

CAN METAGENETIC STUDIES OF SOIL MICROBIAL COMMUNITIES PROVIDE INSIGHTS TOWARD DEVELOPING NOVEL MANAGEMENT APPROACHES FOR ARMILLARIA ROOT DISEASE?

Mee-Sook Kim¹, Amy L. Ross-Davis², Jane E. Stewart³, John W. Hanna², Marcus V. Warwell², Paul J. Zambino⁴, Christy Cleaver⁴, GERAL I. McDONALD², Debbie S. Page-Dumroese², Bruce Moltzan⁵, and Ned B. Klopfenstein²

Armillaria Root Disease

Armillaria root diseases are among the most damaging and broadly distributed group of forest diseases in the world (Lockman *et al.* *in press*). Armillaria root disease is typically more severe in highly susceptible tree species and in trees that are maladapted due to rapidly changing climatic conditions (Ayres and Lombardero 2000, Kliejunas *et al.* 2009, Sturrock *et al.* 2011). Unfortunately, Armillaria root disease is notoriously difficult to manage in forests. Because Armillaria root disease impacts are dependent on complex ecological interactions among the host, soil microbial community, and environment, we propose the development of metagenetic tools to examine soil interactions that suppress (or enhance) Armillaria root disease. Tools to identify these interactions are paramount to develop effective management approaches for Armillaria root disease.

Potential Application of Metagenetics in Disease Management

Several studies have previously demonstrated the potential biological control of Armillaria root disease (e.g., Reaves *et al.* 1990, Raziq 2000, DeLong *et al.* 2002, Chapman *et al.* 2004, Raziq

and Fox 2005). Previous evaluations of biological control have typically focused on myco-parasitic fungi (e.g., *Trichoderma* spp) or saprophytic wood-decay fungi (e.g., *Ganoderma* spp, *Hypholoma* spp). However, they focus on only one or a few microbes, and applications are restricted by the need for massive inoculum. More importantly, inoculations with biocontrol agents must consider the risks of introducing biological organisms in nature, and navigate a lengthy and expensive process to obtain federal licensing and permits for applications within even small areas. Metagenomics and metagenetics have illuminated our understanding of how microbial communities relate to health and disease. An alternative approach to developing biological control methods for Armillaria root disease is based on the natural microbial community of the soil, which can be assessed using recently developed metagenetic technology. With this approach, 1,000s to 10,000s of microbial taxa are identified or assigned to Operational Taxonomic Units from a 1-g sample of soil (or other substrate) with accompanying environmental and soil properties. Through replication, key microbial taxa associated with suppressed disease activity are identified, along with the environmental factors that favor those microbial taxa. Subsequent tests can use management methods that favor natural microbial communities for biological control of Armillaria root disease.

Approaches

Metagenetic tools can be used to characterize soil microbial communities that may suppress Armillaria root disease. Identifying environmental factors, such as soil temperature, moisture, pH, and

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organic matter, associated with these communities can inform effective management of *Armillaria* root disease. Similar tools have been developed to characterize root disease-suppressive soils in agricultural systems, and identify appropriate disease management practices (e.g., Mendes *et al.* 2011, Damon *et al.* 2012, Bonito *et al.* 2014, Penton *et al.* 2014, Qiu *et al.* 2014, Štursová *et al.* 2014).

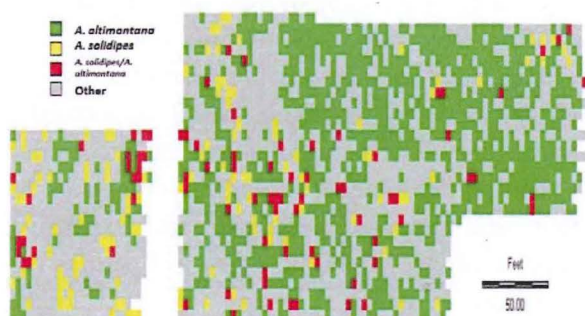


Figure 1. Does *Armillaria altimontana* help protect trees from *A. solidipes* (formerly known as *A. ostoyae*), or does *A. altimontana* reflect environmental conditions that are unfavorable for *A. solidipes*? The map depicts locations of *A. altimontana*, *A. solidipes*, and mixed *A. solidipes* and *A. altimontana* associated with a western white pine (*Pinus monticola*) provenance plantation at the Ida Creek site (Priest River Experimental Forest, northern Idaho). For this proposed study, sampling will focus on individual genets of *A. altimontana* (green areas) and *A. solidipes* (yellow areas).

