ABSTRACT

Phyllachora maydis is a fungal pathogen of *Zea mays* that causes the disease tar spot of maize. Though *P. maydis* was first identified in Mexico in 1904, the pathogen has only been detected in the United States since 2015. Since this introduction to the US, *P. maydis* has been observed in several states across the Midwest and Canada causing devastating yield losses under conducive conditions. *P. maydis* produces black stromata on maize foliage that resemble spots of tar. A secondary necrotic lesion, termed the fisheye lesion, can often form surrounding the tar spot stroma. Much speculation has arisen surrounding the causal agents of the fisheye lesion. Additionally, *P. maydis* is considered an obligate biotroph that only grows on living host tissue and cannot be cultured axenically. Artificial inoculations of *P. maydis* in controlled environments have previously not been reproducible, and previous understanding of this pathogen was scarce. Also, related species within the classified order have not been extensively studied. Therefore, this dissertation has focused on providing fundamental knowledge of the tar spot of maize pathosystem for future investigation.

In Chapter 2, a detection and quantification assay was developed for specificity to *P. maydis.* A Taq-Man qPCR assay was designed to the internal transcribed spacer (ITS) region of *P. maydis.* Specificity to *P. maydis* was confirmed using herbarium specimens of closely related *Phyllachora* spp. and common maize pathogens and endophytic fungi. The assay was also sensitive, being able to reliably detect 100 femtograms of DNA or 150 *P. maydis* spores. Lastly, reproducibility of the qPCR assay was confirmed for future use in various laboratories.

In Chapter 3, an improved *P. maydis* reference genome is provided. Using long-read sequencing, the contiguity and completeness of the *P. maydis* genome was significantly increased. Gene loss within nitrogen assimilation was found in the *P. maydis* genome indicative of its obligately biotrophic lifestyle. Furthermore, the genome annotation was improved with transcript evidence from RNA extracted *in planta.* A survey of the gene expression at this single timepoint was performed, and prediction of carbohydrate active enzymes and effector proteins was established for future elucidation.

Chapter 4 reports maize differential gene expression in response to *P. maydis* over time. An enrichment of defense response genes was found activated in response to *P. maydis.* Specifically, activation of genes involved in biosynthesis of various compounds was observed. This included terpenoids, phenylpropanoids, flavonoids, and lignin which contain compounds

with anti-fungal properties. Additionally, previously identified candidate genes for tar spot resistance loci were found significantly differentially expressed.

Lastly, in Chapter 5, the fungal and bacterial communities associated with tar spot and fisheye lesions were investigated across the US and Mexico. Bacterial communities did not show significant differences when compared by lesion type but were significantly different by location. Fungal communities showed clear differences between lesion types. Interestingly, different fungi were determined as indicator taxa of fisheye lesions between countries.

In conclusion, the first molecular detection assay was developed for future study on the epidemiology of *P. maydis.* The *P. maydis* genome and annotation was improved. Also, the first differential expression analysis from maize in response to tar spot is reported. Lastly, the microbial communities in fisheye lesions were investigated across countries. Overall, these studies provide tools and initial knowledge on the tar spot of maize pathosystem.