Title: Establishment, Spread, and Impact of Non-Native Microbes in Non-Model Perennial Plants

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Project Goals: The Secure Ecosystem Engineering and Design (SEED) Science Focus Area (SFA), led by Oak Ridge National Laboratory, combines unique resources and expertise in the biochemistry, genetics, and ecology of plant-microbe interactions with new approaches for analysis and manipulation of complex biological systems. The long-term objective is to develop a foundational understanding of how non-native microorganisms establish, spread, and impact ecosystems critical to U.S. Department of Energy missions. This knowledge will guide biosystems design for ecosystem engineering while providing the baseline understanding needed for risk assessment and decision-making.

Abstract text:

Biofeedstock plants, such as poplar trees (*Populus sp.*), grow in unison with soil microbes that can aid plant productivity. Commercially available biostimulants and biopesticides containing bacterial species are marketed for enhancing plant growth and pest resistance. However, the establishment and spread of beneficial bacteria in biofeedstock plants may be constrained by a variety of factors including microbe and host plant gene-gene interactions, characteristics of the invading organisms or community, and traits of the resident microbial community. We will investigate how genetics and ecology of non-model bacteria drive their establishment, persistence, and the productivity of biofeedstock perennial plants.

Bacillus species are ubiquitous bacteria in soils, sometimes forming beneficial mutualisms that promote plant growth (Xie, Zhang, and Pare, 2009). Establishment depends on gene-gene interactions between the bacteria and host plant. We will examine establishment success among *Bacillus* species and genotypes that are introduced to soils planted with either *Populus triochocarpa* (genotype 93-968), *P. deltoides* (ILL-101 and D124), a F1 hybrid cross (52-225), or a no-plant control. We will introduce multiple genotypes of three *Bacillus* species: *B. velezensis* (GB03, FZB42, BK100), *B. subtilis* (168, RO-NN-1), *B. amyloliquefaciens* (DSM7), and a mixture of all six genotypes mixed at equal proportions. This study will be conducted in the greenhouse in a fully factorial experiment: 8 *Bacillus* groups (6 genotypes, 1 mixture, 1 no-*Bacillus* control) x 5 plant groups (4 genotypes, 1 no-plant control) x 5 replicates, for a total of 200 pots. Establishment success will be measured using targeted qPCR on extracted DNA from soils. Plant growth parameters – photosynthetic rate, number of leaves, stem height, SPAD greenness index, foliar N content, and above and belowground biomass – will be measured to quantify the effect of microbial invaders on plants. We will quantify how microbial invaders affect the native soil microbiome by characterizing community composition via 16S and ITS2

amplicon sequencing and metaproteomics. We hypothesize that: (1) *B. velezensis* (GB03) will promote plant growth more than the other genotypes; (2) *Populus deltoides* (D124) will have the least successful microbial invasions because it is known to be resistant to other microbial invaders (e.g., *Septoria*); and (3) microbial invasions will be more successful in a diverse community than with individual genotype invasions. This controlled experiment will allow us to quantitatively disentangle how gene-gene interactions and genetic diversity drive microbial invader establishment and persistence.

In a concurrent field study, we will investigate the establishment and systemic spread of *Bacillus velezensis* (GB03). This genotype is applied as a commercial biofungicide in ornamental and fruit crops but has not yet been studied in a *Populus* system. It is applied to the soil and can spread from roots to leaves where it interacts antagonistically with fungal pathogens. We will study how the efficacy of *B. velezensis* varies among *Populus* species and genotypes (two *P. trichocarpa* genotypes, two *P. deltoides* genotypes, and two F1 hybrid genotypes). Invasion ecology theory suggests that propagule pressure increases establishment, and we will introduce *B. veleznesis* at four levels of propagule pressure: 0 (control group), 50% of the commercially recommended rate (3.4 x 10^9 CFU/gallon), the recommended rate (6.9 x 10^9 CFU/gallon), and double the recommended rate (13.8 x 10^9 CFU/gallon). We will track establishment and spread of *B. velezensis* over time by periodically sampling soil, root, and leaf tissues and quantify abundance through targeted qPCR. We hypothesize that (1) *P. trichocarpa* will support *B. velezensis* introductions; and (2) the establishment and rate of systemic spread will increase with increased propagule pressure. Our field study will be a fully factorial cross of 6 tree genotypes x 4 propagule pressure levels x 3 destructive harvest points (720 plants total). Harvests will continue non-destructively once plants are 6 months post-planting. This is the first temporal study of biofungicide systemic spread in a biofeedstock plantation and has applications for national energy security.

References:

Xie, X., Zhang, H., and Pare, P. 2009. Sustained growth promotion in *Arabidopsis* with long-term exposure to the beneficial soil bacterium *Bacillus subtilis* (GB03). Plant Signalling and Behavior 4(10):948-953.

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