Suppression of *Pythium* damping-off with compost and vermicompost

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1. Project Summary

This project is part of a larger vermicompost/ liquid vermicompost extract study taking place at Cornell that involves researchers from several departments, extension staff, as well as both conventional and organic fruit and vegetable growers. Our collaborators in horticulture are assessing the use of vermicompost for nutrient management in vegetable transplants. An economist is investigating the financial benefit of vermicompost as an amendment for plant production. We're working with Cornell Waste Management Institute to create an extension website and video that covers all aspects of the greater project. We have received both state and federal funding for the larger project.

Composts and vermicomposts are microbiologically rich amendments that promote plant growth and can suppress plant diseases. However, the inconsistency of disease suppression prevents growers from fully harnessing this potential benefit. Commercial testing of composts is available, but there remains a great need for scientifically based tests that are verified in the public sphere to determine if a specific material can suppress plant diseases. This section of the larger project focuses on increasing our understanding of the complex microbial mechanisms behind compost-mediated disease suppression in order to develop new techniques to predict compost suppressiveness.

So far we have confirmed that vermicomposted dairy manure and non-aerated liquid vermicompost extract consistently suppress *Pythium* damping-off in cucumber, and that this observed suppression is biologically based. We've developed a novel zoospore attraction assay and generated preliminary data showing that seed colonizing microorganisms from vermicompost interfere with the pathogen's ability to chemically sense the presence of a seed. As part of the larger project, experiments are underway to explore this phenomenon in greater detail and identify the vermicompost-derived microbes that are involved in the suppression of disease.

2. Introduction to Topic

Plant diseases, especially soil-borne and seed infecting pathogens, are a serious issue for both greenhouse and field production of many horticultural crops. Organic growers have limited options for control of these diseases since most of the effective fungicides, fumigants and seed treatments are synthetic, toxic and potentially polluting. OMRI-listed biopesticides are available as substitutes for synthetic inputs. However the cost of developing and registering new products with the EPA is quite high and as a result relatively few products are available (Nelson 2004b). Furthermore, many of the formulations are not consistently effective under variable field conditions. Relying on

application of a single antagonistic organism to control plant disease can reduce pesticide use, but is in some ways an extension of the conventional pest control mentality which does not consider the broader soil ecosystem.

Organic farmers and ecologically-minded agricultural scientists have long recognized the importance of maintaining healthy soil for producing healthy crops. Using compost to increase soil organic matter and promote healthy and productive soils has been a cornerstone of the organic philosophy. Sir Albert Howard, a mentor of J.R. Rodale, described the importance of compost applications in maintaining soil health as early as the 1940's (Howard 1943). Many growers today use compost to increase organic matter and nutrient cycling and to suppress soil-borne plant diseases. However, the inconsistency of disease suppression associated with compost applications has long plagued growers and researchers alike.

Land grant universities offer soil testing for growers, but most tests only look at chemical nutrients and ignore the biological characteristics of the soil.[1] Many organic growers and compost producers therefore rely on commercially available tests to determine if a certain compost batch will be suppressive. Unfortunately most of the commercially available testing methodologies are not based on publicly available peer-reviewed data or a current understanding of microbiology and thus have no valid scientific link to disease suppression. Biologically mediated disease suppression is a complex phenomenon. We need to understand more about how composts suppress plant disease before we can accurately predict which composts will effectively suppress plant diseases.

A. Predicting suppression

The idea that some soils and composts can naturally suppress soil-borne plant diseases is certainly not a new one. Work in this field dates back to the late 1800's (Howard 1943; Howard 1945; Huber and Schneider 1982). As early as 1959, experiments confirmed the biological nature of the observed disease suppression in several soil systems (Menzies 1959). Composts have been well studied for their disease suppressive properties (Hoitink and Kuter 1986; Weltzien 1989). However, even after decades of study, both a comprehensive understanding of how disease suppression occurs, and the ability to manipulate agricultural management practices to create consistently suppressive soils and composts, remain elusive (Janvier et al. 2007). In order to effectively utilize composts, vermicomposts and compost teas as pesticide alternatives, we need to be able to predict their effects on plant disease.

i. Single organism biocontrol

One area of study where the details have been well established is single organism biological control of plant diseases. The biocontrol industry has used this groundwork of scientific understanding to commercialize formulations of individual biocontrol agents. Internationally there are 25 microbial species now registered for the control of a specific group of plant pathogens, the oomycetes (Nelson 2004b). Using the plant disease *Pythium*

[1] A notable exception is the NESARE funded Soil Health Project which offers a soil testing service through Cornell University http://www.hort.cornell.edu/soilhealth/

damping-off as an example, there are four mechanisms by which individual species of beneficial microorganisms can prevent this pathogen from causing disease in a plant: 1) antibiosis, 2) parasitism, 3) competition for nutrients and 4) induced systemic resistance (ISR).

- **Antibiosis** refers to the ability of the biocontrol microorganism to produce antibiotics. In one case a biocontrol bacterium *Bacillus subtilis* produces an antibiotic, zwittermicin A, that is toxic to the plant pathogen *Pythium torulosum* (Shang et al. 1999).
- **Parasitism** is where one organism actually eats another. In one case a biocontrol fungus, *Trichoderma harzianum* winds around the hyphae of *Pythium ultimum*, punctures its cell wall and consumes the plant pathogen (Benhamou and Chet 1997).
- **Competition for nutrients or chemical signals** in seed exudates is well documented. In the case of the biocontrol bacterium *Enterobacter cloacae*, it degrades the specific fatty acids that *Pythium ultimum* needs to germinate, so that even though it doesn't kill the pathogen, there is no plant infection (van Dijk and Nelson 2000).
- With **induced systemic resistance** (ISR) a beneficial bacterium, *Pseudomonas corrugata*, stimulates what can be loosely considered the plant's 'immune system' so that it can protect itself from *Pythium aphanidermatum* (Chen et al. 2000). This is distinct from Systemic Acquired Resistance (SAR) which can occur when a plant is exposed to a low level of a specific pathogen and then acquires resistance to that same pathogen in the future. ISR and SAR function through different phloem mobile chemical signals produced in plant tissue and are entirely distinct pathways (Hammond-Kosack and Jones 2000).

ii. Multiple organism biocontrol

In contrast to the situation with individual microorganisms described above, the suppression of plant diseases with complex communities of microbes is not well understood. Successful commercialization of products containing unspecified groups of microorganisms (composts, vermicomposts, compost teas) for plant disease control has been more difficult due to this general lack of understanding. In a situation where one gram of compost can contain 10^9 (that's 1,000,000,000) bacteria and 10^6 (that's 1,000,000) fungi, it's not surprising that ecological interactions between these organisms are complex. While scientists can see these microbial cells under a microscope, a staggering majority of them, some estimates are up to 99.9%, cannot be isolated and grown in culture with current laboratory techniques. In microbial ecology this phenomenon is called "the great plate count anomaly". Microbial physiology can only be assessed when organisms are in culture. In other words, we know they're there, but we only know what 0.1 – 1% of them are actually doing in the soil ecosystem. Work with soil DNA has led to estimates of up to 1,000,000 microbial species per gram of soil (Torsvik et al. 1990; Gans et al. 2005). Sequencing genes of taxonomic and metabolic importance from soil DNA extracts can provide useful information, but we are still limited by the quality of our sequence

databases. Given this incredible biological complexity and the limitations to scientific understanding of soil microbiology, a clear scientific understanding of how microbial communities in composts suppress diseases remains elusive.

