PA Vegetable Marketing and Research Program: PA Vegetable Growers Association 2016 Final Grant Report

TITLE: Deploying microbes as a seed treatment for protection against soil-borne plant pathogens

PERSONNEL: Rick Carr
Compost Production Specialist
Rodale Institute
611 Siegfriedale Road
Kutztown, PA 19530
610-683-1415
rick.carr@rodaleinstitute.org

Dr. Kristine Nichols (co-PI)
Chief Scientist
Rodale Institute
611 Siegfriedale Road
Kutztown, PA 19530
kristine.nichols@rodaleinstitute.org

Dr. Eric Nelson (co-PI)
Retired Professor
Department of Plant Pathology & Plant-Microbe Biology
Cornell University
323 Plant Science Building
Ithaca, NY 14853
ebn1@cornell.edu

INTRODUCTION

Public demand for organically-grown food has stimulated research into the use of organic amendments such as composts and vermicomposts for disease management [1]. Many scientists and growers alike have long recognized the importance of maintaining healthy soil for producing healthy plants. Compost, vermicompost and other similar organic soil amendments are commonly used for improving the nutrient and microbial diversity of agricultural soils. In general, these materials can improve the health of the soil by increasing organic matter, plant nutrients, and microbial biomass, all of which complement the enhancement of soil processes such as erosion reduction. The use of these materials in agriculture to suppress plant diseases has been well documented [2-4]. However, their mixed effectiveness in laboratory and field

applications [4, 5] has stifled the wide-spread adoption of biocontrol materials suitable for organic agriculture. Continued research that focuses on compost-mediated disease suppression will only increase our understanding and the efficacy of organic amendments as effective tools against plant diseases in organic agriculture.

The variable efficacy of biological control methods in field and greenhouse operations has been a long standing concern among growers and agricultural scientists. Biological control products usually have a narrow spectrum of control, which means that a particular material might be effective in controlling disease in one plant-pathogen interaction but not effective in another [2, 5, 6]. It has been well documented that the microorganisms associated with organic amendments are responsible for much of the observed disease suppression [3, 7, 8]. Studies from Nelson and colleagues [9-12] have elucidated some of the mechanisms responsible for suppression of *Pythium* damping-off using municipal biosolids compost. One important finding was that suppression is biologically mediated, meaning that the microbes found in the compost are responsible for the observed levels of disease suppression. Furthermore, these data indicate that only a subset of microbes from the bulk material colonizing the seed surface are responsible for suppression [13, 14]. Recent research also has revealed a biochemical nature of disease suppression by which anti-microbial toxins are produced while affecting pathogen development and plant infection [10].

There are several different approaches for the application of composted substrates. Solid materials can be amended to soil or potting media [15], or added to water where microbes and soluble plant nutrients can be extracted from the solid material [16] that later can be used as soil and foliar applications, commonly referred to as compost extracts and teas. Both solid and liquid materials have been shown to suppress plant diseases [3, 8, 16-19]. One advantage of using liquid extracts is that they maintain similar characteristics in disease suppression compared to the solid material [12] but have added dispersal issues. A potentially promising application approach for liquid extracts involves production of a fine, dry powder through freeze-drying of the extracts after they are frozen. The freeze-dried compost exact (FDCE) should retain all of the beneficial properties of the liquid extracts and they would be more easily transported. When needed, the powder can be reconstituted back into its original liquid form at the desired concentration. Most importantly, given the findings from our preliminary studies and that seed companies commonly use seed coat applications for disease control, FDCE has the potential to be applied as a seed coating for a more directed application in order to achieve suppression of seed and seedling diseases. An effective seed treatment using FDCE would be acceptable for organic agriculture and would provide organic growers with additional means for controlling soil-borne plant diseases.

