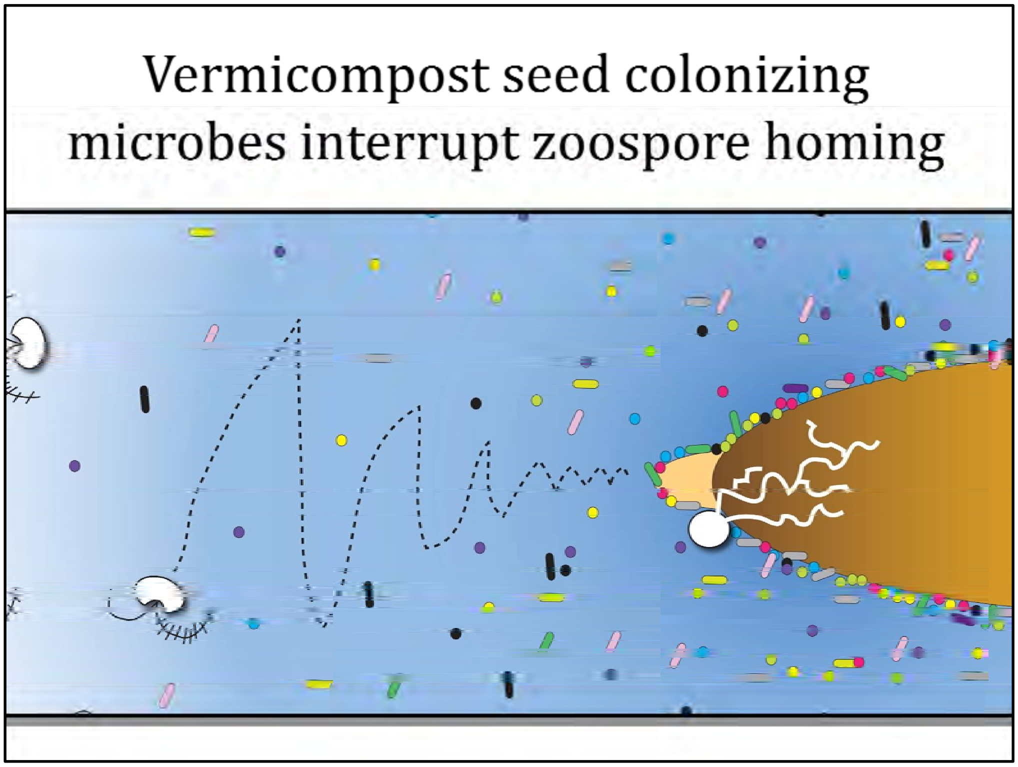


Microbially modified seed exudates were infused into agar discs on glass slides. We then submerged the slides in a zoospore suspension for 30 minutes. Encysted zoospores were counted under the microscope for each treatment.



The x axis shows the treatments; exudates from seeds sown in sand or three batches of vermicompost and a water control with no seeds. The y axis shows the total number of encysted zoospores in 4 fields of view. While high numbers of zoospores encysted on exudates from seeds sown in sand, very few to no zoospores encysted on exudates from seeds sown in vermicompost.


Cucumber seed exudate
SAND
Encysted zoospores
VERMI-COMPOST



This experiment provides one line of evidence that seed colonizing microbial communities from vermicompost are interfering with the zoospore's ability to find the seed by chemically modifying seed exudates. We're currently working to document other aspects of this phenomenon and uncover the microbial mechanisms behind these zoospore responses. Our goal is to increase our understanding of how these materials suppress disease in a single pathosystem. Understanding one system with a high level of detail will provide clues to understanding disease suppression in all systems which will help us develop ways to more effectively use compost for plant disease management.

Conclusions

- Future predictive factors:
  - May be pathosystem specific
  - Global predictors of suppression may not be feasible
- There’s a lot we don’t know, but if we keep working together to figure it out, these materials can be more effective for biologically based disease management

Because of the high variability in compost-mediated suppression of different pathogens, finding measurements that can predict overall suppressiveness may be unrealistic. In the future, predictive factors may be pathosystem specific. Opportunities for collaboration exist among growers, compost producers and researchers to continue to improve the efficacy of compost use for plant disease suppression.

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