Much of the research in this field relies on the unsupported assumption that the more individual biocontrol organisms a compost contains, the better it will be at suppressing diseases (de Brito Alvarez et al. 1995). In fact the situation is far more complicated. In ecology and the emerging field of systems thinking, 'emergent properties' is an important concept which basically means the whole is greater than the sum of its parts. Work in the Nelson research group has shown that some composts containing high numbers of individual biocontrol organisms do not suppress disease, while others with low numbers are suppressive (McKellar and Nelson 2003). This means the microorganisms in the suppressive compost may not individually be suppressive but are working together in ways that we do not yet understand to prevent plant disease.

Microbiological testing of composts to predict disease suppression is commercially available, but none of the methods used by these labs have been shown in the peer reviewed scientific literature to be an effective way to consistently predict disease suppression. More detailed information on attempts to correlate microbiological measurements with disease suppression in soils and composts can be found in the following reviews (Reeleder 2003; Garbeva et al. 2004; Mazzola 2004; Janvier et al. 2007; Bonanomi et al. 2010).

- **Presence of known biocontrol organisms:** Assessing the presence of groups of microorganisms known to be suppressive when applied individually is a common method. The presence of individually suppressive microorganisms does not necessarily correlate with the overall suppressiveness of a specific compost, sometimes it does (Boehm et al. 1993; Postma et al. 2005) and sometimes it doesn't (McKellar and Nelson 2003). The same is true for in vitro inhibition assays. Bacteria isolated from composts can be grown on a Petri dish with a single plant pathogenic fungus to determine if they inhibit fungal growth (de Brito Alvarez et al. 1995). However, these assays cannot predict whether or not this organism can effectively prevent plant disease on its own in the presence of the host or in the soil environment.
- **Microbial diversity:** Many assume that high overall microbial diversity will lead to suppressive compost, but the relationship between microbial diversity and disease suppression is complex. In some cases disease suppression is correlated with high microbial diversity (Postma et al. 2005), in other cases it is correlated with low microbial diversity (Boehm et al. 1993; van Elsas et al. 2002), and this relationship can change over time in the same material (Hallmann et al. 1999).
- **Microbial activity:** Similarly to microbial diversity, in some cases high microbial activity is correlated with suppression (van Os and van Ginkel 2001; Hunter et al. 2006), and in other cases high microbial activity is correlated with increased disease (Erhart et al. 1999). This correlation can be highly variable and situation-specific (Knudsen et al. 1999) depending on the pathogen tested (Scheuerell et al. 2005) and soil management (Ghini and Morandi 2006).

- **Total-Active fungal and bacterial ratios:** Another technique involves using microscopy to measure the ratio of active to total bacteria and fungi. This is an important tool in soil ecology for measuring the biological impacts of soil disturbances (Klein and Paschke 2000). However, use of these measurements as a predictive tool for disease suppression is almost exclusively confined to the private sphere so data are not publicly available for scrutiny. In the scientific literature there is only one instance applying similar techniques to disease suppression, i.e. microscopic measurement of total and active bacterial cells with fluorescent staining. In this case, these measurements only correlated with the suppressiveness of compost teas if the teas were made without molasses-based additives (Scheuerell and Mahaffee 2004).
- **Heterotrophic plate counts:** Enumeration of culturable heterotrophic bacteria (colony forming units: CFU) is available commercially, and is used widely in research, but is not a consistent predictor of disease suppression (Scheuerell and Mahaffee 2004; Ghini and Morandi 2006).

B. Vermicompost and non-aerated liquid extracts of vermicompost

Vermicompost is used in horticulture primarily as an organic transplant media amendment partly due to its fine structure and higher proportion of plant available N compared to other types of compost made from the same starting materials (Leonard and Rangarajan 2007). Liquid extracts of compost can supplement seedling nutrient management by being piped directly into greenhouse irrigation as pioneered by Elzinga and Hoeksema Greenhouses (see Appendix 3). However, some of the commercially available aerated compost 'tea' brewers can run upwards of $20,000 which may exclude their use by smaller producers. As part of this study, we wanted to develop a low cost way to produce non-aerated liquid vermicompost extracts that were useful for greenhouse nutrient management and suppressed *Pythium* damping-off when used as a container drench.

C. *Pythium* damping-off

Pythium aphanidermatum is a pathogenic oomycete (a group of organisms previously considered to be fungi) with a host range of over 50 greenhouse and field crop species. It can cause pre- and post-emergence seedling damping-off as well as severe root and fruit rots. In conventional production, fungicide treated seeds are an effective way of preventing *Pythium* damping-off, however certified organic growers have limited options for preventing this disease. *P. aphanidermatum* has a unique life cycle where multiple stages (oospores, sporangia & zoospores) can infect hosts (Figure 1). As seeds germinate they passively release a soup of chemicals known as seed exudates which serve as chemical cues for motile zoospores and surrounding microorganisms (Figure 2). Once zoospores reach their host, they encyst, germinate and initiate infection (Figure 3). Since there are so many cases of composts suppressing *Pythium* damping-off in the scientific literature, we were curious to know how this suppression occurs with the long term goal of increasing

the efficiency of this practice. We set out to understand how seed colonizing microbes from a suppressive vermicompost could interfere with zoospore pre-infection events.

Figure 1. *Pythium aphanidermatum* life cycle (Matthews 1931)

