SEED COLONIZING MICROBES ALTER ZOOSPORE CHEMOTAXIS AND ENCYSTMENT OF THE OOMYCETE PLANT PATHOGEN *PYTHIUM APHANIDERMATUM*

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INTRODUCTION

Pythium aphanidermatum is an oomycete plant pathogen that produces motile zoospores capable of locating and infecting germinating seeds in the soil environment (Figure 1). Zoospores respond chemotactically to a wide range of chemical cues present in seed exudates (Donaldson and Deacon, 1993). Once zoospores arrive at the seed surface, they undergo distinct stages of pathogenic development, each of which are regulated by the plant host (Figure 2) (Nelson, 2006). The presence of certain species of bacteria on the host surface can inhibit zoospore pathogenesis via the production of antibiotics (Shang, et al., 1999). In other cases, zoospore chemotaxis is interrupted by root colonizing bacteria, presumably due to the degradation of necessary chemical cues (Islam, 2010, Zhou and Paulitz, 1993, Heungens and Parke, 2000). While there is limited evidence that individual species of microbes can interrupt zoospore pathogenesis, the interactions between complex communities of plant associated microorganisms and zoospores of plant pathogenic oomycetes have not yet been explored. Seed-colonizing microbes have been shown to interfere with sporangial germination in a closely related species, *Pythium ultimum,* which prevented the occurrence of disease (Chen and Nelson, 2008). Our goal was to investigate the effects of seed-colonizing microbes on *P. aphanidermatum* zoospore pathogenesis in a disease suppressive substrate; vermicomposted dairy manure by answering the following questions:

1) **When do zoospores arrive on the seed surface in different substrates?**
2) **When does a suppressive bacterial community develop on the seed surface? and**
3) **How do zoospores respond to microbially modified seed exudates?**

Figure 1. Conceptual diagram of spermosphere interactions between the host (seed), the pathogen (P. aphanidermatum zoospores) and the suppressive seed colonizing community (derived from vermicompost)

Zoospores respond chemotactically to chemical cues in seed exudates. Upon reaching the seed, zoospores encyst and germinate, initiating infection.

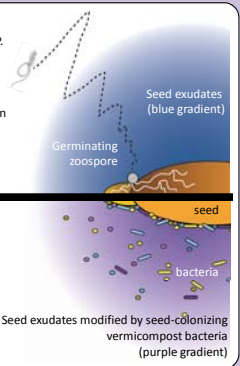
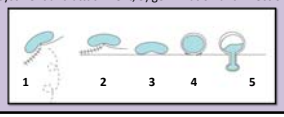


Figure 2. Stages of zoospore pathogenesis; 1) chemotaxis, 2) docking, 3) shedding flagella, 4) encystment and attachment, 5) germination and infection



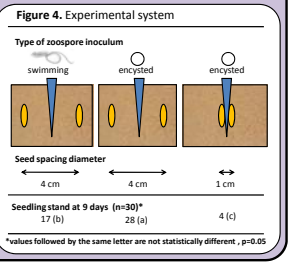
EXPERIMENTAL SYSTEM

Pathogen: *P. aphanidermatum* (*Pa*) zoospore inoculum
Host: Cucumber (*Cucumis sativum* cv 'Marketmore 76')
Suppressive microbial community: Vermicomposted dairy manure (VC)

Bioassays were conducted in an apparatus that held matric potential at a constant -3.5 kPa in a growth chamber at 27°C and 18 h photoperiod (Figure 3). Cucumber seeds were embedded into nylon mesh and sown in test media; sterile sand or sand amended with 40% v:v vermicomposted dairy manure. Funnels were flooded, drained and 4 x 10^6 zoospores were added to the center of the substrate. After 24 h seeds were transferred to funnels containing sterile sand to prevent the formation of secondary zoospores. Seedlings were assessed for disease symptoms at 9 d. To ensure that zoospores were actively swimming to reach the host, inocula of swimming and mechanically encysted zoospores were compared (Figure 4). **While mechanically encysted zoospores were viable, they caused few to no disease symptoms in seeds sown with a 4 cm d. In contrast, swimming zoospores were able to reach germinating seeds within 24 h and cause ~50% mortality.**

Figure 3. Apparatus to control matric potential

Figure 4. Experimental system



1) WHEN DO ZOOSPORES ARRIVE ON THE SEED SURFACE IN DIFFERENT SUBSTRATES?

Methods: Seeds were removed at 24 h, half were transplanted to sterile sand for assessment of disease symptoms at 9 d and half were removed for DNA extraction and subsequent qPCR analysis (Table 1). Vermicompost had no effect on DNA extraction or amplification (data not shown).

Results: Seeds sown in vermicompost had no detectable *Pa* DNA at 24 h and no mortality at 9 d, while *Pa* DNA was present on seeds sown in sand which had ~40% mortality at 9 d (Figure 5, Table 1).