Researchers from Rodale Institute and Cornell University have been exploring the use of FDCE for suppressing infections caused by *Pythium aphanidermatum* in cucumber. Their working hypothesis is if (1) solid vermicompost can consistently suppress cucumber seed and seedling infections caused by *P. aphanidermatum* [9, 12, 20], (2) liquid vermicompost extracts can similarly suppress *P. aphanidermatum* [10, 12], and (3) vermicompost extracts can be freeze-dried into a powder, reconstituted back to its liquid state and still suppress *P. aphanidermatum* (unpublished data), then (4) can the specific subset of microbes associated with seed colonization and suppression be deployed as a seed treatment such that we still achieve plant protection from

soil-borne pathogens? In addition, can this seed treatment application be developed for organic production as an effective tool for disease management? We are confident that if the disease-suppressive microbes found in freeze-dried, liquid extracts can be directly applied to seeds before sowing then disease control may be more effective at controlling seed rotting pathogens than broadcast applications of composted substrates.

Preliminary data suggests that there is a dose response in the rate of application when attempting to suppress *P. aphanidermatum* (Figure 1). An application rate of 1.5% FDCE by weight on cucumber seeds show almost no sign of suppression, but when increased to 6% by weight, seed germination increased to 28%. This research proposal intends to expand on the preliminary data already gained and explore higher application rates (10-50% FDCE by weight).

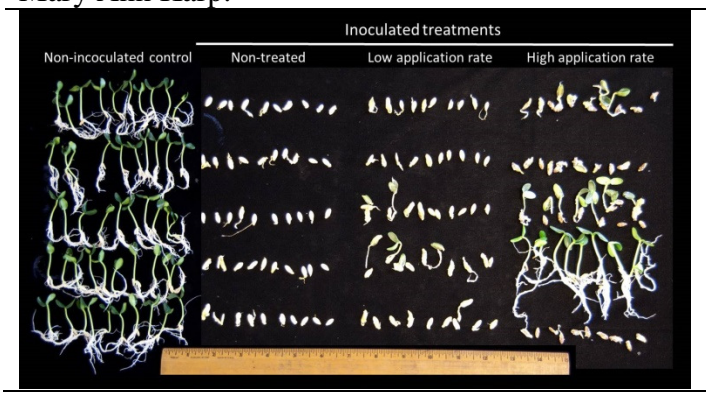


Figure 1. Microbial seed treatment using freeze-dried compost extract (FDCE) on cucumber seeds shows a dose response in the rate of application when attempting to suppress Pythium damping-off. Low application = 1.5% FDCE by weight; high application = 6% FDCE by weight. Photo credits: Mary Ann Karp.

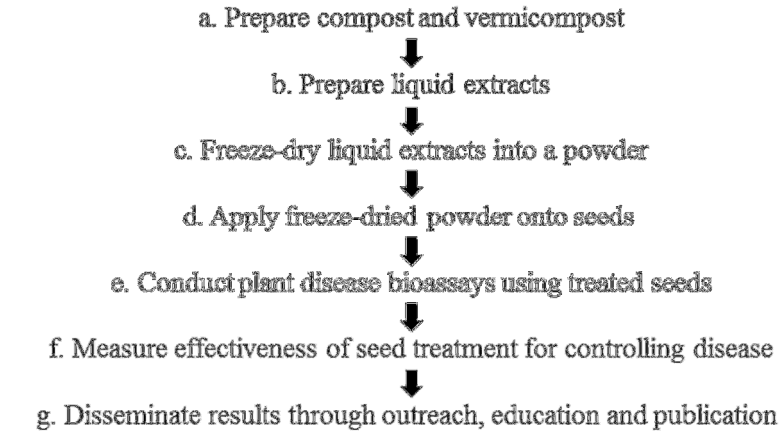
OBJECTIVES

1. Vary the rate of FDCE application on cucumber seeds.
2. Conduct plant disease bioassays to measure suppression of *P. aphanidermatum* using seed treatments of FDCE.
3. Determine efficacy of FDCE suppressing Pythium damping-off.

WORK STATEMENT

This project is a continuation of the work from an on-going project to evaluate the use and effectiveness of applying freeze-dried compost extracts on the seed coat of cucumber to suppress infections caused by *P. aphanidermatum*. The general research approach is outlined in Figure 2. All compost extract production, freeze-drying and data analysis took place at Rodale Institute. Seed treatment application has not been completed but will take place at Cornell University. Plant

Figure 2. Generalized approach for exploring the use of freeze-dried compost or vermicompost extracts as a seed treatment application for controlling soil-borne plant pathogens.



disease bioassays also have not been completed but will take place at Rodale Institute.