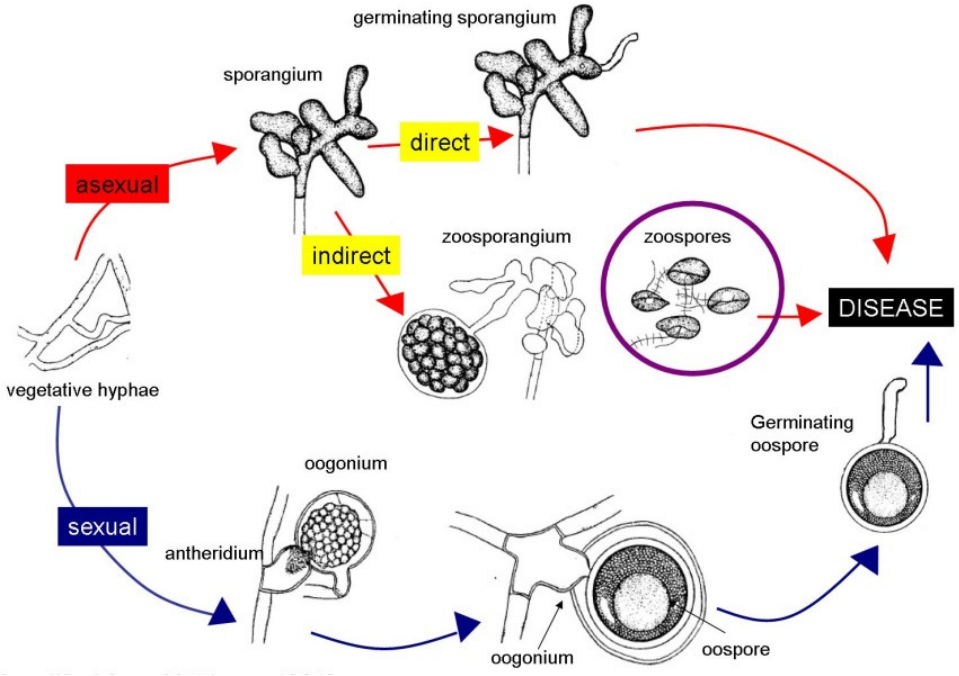


Figure 2. Schematic of the cucumber spermosphere showing the interaction between germinating seeds (exudates represented as blue gradient), seed colonizing microbes and a *Pythium* zoospore swimming towards its host. (Spermosphere = a short-lived, rapidly changing, and microbiologically dynamic zone of soil surrounding a germinating seed)

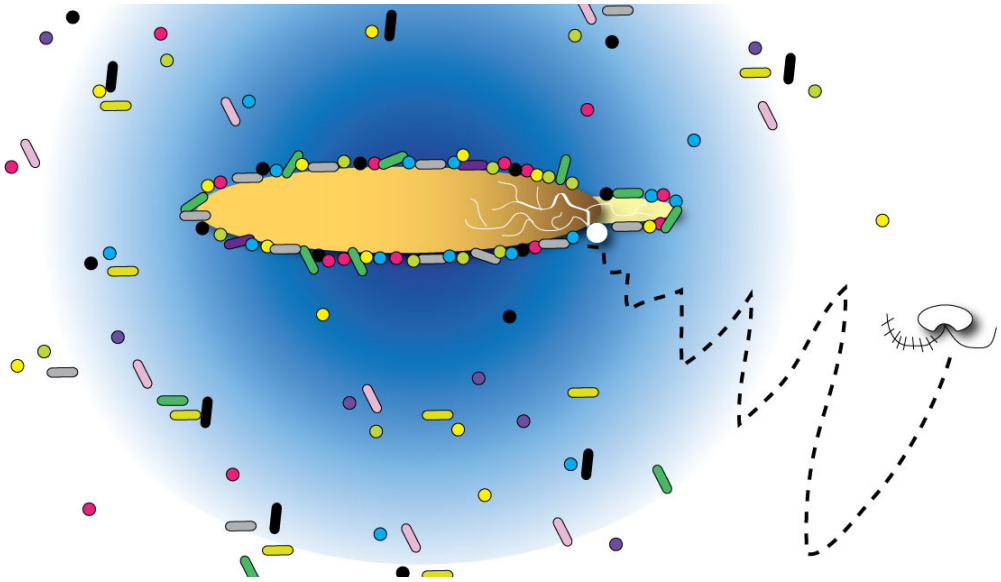
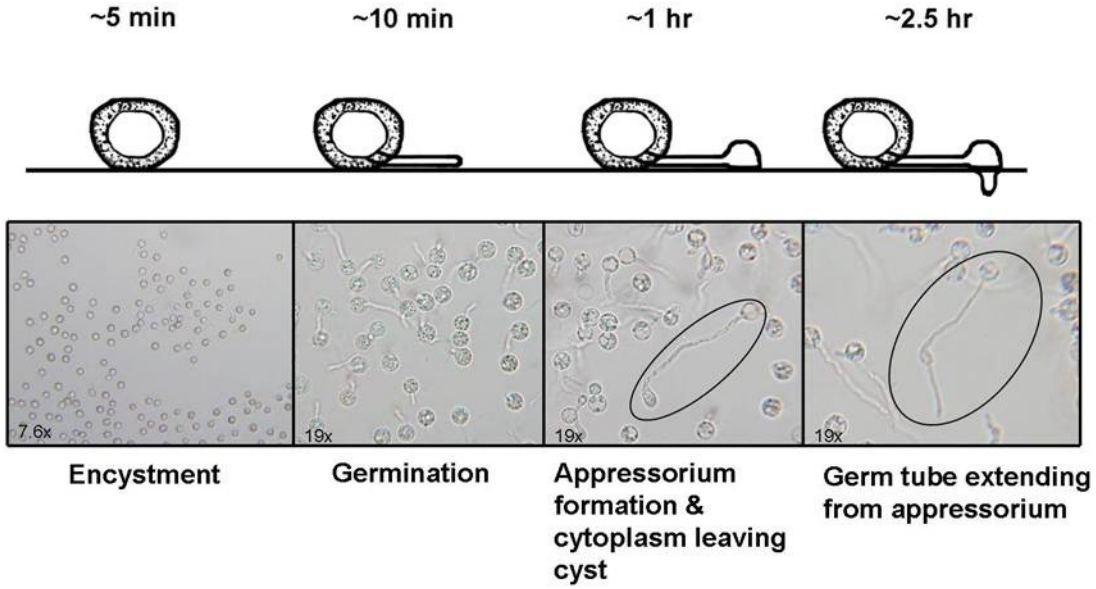


Figure 3. Responses of *P. aphanidermatum* zoospores to cucumber seed exudates



3. Objectives Statement

From our original proposal in 2006:

The overall goal of this project is to increase the efficacy and consistency of using composted animal manures to enhance disease suppression in organic vegetable and fruit production systems. The specific objectives of this portion of the larger project include;

A. To increase our understanding of how microbes present in compost and vermicompost prevent plant diseases, specifically *Pythium* damping-off
    a. To identify key microbial species associated with disease suppression
    b. To identify potential mechanisms of disease suppression
B. To use this understanding to develop tools for predicting whether or not a compost or vermicompost will suppress *Pythium* damping-off
C. To document the effect of composting process (vermicompost vs. thermogenic or "hot" compost) on disease suppressive properties of the finished material

As our project progressed and we obtained other sources of funding we dropped objective C. because we had difficulty finding sources of high quality vermicompost and thermophilic compost made from the same feedstock at the same facility to make a comparison of these materials. Our original project spanned 3 years, but we only ended up applying for the first two years of funding. So while we've made significant progress on objectives A. a. and B., we have nothing definitive to report at this time. We have added an additional objective as a consequence of the additional funding we were able to secure:

D. To develop a cost effective method of producing non-aerated vermicompost extracts that provide essential plant nutrients and suppress *P. aphanidermatum* when used as a soil drench.

4. Materials and Methods

Vermicompost materials

Vermicomposted dairy manure, Worm Power™, was obtained from RT Solutions, LLC in Avon, NY. This material is OMRI listed for use in certified organic agriculture. Separated dairy manure solids from Coyne Farm were hot composted with forced air in an indoor facility to meet the time – temperature requirements for pathogen and weed seed destruction. Uncured thermophilic compost was then fed in thin layers to composting earthworms in a continuous flow-through vermicomposting system. Finished vermicompost was scraped from the bottom of the worm beds and screened. The entire process from raw manure to finished vermicompost takes around 75 days. For more information about the vermicomposting process at this facility please see the educational materials on the RT Solutions website (www.wormpower.net). Vermicompost was stored frozen and thawed for 24 hours before use in bioassays.

