

ABSTRACT

INVESTIGATING MANAGEMENT AND GENETICS OF SOYBEAN SUDDEN DEATH SYNDROME PATHOGENS *FUSARIUM VIRGULIFORME* AND *F. BRASILIENSE*

By

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Annual soybean production in the U.S. is worth nearly \$40 billion, valued for its oils and protein content. Many pathogens and pests cause significant soybean yield losses each year, but one of the top threats is sudden death syndrome (SDS). At least five fungal species cause soybean SDS globally, but only two have been found in the U.S.; *Fusarium virguliforme* and *F. brasiliense*. These soil-borne pathogens infect root tissues and cause root rot, with continued infection leading to foliar interveinal chlorosis, interveinal necrosis, leaf drop, and yield loss. The pathogens are strong saprophytes that can overwinter in soybean and corn residue, so successful management is difficult. Long-term crop rotations and seed treatments with fungicides show some efficacy, but these strategies can be costly for growers. Growers desire genetic resistance to SDS, but no soybean germplasm has shown 100% resistance to SDS to date. Therefore, the overall goals of projects presented in this dissertation were to help improve SDS management and explore the biology and genetics of *F. virguliforme* and *F. brasiliense*.

To achieve these goals, I developed a risk prediction tool for integration with current SDS management strategies (Chapter 2). This study revealed that pathogen data collected from soil at-planting can be used to accurately model spatial distributions pathogens and model future SDS development and yield loss at a field level. This risk prediction study used a qPCR assay specific for *F. virguliforme*, but a similar qPCR assay for *F. brasiliense* did not exist. Therefore, I developed a qPCR assay that can distinguish *F. brasiliense* from close relatives (Chapter 3).

This tool that can be used to generate SDS-prediction models for *F. brasiliense* and I predict will be valuable in diagnostic labs across the country to distinguish between these two species.

To advance our understanding of the biology and genetics of these pathogens, I developed a new protoplast generation and transformation method to generate fluorescent strains of each pathogen (Chapter 4). This chapter is the first to report genetic transformation in *F. brasiliense*. Furthermore, I used the fluorescent strains to investigate the synergistic role of soil-borne nematodes in SDS (Chapter 5). The interactions between these fungal pathogens and nematodes *in vitro* show that *F. virguliforme* and *F. brasiliense* can colonize immobile nematodes, but suggest that they are not actively vectored into soybean roots by nematodes.

The genetic mechanisms of SDS development are poorly understood, so I developed high quality genome sequences for *F. virguliforme* and *F. brasiliense* (Chapter 6) and investigated two recognized effector proteins; FvTox1 and FvNIS1 (Chapter 7). The genome assemblies developed here have significantly improved continuity, with improved genome assembly metrics like contig length (N50) and contig number. However, whole-genome alignments between *F. virguliforme* and *F. brasiliense* from soybean (*Glycine max*) or dry bean (*Phaseolus vulgaris*) did not reveal obvious mobile pathogenicity chromosomes that have been observed in the close relative *F. oxysporum*. However, these genome resources should facilitate discovery of new fungal effector proteins like FvTox1 and FvNIS1. Interestingly, my results show that FvNIS1 is able to induce a hypersensitive response in tobacco, while FvTox1 is not, suggesting a conserved mechanism between soybean and tobacco for FvNIS1 recognition.

Overall, this work provides valuable tools for managing and studying SDS-causing fungi, while also revealing insights into the genetics and genomics of the SDS-causing pathogens *F. virguliforme* and *F. brasiliense*.