Title: Development of CRISPR-Cas editing tools in *Sphaerulina musiva* towards controlling its establishment and pathogenicity in *Populus* ecosystems

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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:

The genus *Populus* are economically important biofuel crops cultivated worldwide, but mainly in the Northern hemisphere to fulfill the demands for bioenergy and fiber production. Poplars and their hybrids' widespread distribution and usage is limited by their vulnerability to various diseases, of which the leaf spot and canker disease caused by the invasive fungal pathogen *Sphaerulina musiva* is the most detrimental. Human-mediated transport and introduction has and will continue to result in the establishment and spread of this invasive species throughout the United States. For this reason, we need to understand the biotic and abiotic determinants for the establishment, spread, and virulence of *S. musiva -Populus* pathosystem.

In this project, we established the first CRISPR-Cas9 gene-editing protocol to successfully transform *S. musiva*. We are leveraging from this established tool to advance our understanding of genomic factors affecting above- and below-ground establishment of *S. musiva* and its virulence on *Populus* trees. Firstly, we generated and validated knockout strains of *S. musiva*, to examine the role of the effector gene *ecp2* in the pathogenicity behavior of this fungus on *Populus.* This has been done through pathogenicity experiments conducted on detached *Populus* leaves. Later, we established a closed system *in planta* to identify and characterize more genetic markers implicated in the establishment of *S. musiva* within native *Populus* soil microbial communities through RNA sequencing analysis. Ultimately, transcriptomic data from this experiment, will be complemented

with additional knockouts to confirm the function of the identified genetic markers in the establishment of this pathogen within the native soil microbiota.

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