Non-aerated vermicompost extract was produced by mixing vermicompost with water at a 1:60 ratio in a 100 gallon plastic tub. Water was circulated briefly twice a day with a sump pump over the extraction period, but was never actively aerated. Finished extract was strained through 4 layers of cheesecloth and stored at room temperature in 5 gallon buckets with vented lids before use in bioassays. Three samples were sent to the Pennsylvania State University Agricultural Analytical Services Lab (University Park, PA) for nutrient analysis.

Disease suppression bioassays – solid vermicompost

A disease suppression bioassay was developed that controls for the environmental factors of temperature, light, moisture (including soil matric potential), and inoculum level based on previous work (Mandelbaum et al. 1993; Chen and Nelson 2008) (Figure 4.). Fritted glass Buchner funnels are connected to tubing and filled with deionized water. Glass fiber filters were laid over the fritted glass, followed by 25 mL sterile quartz sand wet sieved to 0.5 – 1.0 mm (or 40% vermicompost/sand mixture), 10 surface sterilized cucumber seeds (Marketmore 76, Johnny's) and an additional 25 mL sand. Sterile vermicompost was autoclaved for 40 min for three consecutive days. Flasks were raised to the same shelf as the funnels, creating a vacuum where the water column in the tubing passively waters the sand/seed matrix from below through the fritted glass. Once funnels are equilibrated at a flooded stage (30 min), *Pythium aphanidermatum* (isolate Pa58) zoospores were added to the funnel. Zoospore suspensions were produced by leeching 7 d old V8 cultures of Pa58 in sterile water for 18 h, refreshing the water then harvesting after 8 h. Zoospores were enumerated with a haemocytometer and suspensions of $3.6 * 10^4$ zoospores mL^{-1} were prepared. 50 mL zoospore suspension was added to each inoculated funnel. Flasks were then returned to the lower shelf causing water to drain out of the funnels, and matric potential to equilibrate at -3.5 kPa regardless of the physical attributes of the material tested. *Pythium* spp. are highly sensitive to changes in soil water, so

controlling this variable is crucial (Stanghellini and Burr 1973; Lifshitz and Hancock 1982; Mondal et al. 1995).

Figure 4. Apparatus for controlling matric potential for disease suppressive bioassays. Fritted glass Buchner funnels being passively watered through gravity on a water column under vacuum.

Seedlings were harvested after 7 days at 27°C and 18 hour days in a climate controlled growth chamber. Seedling height was measured and seedling disease rated on a scale of 0-5 with 5 being healthy. For each treatment, 3 funnels containing 10 seeds each were run without inoculum and 3 funnels containing 10 seeds each were run with inoculum. The experiment was then replicated in time over three weeks. Data is clustered by rep and funnel, so a mixed model is used in SAS to evaluate treatment differences.

Disease suppression bioassays – non-aerated liquid vermicompost extracts

Surface sterilized cucumber seeds (Marketmore 76, Johnny's) were sown in sterile quartz sand wet sieved to 0.5 – 1.0 mm d in 12-well tissue culture plates. Each well contained 6 mL sand, 1 seed and the following amendments (Table 1.). Plates were incubated in a moist chamber at 27^{0}C with 18 h days for 7 d. Seedlings were harvested and rated for disease symptoms.

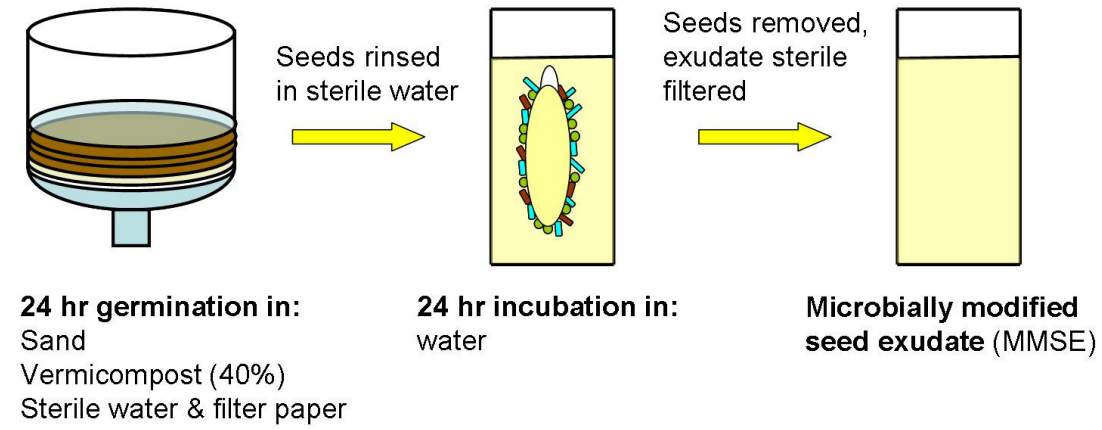
Table 1. Treatments in non-aerated vermicompost extract (NVE) disease suppression bioassays.

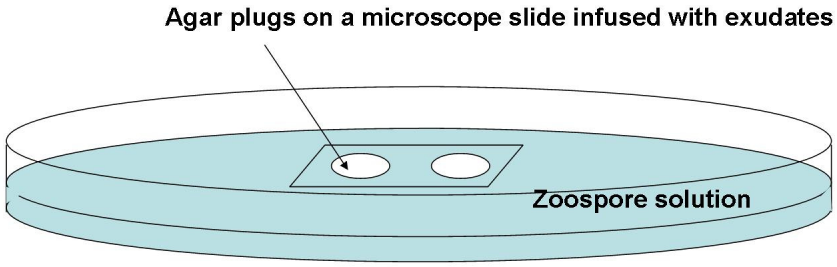
Treatment	Water mL	NVE mL	Zoospores ML
Control non-inoculated	1.75	-	-
Control inoculated	1.25	-	0.5
NVE non-inoculated	1.0	0.75	-
NVE inoculated	0.5	0.75	0.5

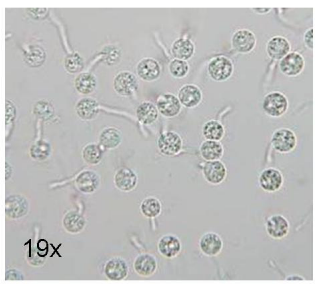
Zoospore attraction assays

P. aphanidermatum zoospore response to microbially modified seed exudates was measured using zoospore attraction assays that were developed specifically for this study based on other work done in this field (Heungens and Parke 2000; Islam et al. 2004) (Figure 5). Microbially modified seed exudate (MMSE) was prepared by sowing surface sterilized seeds in fritted glass Buchner funnels as described in the previous section. Seeds were allowed to germinate for 24 hours in sand and 40% v:v vermicompost : sand at 27^0C . Seeds were then removed, rinsed and incubated in sterile water for 24 h at 27^0C. The resulting seed exudates were filtered to 0.2 µm. 5 µL MMSE was added to agarose plugs on a microscope slide and allowed to absorb for 10 minutes. Slides were then incubated in a suspension of Pa58 zoospores (3.6 x 10^4 zoospores mL^{-1}) for 30 minutes. Slides were removed and imaged at 19x to enumerate encysted zoospores.