RESULTS AND DISCUSSION

Compost production

Compost was prepared from diary bedpack manure and leaf feedstocks. Table 1 shows data collected during compost production that meet National Organic Program certification (NOP §205.203). Windrow temperature during active composting is displayed in Figure 3. Approximately 5 months after composting, 1 gallon samples were collected from the windrow, screened to ¼ inch and then frozen at -20°F for further testing. The average windrow temperature at the time of collection was below 95°F.

Compost extract production

Frozen samples of compost were removed from the freezer and thawed for 24 hours at temperatures between 60-65°F before preparing liquid compost extracts. Two gallons of compost was added to 48 gallons of dechlorinated water in a 60-gallon, food-grade plastic vessel (Figure 4) to make a 1:25 compost:water (v:v) extract. The solution was recirculated using a sump pump for 30 minutes every 12 hours for 7 days, at which point the solution was removed, screened to 0.5 mm, and then 250 ml subsamples were placed into 600 ml freeze-dry glassware and placed in the freezer at -20°F for a minimum of 24 hours before freeze-drying.

Alternatively, compost extract was prepared as stated previously and then 25 ml subsamples were screened to 0.5mm, placed into 32 ml centrifuge tubes and then centrifuged at 15000 rpm for 15 minutes. The supernatant was discarded and the pellet was frozen at -20°F in the centrifuge tube for a minimum of 24 hours before freeze-drying.

Table 1. Composting time and temperature data collected for National Organic Program certification (NOP §205.203).

Started building on:	5/23/2016				
Finished building on:	5/24/2016				
Day one meeting temps:	5/26/2016				
Day 15 meeting temps:	6/9/2016				
Five turns while meeting temps:	5/27/2016	5/31/2016	6/2/2016	6/3/2016	6/4/2016
Began moving off the yard:	NA				

		Buckets		
Contents:	leaves from 2015	15	=	33%
	Burkholder's dairy bedpack manure	30	=	67%
	total =	45		100%

Total number of turns = 17

Windrow dimensions:	5/25/2015		
length (m) =	12.3	40.5	ft
width (m) =	4.0	13.0	ft
height (m) =	1.5	5.0	ft

volume = 1/2*length*width*height = 37 m^3
48 yd^3
44 tons

Windrow dimensions:	8/10/2016		
=	months after building		
length (m) =	11.6	38	ft
width (m) =	2.8	9.2	ft
height (m) =	0.8	2.6	ft

volume = 1/2*length*width*height = 12.7 m^3
16.5 yd^3
15.2 tons

Percent change in volume = 66% decrease in volume

Figure 3. Compost windrow temperature during active composting.

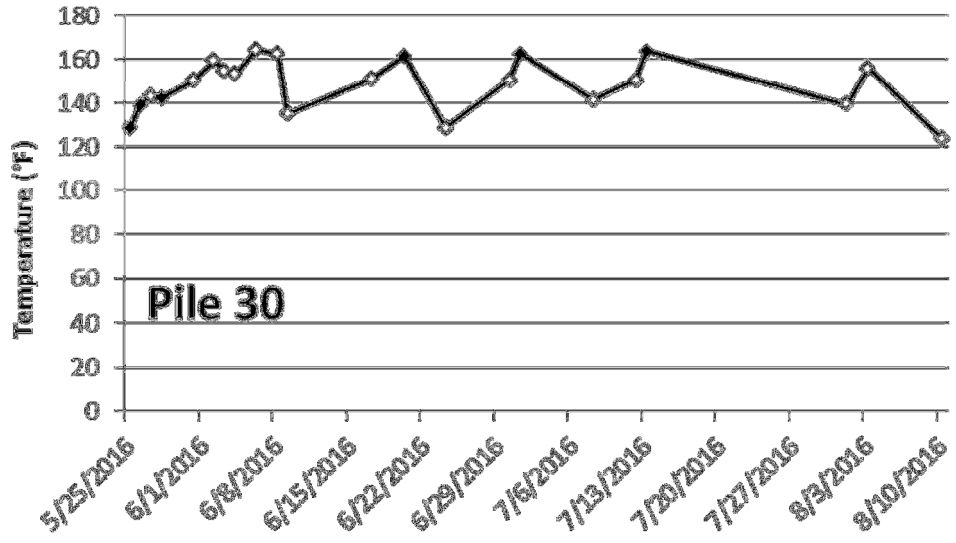


Figure 3. Compost extract production vessels at Rodale Institute, Kutztown, PA.