Proposed Methods

The proposed tool will be assessed on six plots of each *A. altimontana* and *A. solidipes* for a total of 12 plots established within the Priest River Experimental Forest in northern Idaho (Figure 1). Total DNA will be extracted from each soil sample. Double-barcoded and pooled PCR products will be sequenced for multiple amplicons to assess the eukaryotic and prokaryotic microbial communities. Forest soil microbial communities will be analyzed to 1) determine differences among subplots, 2)

assess how differences relate to the soil properties (chemical and physical), and 3) examine how communities compare between plots associated with saprophytic *A. altimontana* and pathogenic *A. solidipes*.

Anticipated Outcomes

This project will develop metagenetic tools to characterize soil microbial communities and soil properties/environmental factors related to *Armillaria* root disease (i.e., trees colonized by saprophytic *A. altimontana* and pathogenic *A. solidipes*). This study will contribute tools for developing management practices that suppress *Armillaria* root disease and enhance forest resilience.

REFERENCES

- Ayres, M.P. and Lombardero, M.J. 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *The Science of the Total Environment* 262:263-286.
- Bonito, G. *et al.* 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Molecular Ecology* 23:3356-3370.
- Chapman, B. *et al.* 2004. Early results from field trials using *Hypholoma fasciculare* to reduce *Armillaria ostoyae* root disease. *Canadian Journal of Botany* 82: 962-969.
- Damon, C. *et al.* 2012. Metatranscriptomics reveals the diversity of genes expressed by eukaryotes in forest soils. *PLoS One* 7(1):e28967.
- DeLong, R.L. *et al.* 2002. Fluorescent pseudomonad population sizes baited from soils under pure birch, pure Douglas-fir, and mixed forest stands and their antagonism toward *Armillaria ostoyae* *in vitro*. *Canadian Journal of Forest Research* 32:2146-2159.

Kliejunas, J.T. *et al.* 2009. Review of the Literature on Climate Change and Forest Diseases of Western North America. General Technical Report. PSW-GTR-225. Albany, CALIFORNIA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Lockman, I.B. *et al.* Root diseases of forests across the U.S.A. Gen. Tech. Rep. RMRS. Ft. Collins, Colorado: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. *in press*

Mendes, R. *et al.* 2011. Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* 332:1097-1100.

Penton, C.R. *et al.* 2014. Fungal community structure in disease suppressive soils assessed by 28S LSU gene sequencing. *PloS one* 9(4):e93893.

Qiu, M. *et al.* 2014. De-coupling of root-microbiome associations followed by antagonist inoculation improves rhizosphere soil suppressiveness. *Biology and Fertility of Soils* 50(2):217-224.

Raziq, F. 2000. Biological and integrated control of Armillaria root rot. Pages 183-201 In: Fox, R.T.V., ed. *Armillaria root rot: Biology and control of the honey fungus*. Intercept, Andover, UK.

Raziq, F. and Fox, R.T.V. 2005. Combinations of fungal antagonists for biological control of Armillaria root rot of strawberry plants. *Biological Agriculture and Horticulture* 23:45-57.

Reaves, J.L. *et al.* 1990. The effects of *Trichoderma* species isolated from burned and non-burned forest soils on the growth and development of *Armillaria ostoyae* in culture. *Northwest Science* 64:39-44.

Sturrock, R.N. *et al.* 2011. Climate change and forest diseases. *Plant Pathology* 60:133-149.

Štursová, M. *et al.* 2014. When the forest dies: the response of forest soil fungi to a bark beetle-induced tree dieback. *The ISME Journal* 8:1920-1931.