Figure 5: Schematic of the set up for zoospore encystment assays.







Slides are removed after 30 min, imaged and encysted zoospores are counted

5. Project Results

Vermicompost materials

Non-aerated vermicompost extracts are a valuable source of micronutrients but contained relatively low levels of plant available nitrogen (Table 2).

Table 2. Plant nutrient values (average of three samples) in non-aerated vermicompost extract produced with a 1:60 vermicompost : water ratio compared to conventional fertilizer. (Thanks to N. Mattson, Department of Horticulture, for assistance with the comparison)

Nutrient	NVE	Scott's 20-10-20		Units
		100 ppm N	200 ppm N	
ammonium N	2.600	40	80	ppm
nitrate N	13.313	60	120	ppm
P	66.667	22	44	ppm
K	293.333	83	166	ppm
Ca	46.667	0	0	ppm
Mg	10.000	0.75	1.5	ppm
S	20.000	0	0	ppm
Na	56.117	0	0	ppm
Al	2.663	0	0	ppm
Fe	7.613	0.25	0.5	ppm
Mn	0.267	0.125	0.25	ppm
Cu	0.703	0.0625	0.125	ppm
Zn	1.147	0.125	0.25	ppm
B		0.0625	0.125	ppm
Mo		0.025	0.05	ppm

Disease suppression bioassays – solid vermicompost

Vermicompost suppressed *P. aphanidermatum* damping-off in cucumber seedlings when amended to sand at 40% v:v (Figures 6 & 7). This suppression was biologically based since heat sterilized vermicompost offered little protection from the pathogen. Vermicompost from this facility is consistently suppressive with three years of samples providing significant protection.

Figure 6: Solid vermicompost disease suppression bioassays in fritted glass Buchner funnels. Average disease rating of 7 day old cucumber seedlings NIN (gray) = non-inoculated, IN (black) = inoculated with 50 mL 3.6 x 10^4 zoospores mL^{-1}. Disease rating: 5 = healthy, 0 = completely rotted. Bars with the same letter are not significantly different ($p>0.05$). CDVC = Coyne Dairy Vermicompost batches 1 (2006), 2 (2007) and 3 (2008). (St) = sterilized.

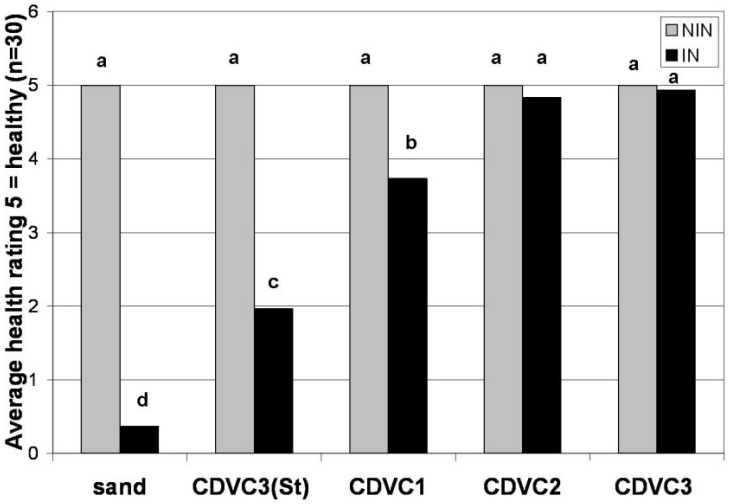
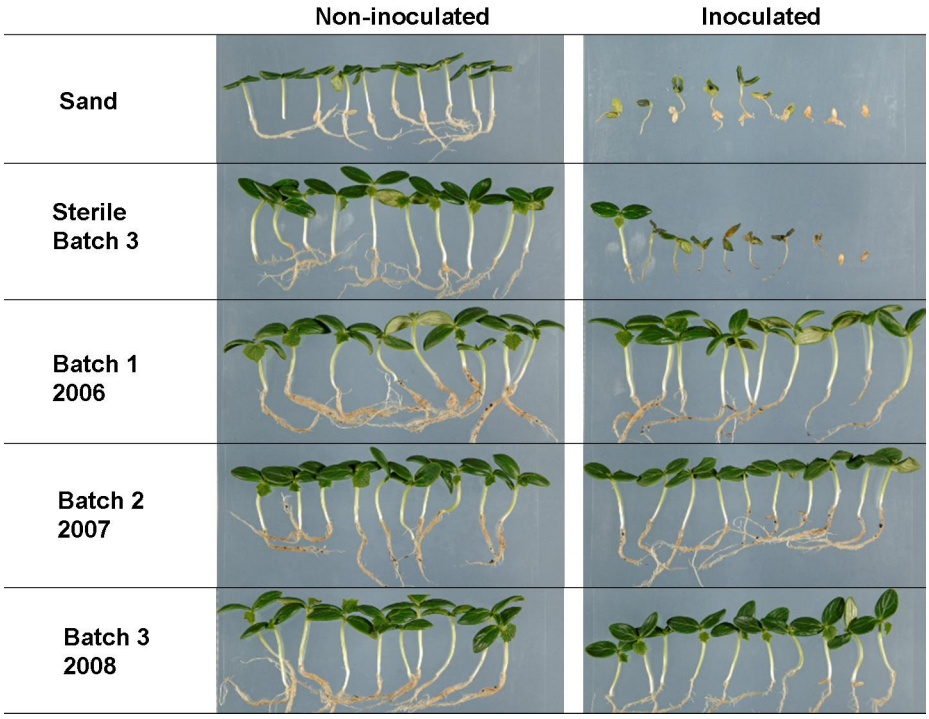