Freeze-dried compost extract (FDCE) production

Frozen compost extract was freeze-dried using a Labconco Freeze-Dry System. Extract samples frozen as 250 ml required approximately 5 days to freeze-dry and yielded an average of 0.002 g/ml while samples that had been centrifuged required 12 hours to freeze-dry and yielded approximately 0.05 g/tube. Centrifuging significantly increased the efficiency of generating FDCE to be used as a seed treatment application.

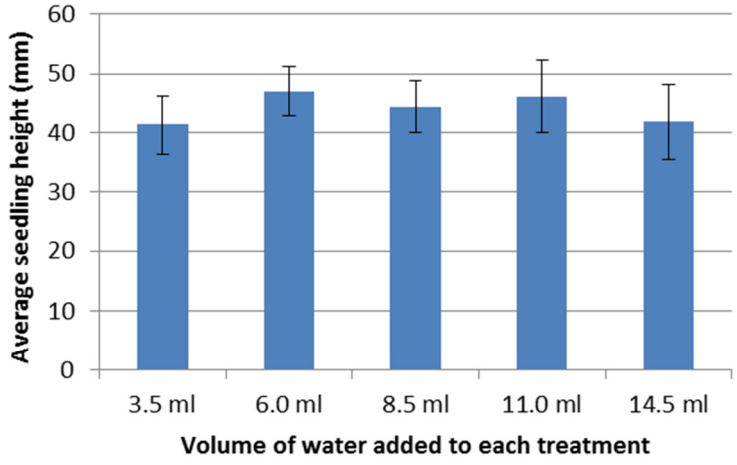
Project leaders are still in the process of generating enough FDCE to vary the rate of treatment application to the seed surface. The objective is to generate enough FDCE to apply at rates of 10% and 15% FDCE by weight of seed. This task should be completed by the middle of February, 2017 at which point seeds and the microbial seed treatment will be sent to Cornell University for application. Afterwards, the treated seeds will be returned to Rodale Institute and tested for their efficacy in suppressing Pythium damping-off caused by *Pythium aphanidermatum*.

Plant growth bioassay data

Plant growth bioassays were completed using cucumber in sand to determine optimal growth under laboratory conditions. Sand was sieved to 0.5-1.0 mm and then heat sterilized. Cucumber seeds (Marketmore 76 organic, High Mowing Seeds) were sorted to remove damaged or discolored seeds, weighed to 0.02-0.03 g/seed, surface disinfested with 0.5% sodium

hypochlorite for 2.5 minutes, rinsed with sterile water, and air dried prior to use in bioassays. Five seeds were sown on top of 25 ml of sterile sand in 100 ml glass containers and then another 25 ml of sterile sand was added on top of the seeds. The amount of sterile water added to each container was varied between 3.5-22 ml. Containers were covered with parafilm and then place in an incubator for 7 days at 24°C with 14 h daylight. After incubation, seeds and seedlings were destructively harvested and percent germination and seedling height was measured. Figure 4 shows select data from plant growth bioassays. Based on these data, it was determined that 7.5 ml of water was needed to create optimal cucumber growth under laboratory conditions.

Figure 4. Plant growth bioassay results. Five cucumber seeds per treatment were sown on top of 25 ml of sterile sand in 100 ml beakers and then another 25 ml of sterile sand was placed on top of the seeds. Sterile water was added to each container and then incubated for 7 days at 24°C with 14 h daylight. Bars represent standard deviation.



Disease bioassay data

Plant disease bioassays have not been completed. This task is scheduled to conclude by the end of March 2017. Upon completion of disease bioassays, a follow-up report shall be submitted to the PA Vegetable Growers Association so that they may review the success of the project.