Figure 5. Representative 9 day old seedlings after inoculation with *Pa* zoospores

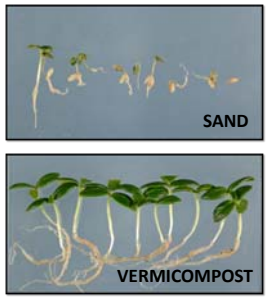


Table 1. Seedling survival and pathogen biomass* after inoculation with *Pa* zoospores

	Sand	VC
Pa58 DNA per seed (fg)	74.7 (a)	0.0 (b)
Seedling stand at 9 d (n=30)	18 (B)	30 (A)

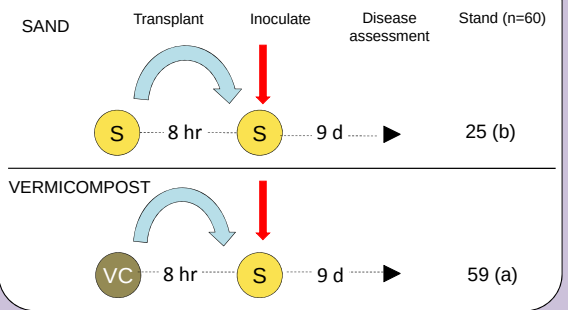
* Pathogen biomass determined with qPCR analysis

2) WHEN DOES A SUPPRESSIVE BACTERIAL COMMUNITY DEVELOP ON THE SEED SURFACE?

Methods: Cucumber seeds were sown at 4 cm d in sand or sand amended 40% v:v with vermicomposted dairy manure (VC). At 8 h, seeds were removed, transplanted into sterile sand and point source inoculated with 4 x 10^6 *Pa* zoospores. Disease symptoms were assessed at 9 d (Figure 3).

Results: Seeds originally sown in sand and inoculated at 8 h showed ~50% mortality after 9 days while seeds originally sown in vermicompost showed 2% mortality. These results indicate that the microbial community present on the surface of the seed after 8 h of germination in vermicompost provides almost complete protection from disease symptoms when exposed to *Pa* zoospores.

Figure 6. Transplant bioassay schematic and results. S = sand and VC = vermicompost



3) HOW DO ZOOSPORES RESPOND TO MICROBIALLY MODIFIED SEED EXUDATES?

Methods: After 24 h seeds were removed from substrate and incubated in sterile water for an additional 24 h. The resulting seed exudates were filtered to 0.2 µm to remove bacterial cells and stored at -20°C until use (Figure 7). Microbially modified seed exudates (MMSE) and a water control were infused into agar discs on a microscope slide and submerged in a zoospore suspension (8 x 10^4 zoospores mL^{-1}) for 30 min. Slides were removed, imaged at 7.6 and germinating zoospores were enumerated. The experiment was repeated 6 times.

Results: Exudate from seeds sown in sand attracted orders of magnitude more zoospores than exudate from seeds sown in vermicompost or the water control (Figure 8).

Figure 7. Schematic of zoospore encystment assay

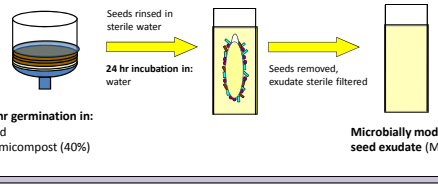
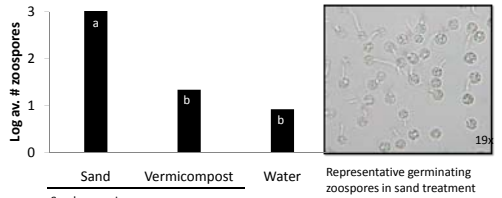


Figure 8. Average number of zoospores in 4 fields of view in 6 reps. ANOVA p = 0.001



CONCLUSIONS

When *P. aphanidermatum* zoospores swim 2 cm through a sand matrix to reach their host, they arrive at the seed surface within 24 h when seeds are sown in sand, causing significant seedling mortality. However, a 40 % amendment of vermicompost provides almost complete protection from disease symptoms (Figure 5, Table 1). When transplanted to sand after only 8 h of germination in vermicompost, seeds are protected from infection (Figure 6), indicating the seed colonizing microbial community plays a crucial role in suppressing disease. When seed exudates are modified *in vitro* by seed-colonizing microbes from a suppressive growing medium, zoospores no longer respond to them chemotactically (Figure 8). It appears that seed-colonizing microbes from vermicompost are interrupting chemical signaling between the host and the pathogen which prevents the occurrence of disease symptoms (Figure 1). These plant-associated microbes are not entirely dissimilar from human gut probiotics, some of which directly interfere with pathogen induced up-regulation of the host's inflammatory response (Zanello et al. 2009).

REFERENCES

Chen, M.-H. and Nelson, E.B., 2008. **Phytopathology** 98, 1012-1018.
Donaldson, S.P. and Deacon, J.W., 1993. **New Phytologist** 123, 289-295.
Heungens, K. and Parke, J.L., 2000. **AEM** 66, 5192-5200.
Hoitink, H.A.J. and Kuter, G.A., 1986. Effects of composts in growth media on soilborne pathogens. In: Chen, Y. and Avnimelech, Y. (Eds.), **Role of Organic Matter in Modern Agriculture**. Nijhoff Publishers, Boston, pp. 289-306.
Islam, M.T., 2010. **World Journal of Microbiology & Biotechnology** 26, 629-637.
Nelson, E., 2006. Rhizosphere regulation of preinfection behavior of oomycete plant pathogens. In: Mukerji, K.G., Manoharachary, K.G. and Singh, J. (Eds.), **Microbial Activity in the Rhizoshere**. Springer Berlin Heidelberg, Berlin, pp. 311-343.
Shang, H., Chen, J., Handelsman, J. and Goodman, R.M., 1999. **Current Microbiology** 38, 199-204.
Zanello, G., Meurens, F., Berri, M. and Salmon, H., 2009. **Current Issues in Molecular Biology** *11, 47-58.*
Zhou, T. and Paulitz, T.C., 1993. **Phytopathology** 83, 872-876.

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