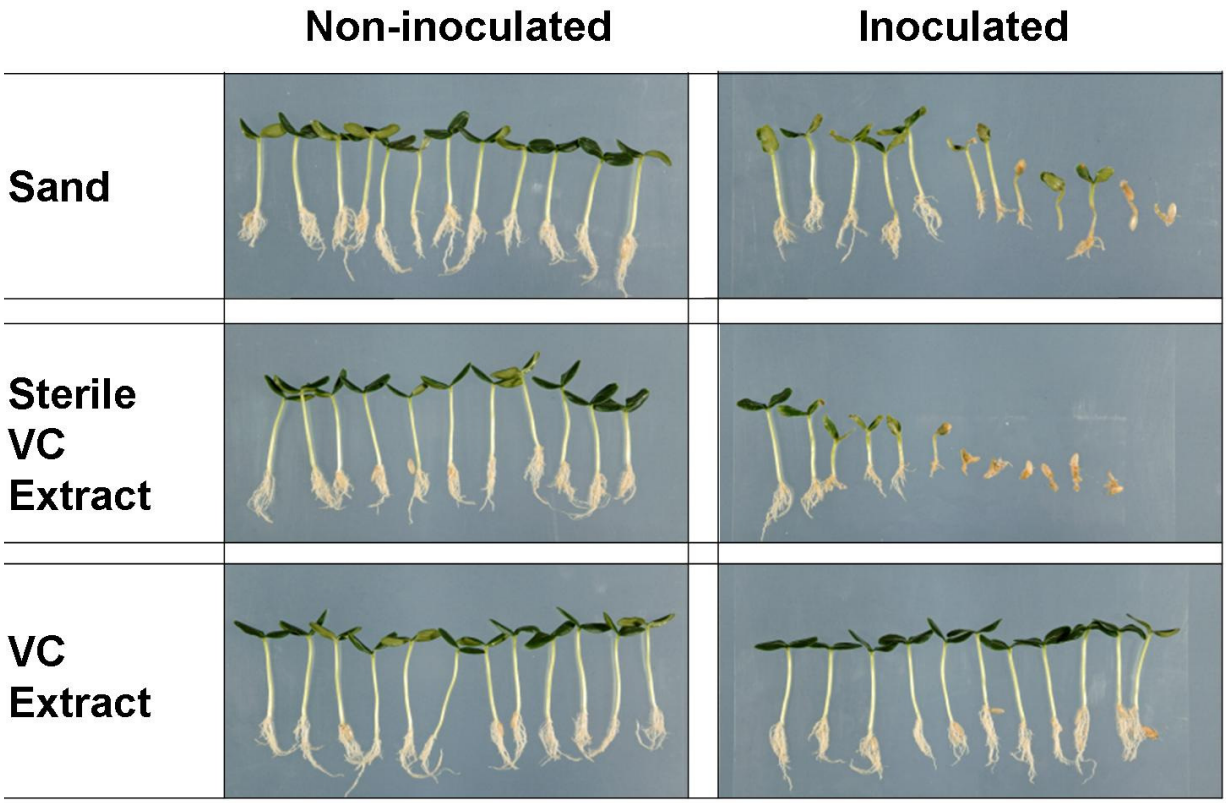
Figure 7: Representative 7 day old cucumber seedlings showing the difference in disease symptoms between sand and vermicompost-amended sand. All inoculated treatments received 50 mL of 3.6 x 10^4 zoospores mL^{-1}. Photo credit: K. Loeffler



Disease suppression bioassays – non-aerated liquid vermicompost extract

Non-aerated liquid vermicompost extract suppressed *P. aphanidermatum* damping-off in cucumber seedlings when amended to sand at 1:8 v:v (Figure 8). This suppression was biologically based since filter sterilized extract offered no protection from the pathogen. Three separate batches of NVE were tested a total of three times each, and every batch was significantly suppressive (data not shown).

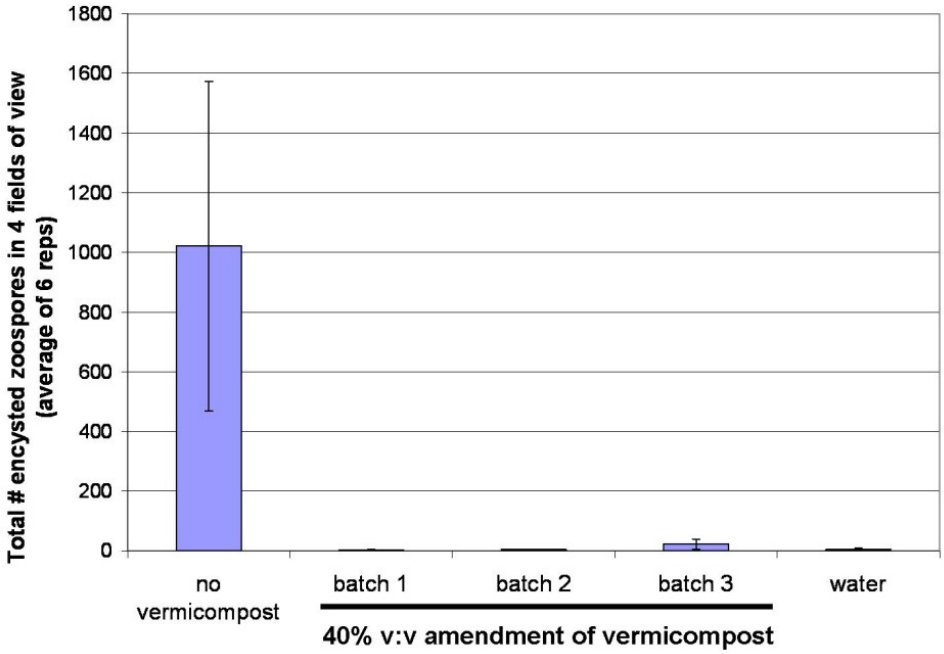
Figure 8: Representative 7 day old cucumber seedlings showing the difference in disease symptoms between sand and vermicompost-amended sand. All inoculated treatments received 500 µL of 3.6×10^4 zoospores mL^{-1}. Photo credit: K. Loeffler



Zoospore attraction assays

Exposing cucumber seed exudates to the vermicompost-derived seed colonizing microbial community reduced their attractiveness to *P. aphanidermatum* zoospores. Each replicate of the experiment exposed zoospores to all treatments: exudate from seeds sown in sand and vermicompost and water with no exposure to seeds. Zoospores selectively encysted on exudate from seeds sown in sand in large numbers, while hardly any zoospores encysted on microbially modified seed exudate or water (Figure 9).

Figure 9: Zoospore attraction assay results. Total number of encysted zoospores in 4 fields of view when exposed to seed exudates, MMSE and water (6 replicates). Error bars are standard deviations from the mean.



6. Conclusions and Discussion

Vermicompost materials

Vermicompost and non-aerated liquid vermicompost extracts (NVE) can be valuable components of an organic nutrient management strategy for greenhouse crops. Vermicompost performs best when mixed with blood meal, green sand and rock phosphate (Leonard and Rangarajan 2007; Leonard et al. 2008). Since nutrient content in vermicompost can vary, we encourage growers to test a variety of amendment rates with each new crop. Amendment rates can also vary according to crop when using the same batch of vermicompost. We found that although a 20% amendment performed well for tomato production, it was too high a rate for cabbage which performed better at 10%. Since NVE can be made on site for relatively low input ($250 for a 100 gallon drum, sump pump, PVC pipe and timer), it may be allowable under current NOP regulations. Check with your certifier to be sure. This specific extract is currently being marketed by RT Solutions, LLC as Worm Power Shower ™, but has not yet gone through testing by OMRI to be officially listed as an allowable product. NVE would also need to be combined with another nutrient source such as blood meal that is high in N in order to provide balanced plant nutrition. However, the batches we made were a good source of micronutrients. High sodium levels may be a problem for crops sensitive to salinity. Batches of NVE made with vermicompost : water ratios of 1:5 to 1:25 were phytotoxic (data not shown). This study used a 1:60 ratio. Different types of compost are likely to produce liquid extracts that have a wide range of

chemical characteristics. We encourage growers to experiment with multiple recipes to find one that is appropriate for the type of compost – vermicompost and crop species.

