IMPROVED DETECTION AND MANAGEMENT OF *PHYTOPHTHORA SOJAE*

By

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ABSTRACT

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Phytophthora spp. cause root and stem rots, leaf blights and fruit rots on agricultural and economically important plant species. Symptoms of *Phytophthora* infected plants, particularly root rots, can be difficult to distinguish from other oomycete and fungal pathogens and often result in devastating losses. *Phytophthora* spp. can lie dormant for many years in the oospore stage, making long-term management of these diseases difficult. *Phytophthora sojae* is an important and prevalent pathogen of soybean (*Glycine max* L.) worldwide, causing Phytophthora stem and root rot (PRR). PRR disease management during the growing season relies on an integrated pest management approach using a combination of host resistance, chemical compounds (fungicides; oomicides) and cultural practices for successful management. Therefore, this dissertation research focuses on improving the detection and management recommendations for *Phytophthora sojae*.

In Chapter 1 I provide background and a review of the current literature on *Phytophthora sojae* management, including genetic resistance, chemical control compounds (fungicides; oomicides) and cultural practices used to mitigate losses to PRR. In my second chapter I validate the sensitivity and specificity of a preformulated Recombinase Polymerase Amplification assay for *Phytophthora* spp. This assay needs no refrigeration, does not require extensive DNA isolation, can be used in the field, and different qPCR platforms could reliably detect down to 3.3-330.0 pg of *Phytophthora* spp. DNA within plant tissue in under 30 minutes. Based on the limited reagents needed, ease of use, and reliability, this assay would be of benefit to diagnostic

labs and inspectors monitoring regulated and non-regulated *Phytophthora* spp. Next, I transitioned the Habgood-Gilmour Spreadsheet ('HaGiS') from Microsoft Excel format to the subsequent R package 'hagis' and improved upon the analyses readily available to compare pathotypes from different populations of *P. sojae* (Chapter 3; 'hagis' beta-diversity). I then implemented the R package 'hagis' in my own *P. sojae* pathotype and fungicide sensitivity survey in the state of Michigan, identifying effective resistance genes and seed treatment compounds for the management of PRR. This study identified a loss of Rps1c and Rps1k, the two most widely plant *Phytophthora sojae* resistance genes, as viable management tools in Michigan and an increase in pathotype complexity, as compared to a survey conducted twenty years ago in Michigan (Chapter 4). In Chapter 5 I led a multi-state integrated pest management field trial that was performed in Michigan, Indiana, and Minnesota to study the effects of partial resistance and seed treatments with or without ethaboxam and metalaxyl on soybean stand, plant dry weights, and final yields under *P. sojae* pressure. This study found that oomicide treated seed protects stand across three locations in the Midwest, but the response of soybean varieties based on seed treatment, was variety and year specific. Significant yield benefits from using oomicide treated seed were only observed in one location and year. The effects of partial resistance were inconclusive and highlighted the need for a more informative and reliable rating system for soybean varieties partial resistance to *P. sojae*.

Finally, in Chapter 6 I present conclusions and impacts on the studies presented in this dissertation. Overall, the studies presented provide an improvement to the detection, virulence data analysis, and integrated pest management recommendations for *Phytophthora sojae.*

This dissertation is dedicated to my family.
To my parents, who provided an environment where their children could be curious and explore the natural world around them.
To my brother, for always being there to talk and being a constant source of encouragement.
To my Grandparents, Judy York Hensley and the late Bobby Glenn Hensley, who encouraged my interest in (marine) biology from a young age. I will forever cherish and find comfort in the time I spent with you both as a child.
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As a first-generation college student, and subsequently a first-generation graduate student, the journey through my PhD has been difficult. Through it all my family has been a constant source of love, help, and encouragement. I could not have done this without you.
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Chapter 1: **Literature Review**

Management of Phytophthora Stem and Root Rot of Soybean

This chapter is an overview of the management practices that are used to mitigate losses to Phytophthora stem and root rot of soybeans (PRR), caused by *Phytophthora sojae*. This chapter is split into four parts, 1) Introduction to *P. sojae*, 2) Management practices for PRR such as genetic resistance and chemical control options, 3) Future directions of management for this pathosystem, and 4) Conclusions on management practices for PRR.

