Title: Understanding the establishment of *Sphaerulina musiva* and development of genetic approaches to enhance poplar disease control

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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.

The fungus *Sphaerulina musiva* has been reported to be both an agent of disease and a mutualistic partner of Poplar trees. While *S. musiva* is known to be the causal agent of one of the most detrimental diseases affecting susceptible hybrid poplar (e.g. *P. trichocarpa*) in North America, there have evidence that it can act as a passive endophyte when interacting with *P. deltoides*. This vast diversity of interactions is mainly related to the host genetic variation and susceptibility.

Breeding and cultivation of resistant plant species have been the main approaches used so far to control the damage caused by this pathogen on susceptible hosts. The limited amount of information about genetic functional molecular markers involved in *S. musiva*'s establishment and pathogenicity combined with the lack of optimized genetic tools have greatly hampered the development of disease mitigation strategies at the pathogen level. Therefore, we aim to quantify the genetic and molecular determinants that influence the establishment of *Sphaerulina musiva* within native *Populus* and *Salix* microbial communities.

For our first experiment, we have selected two *S. musiva* strains that represent the extremes of the virulence spectrum for the host *Populus trichocarpa*, with a long-term goal of extending and validating this work on the large collection of North America native population of 122 *S. musiva* isolates. Transcriptomics and proteomic data will be leveraged to identify functional markers that associate with successful establishment to interrogate the establishment and persistence of *S. musiva* in *Populus* and its associated microbiome. The collected omic datasets will be used to design and implement CRISPR RNA-guided gene drive systems to eradicate host-recognition mechanisms between *Sphaerulina* and *Populus* or *Salix* and attenuate its pathogenicity. Our ultimate objective of the CRISPR/Cas9 gene drive is to suppress leaf blight and stem canker

disease caused by *S. musiva* which has adverse outcomes on the production of the ecologically and commercially significant DOE biofeedstock *Populus*.

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