Disease suppression

Both the solid vermicompost and liquid vermicompost extract significantly suppressed *P. aphanidermatum* on cucumber seedlings. These materials could play an important role in the cultural control of *Pythium* damping-off in organic greenhouse crops where fungicide seed treatments are not an option. However, it is important to note that composts suppressive to a single pathogen are often conducive to other pathogens (Bonanomi et al. 2010), so there is no guarantee that these materials will suppress disease caused by other soil borne fungal or oomycete pathogens. Non-aerated extracts may serve as a way to culture the microbiota present in solid vermicompost. We achieved comparable levels of disease suppression with both solid vermicompost and NVE. When the small amount of solid vermicompost used in the production of NVE is considered, the NVE used in our bioassays represents 2,880 times less solid vermicompost than was used in the vermicompost bioassays. It would be interesting to follow up by investigating if the microbes responsible for suppression are the same in these two materials or if the extraction conditions select for a unique microbial community in the NVE. It would also be valuable to see how these materials perform against *Pythium* damping-off in commercial greenhouse production in a variety of transplant media. Measuring disease suppression on farm is complicated because it relies on the presence of an outbreak since few greenhouse managers would allow an inoculation of *Pythium* at their facility. We would encourage any growers struggling with seedling damping-off in their transplant media to submit samples to a diagnostic lab to confirm the presence of *Pythium* spp. and collaborate with their regional cooperative extension on a controlled trial of these or similar materials for disease suppression.

Zoospore attraction assay

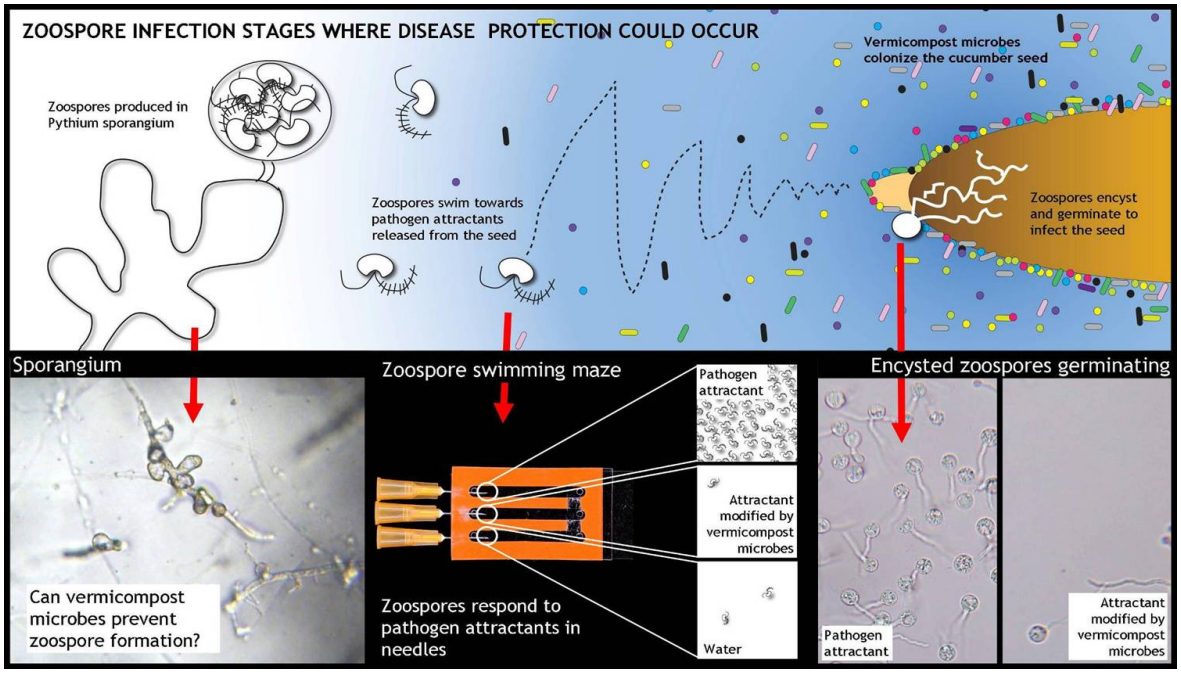
Seed exudates exposed to vermicompost-derived seed colonizing microbes had a profound effect on *P. aphanidermatum* zoospore behavior. Orders of magnitude fewer zoospores encysted on MMSE compared to control seed exudate. There are at least two possible explanations for this change in zoospore homing behavior: 1) the seed colonizing microbes have metabolized or modified the chemical signal used by zoospores to find their hosts, or 2) the seed colonizing microbes have produced a chemical toxin that somehow repels the zoospores even though the homing signal is present. We are currently working to understand this interaction in greater detail. We have re-designed the disease suppression bioassay to include a point source of inoculum so that zoospores must swim 4 cm before finding a host seed. We've developed a quantitative Polymerase Chain Reaction (qPCR) protocol to detect zoospores on the seed surface. Seeds will be inoculated with a point source of zoospores and then removed at various time intervals to assess how many zoospores have arrived on their surface in different treatments (sand, sterile vermicompost, vermicompost). This set of experiments will allow us to confirm or reject our current hypothesis that disease suppression is due to a microbially mediated interruption of zoospore homing behavior.

Additional funding has allowed us to expand the project focus and work on answering other related questions (Figure 10):

- Do vermicompost-derived microbes affect the formation of zoospores? (E. Carr – Technician)
- Which vermicompost-derived seed colonizing bacteria are unique to suppressive communities and not present in conducive communities? (M. Minson – undergraduate Hunter S. Rawlings presidential research scholar)

The results of these combined projects will add significantly to our current understanding of how complex communities of microorganisms can protect plants from seed-infecting oomycete pathogens. Seed colonizing bacterial species associated with suppression in this ongoing study can eventually be screened as potential indicators of *Pythium* suppression, but reliable predictive factors relevant to multiple pathosystems will require years of additional work. This project is an important first step in that process.

Figure 10: Schematic of zoospore pre-infection stages where suppression may occur.



7. Outreach

Publications (2010) **In progress.** Anticipate one applied paper on non-aerated vermicompost extract production and use and one or two more basic papers on the interruption of zoospore homing by vermicompost-derived seed colonizing microbes. The Rangarajan group will most likely publish something on the temperature sensitivity of nutrient release using vermicompost and blood meal mixtures.

In press: Jack, ALH *The suppression of plant pathogens by vermicomposts* in "Earthworms, Organic Waste, and Environmental Management" Edwards, C, Sherman, RL and Arancon, NQ eds. Taylor and Francis

Website (2010) In progress. Will include a 5 – 10 minute video about vermicompost production and use and all of our research reports from the greater project.