LITERATURE CITED

1. Lotter, D.W., 2003. Organic agriculture. Journal of Sustainable Agriculture, 21: p. 59-128.
2. Hoitink, H.A.J. and P.C. Fahy, 1986. Basis for the control of soilborne plant-pathogens with composts. Annual Review of Phytopathology, 24: p. 93-114.
3. Martin, F.N. and J.E. Loper, 1999. Soilborne plant diseases caused by *Pythium* spp: Ecology, epidemiology, and prospects for biological control. Critical Reviews in Plant Sciences, 18: p. 111-181.
4. Litterick, A.M., L. Harrier, P. Wallace, C.A. Watson, and M. Wood, 2004. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and

disease incidence and severity in sustainable temperate agricultural and horticultural crop production - A review. Critical Reviews in Plant Sciences, 23: p. 453-479.
5. Scheuerell, S.J. and W.F. Mahaffee, 2006. Variability associated with suppression of gray mold (*Botrytis cinerea*) on geranium by foliar applications of nonaerated and aerated compost teas. Plant Disease, 90: p. 1201-1208.
6. Scheuerell, S.J., D.M. Sullivan, and W.F. Mahaffee, 2005. Suppression of seedling damping-off caused by *Pythium ultimum*, *P. irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of Pacific northwest compost sources. Phytopathology, 95: p. 306-315.
7. Nelson, E.B. and C.M. Craft, 2000. Microbial strategies for the biological control of turfgrass diseases, in *Fate and Management of Turfgrass Chemicals*. p. 342-352.
8. Szczeck, M.M., 1999. Suppressiveness of vermicompost against Fusarium wilt of tomato. Journal of Phytopathology, 147: p. 155-161.
9. Jack, A.H. and E.B. Nelson, 2008. Modification of seed exudates by seed-colonizing microbes from vermicompost alters pre-infection behavior of *Pythium aphanidermatum* zoospores. Phytopathology, 98: p. S73-S73.
10. Carr, E.A. and E.B. Nelson, 2014. Disease-suppressive vermicompost induces a shift in germination mode of *Pythium aphanidermatum* zoosporangia. Plant Disease, 98: p. 361-367.
11. Chen, M.-H. and E.B. Nelson, 2012. Microbial-induced carbon competition in the spermosphere leads to pathogen and disease suppression in a municipal biosolids compost. Phytopathology, 102: p. 588-596.
12. Jack, A.L. and E.B. Nelson, 2010. Suppression of Pythium damping-off with compost and vermicompost: Final report to the Organic Farming Research Foundation, Cornell University: Ithaca, NY.
13. Chen, M.-H., A.L.H. Jack, I.C. McGuire, and E.B. Nelson, 2012. Seed-colonizing bacterial communities associated with the suppression of Pythium seedling disease in a municipal biosolids compost. Phytopathology, 102: p. 478-489.
14. Chen, M.-H. and E.B. Nelson, 2008. Seed-colonizing microbes from municipal biosolids compost suppress *Pythium ultimum* damping-off on different plant species. Phytopathology, 98: p. 1012-1018.
15. Edwards, C.A., I. Burrows, K.E. Fletcher, and B.A. Jones, 1984. The use of earthworms for composting farm wastes, in *Composting of Agricultural and Other Wastes*, J.K.R. Gasser, Editor Elsevier Applied Science Publishers: Essex, UK. p. 229-253.
16. Scheuerell, S. and W. Mahaffee, 2002. Compost tea: Principles and prospects for plant disease control. Compost Science & Utilization, 10: p. 313-338.
17. Szczech, M. and U. Smolinska, 2001. Comparison of suppressiveness of vermicomposts produced from animal manures and sewage sludge against *Phytophthora nicotianae* Breda de Haan var. nicotianae. Journal of Phytopathology-Phytopathologische Zeitschrift, 149: p. 77-82.
18. Scheuerell, S.J. and W.F. Mahaffee, 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum.* Phytopathology, 94: p. 1156-1163.
19. Weltzien, H.C., 1989. Some effects of composted organic materials on plant health. Elsevier Science Bv.

20. Jack, A.H. and E.B. Nelson, 2012. Seed colonizing microbes from disease suppressive vermicompost alter zoospore chemotaxis, encystment, and germination of *Pythium aphanidermatum.* PLoS ONE, *submitted*.