ISME (2010) (anticipated, abstracts due 3-12-10) International Society of Microbial Ecology, meeting in Seattle, WA. Poster on zoospore work

MOSES (2010) (upcoming) Midwest Organic and Sustainable Education Service 21st annual Organic Farming Conference "*Disease suppressive soils and composts: what does the science tell us*?" with Alex Stone, Oregon State University

Veg Expo (2010) (upcoming) Empire State Fruit and Vegetable Expo & Farmer's Direct Marketing Conference "*What makes compost disease suppressive*?" & "*Using vermicompost in potting media for tomato and pepper transplants*" second presentation with Anu Rangarajan, Cornell University

Ag Forum (2010) Cornell Cooperative Extension Suffolk County 29th annual Long Island Agricultural Forum "*Vermicompost use in greenhouse production: nutrient management and disease suppression*"

CCE (2009) Cornell Cooperative Extension Ulster County – Hudson Valley High Tunnel Production Seminar "*Compost and microbial disease suppression*"

Worm Power (2008) Open house at the vermicomposting facility in Avon, NY. Led guided tours of the facility and staffed a dissecting microscope activity table where visitors viewed vermicompost microarthropods and learned about the soil food web. These events were funded by a NYS Agritourism grant to RT Solutions, LLC.

NC State (2006,8,9) North Carolina State University Vermicomposting Workshop, Raleigh, NC "*Vermicompost-mediated suppression of Pythium damping off*"

APS (2008) American Society of Phytopathology Minneapolis MN "*Modification of seed exudates by seed-colonizing microbes from vermicompost alters pre-infection behavior of Pythium aphanidermatum zoospores*"

CSS (Fall 2008)	Cornell Department of Crop and Soil Science Seminar Series "*Vermicompost: Horticultural applications and impacts on plant-associated microbial communities*" shared talk with Tom Herlihy of Worm Power.
Master Gardener (2007)	Master Gardener Training "*The world beneath our feet: Exploring soil life*" with Joann Gruttadaurio, Cornell University
Expo (2006)	Small Farms Expo: Cornell, Rutgers and Penn State Cooperative Extension Augusta, NJ "*Vermicompost production and use*"

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9. Addenda

Appendix 1.

Personnel and collaborators in the greater vermicompost project with a summary of additional funding received. The authors wish to thank the OFRF for providing seed funding for this project. Initial funds from the OFRF allowed us to generate preliminary data and develop proposals for additional funding of the project.

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New York Farm Viability Institute (2008-2010)"Potential use of vermicompost as a substitute for synthetic inputs to horticulture and nursery production" $120,000

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USDA SBIR Small Business Innovation Research Phase I & II 2008-2011 (ALH Jack, EB Nelson, TE Herlihy) *"Development of plant protection products based on vermicomposted dairy manure"* In collaboration with RT Solutions, makers of Worm Power vermicompost [I $80,000 II $350,000]

NYSTAR NY State Foundation for Science, Technology and Innovation 2008-2011 (ALH Jack, EA Carr, EB Nelson, TE Herlihy) matching funds for USDA SBIR project [$73,000]

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OCIA Organic Crop Improvement Association – Student Research Scholarship to A. Jack [$1,000]
http://www.ocia.org/RE/Scholarship/Scholarship2007.aspx

Appendix 2.

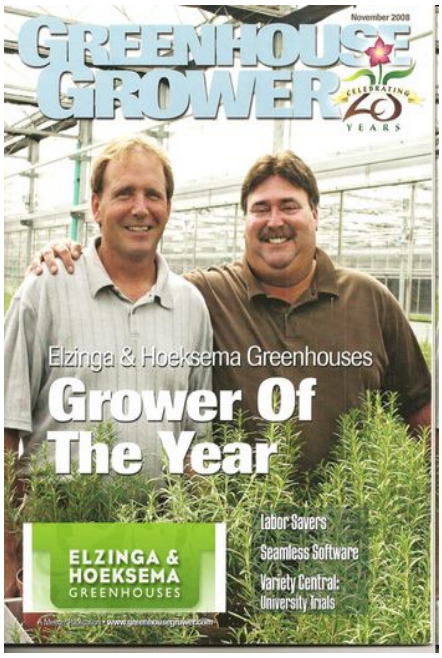
Information on vermicompost as a potting media amendment in organic agriculture from the Rangarajan research group

- Leonard & Rangarajan 2007
- Leonard, Rangarajan & Jack 2008

Appendix 3.

Elzinga and Hoeksema Greenhouses were named as Greenhouse Growers of the Year in 2008. This farm has pioneered the combined use of solid vermicompost (from Worm Power in Avon, NY) and aerated vermicompost extracts in their certified organic greenhouse.

- Greenhouse Grower magazine article
    - http://www.greenhousegrower.com/news/?storyid=743
    - http://www.greenhousegrower.com/specialreports/grower/?storyid=1524
- OMRI article on organic amendments



Appendix 4.

Video microscopy of *P. aphanidermatum* zoospore life cycle

A. Zoospores emerging from a zoosporangium
B. Zoospores interacting with cucumber root border cells
C. Time lapse (4 hr) of encysted zoospores germinating while attached to a cucumber root border cell

Appendix 5.

Photos of field trials and collaborators in greater vermicompost project at Cornell

Appendix 6.

Outreach posters from Cornell Center for Life Science Enterprise events 2008 - 2009 "Public Engagement and Science Communication Symposium" where non-scientist citizens of Ithaca judge outreach posters from research groups funded through the center. Our 2008 poster won the $10,000 prize.

Appendix 7.

American Phytopathological Society abstract

Jack, ALH & Nelson, EB (2008) *Modification of seed exudates by seed-colonizing microbes from vermicompost alters pre-infection behavior of Pythium aphanidermatum zoospores.* Phytopathology 98:6 S73 [meeting abstract]

Suppression of plant diseases with composts is well documented, but the microbial mechanisms involved are poorly understood. For diseases caused by Pythium spp., the spermosphere is a critical habitat for microbial interaction and host infection, leading us to hypothesize that seed colonizing microbes from composts may have important impacts on compost-mediated disease suppression. To test this hypothesis, we established the suppressiveness of vermicomposted dairy manure (VC) in cucumber bioassays with *Pythium aphanidermatum* zoospore inoculum. Seed and seedling health were significantly improved with VC amendments. However, sterilized VC did not provide protection, indicating the observed suppression is biological in nature. Transplant experiments were conducted to establish the temporal pattern of seed colonization and disease suppression. These experiments revealed that *P. aphanidermatum* zoospores were able to colonize/infect seeds within 27 hours of sowing in unamended soil. Vermicompost microbes colonizing seeds within 24 h of sowing were incubated in the presence of seed exudates to obtain microbially-modified seed exudates (MMSE). When given a choice between different exudates, fewer zoospores encysted on vermicompost MMSE than on unmodified exudates, indicating that these exudates were less attractive to zoospores. This result shows that, within a short time frame, seed colonizing VC microbes can interfere with the zoospores' ability to respond to exudates. Interference with one or more zoospore pre-infection events including; attraction, encystment, attachment and germination, is proposed as a potential mechanism for vermicompost-mediated suppression of *P. aphanidermatum* on cucumber.