Introduction to Phytophthora sojae

Uniform and healthy stand establishment is essential to maximizing crop yield. Oomycetes, such as those in the genera *Pythium* and *Phytophthora* constitute a major threat to not only stand establishment but also final yields. Phytophthora stem and root rot, historically attributed to *Phytophthora sojae*, has been a yield limiting biotic factor in soybean production for decades and causes an estimated $357 million in yield loss every year, worldwide. *Phytophthora sojae* infected plants were first observed in 1948 in Indiana and then again in 1951 within Ohio. However, the causal agent was not described until 1958 (Kaufman and Gerdeman 1958). Since then, *Phytophthora sojae* has been found in all major soybean producing regions worldwide (Schmitthenner, 2000). In 1958, this new *Phytophthora* spp. was named *Phytophthora sojae* (Kaufman and Gerdeman 1958) but was renamed the next year to *Phytophthora megasperma* var *sojae* and added to the *Phytophthora megasperma* species complex. This renaming was done as it had slightly smaller, but very similar, oogonia and antheridia compared to previously identified *Phytophthora megasperma* isolates. The *Phytophthora megasperma* species complex is a collection of morphologically similar species that are genetically distinct. These species have large oogonia and antheridia and can infect a wide host range, as most are their own distinct species but are grouped together. *Phytophthora megasperma* f. sp. *sojae* would be kept as a part of the *Phytophthora megasperma* species complex up until 1989 when Faris et al. reinstated the

first name, *Phytophthora sojae* (Faris et al 1989). *P. sojae* is not the only *Phytophthora* spp. differentiated from the *Phytophthora megasperma* species complex causing disease on soybean roots. *Phytophthora sansomeana* E.M. Hansen & Reeser is a recently described species which has been found frequently within rotten soybean roots. While the only agronomic host for *P. sojae* is soybean, *P. sansomeana* can infect a wider range of hosts including soybean, corn, clover, wild carrot, and Douglas fir (Hansen et al 2009).

Phytophthora sojae is an oomycete root rot pathogen of soybean and some species in the *Lupinus* genus (Schmitthenner 2000). Oomycete plants pathogens, such as *Phytophthora sojae*, overwinter within plant debris and soil as the resting spore, the oospore. Oospores of *P. sojae* will germinate in the spring, during times that there is free water within the soil, and produce either vegetative hyphae or sporangia containing flagellated, motile, zoospores. Once released from sporangia, these zoospores are chemotaxic and utilize free water to maneuver themselves towards soybean roots. Zoospores which have encountered a soybean root will encyst and produce penetrative hyphae which will begin the infection of the root cortex and eventually the vascular tissue (Dorrance 2018). It is in these infected roots that the oospore will be formed to overwinter until the next year and the cycle repeats itself. Some *Phytophthora* spp. require two different mating types (heterothallic) to form a fertilized oospore, however, *P. sojae* and *P. sansomeana* are homothallic and can produce a fertilized oospore without another individual. Susceptible soybean cultivars can be infected by *Phytophthora sojae* during any stage of their life cycle, from germination to senescence. Typical *P. sojae* symptoms can be an early season pre- and post-emergence damping off, a mid-season stem rot, as well as plant stunting. Young, *P. sojae* infected, seedlings will be necrotic and may appear to have died very suddenly, usually after periods of heavy rain or soil saturation. Mature plants infected with *P. sojae* can display a

chocolate-brown stem lesion originating from or below the soil line that causes the stem to look and feel water soaked.

Management Practices

Gene-for-gene resistance

Phytophthora sojae has been managed primarily via deployment of resistance genes (Rps genes, **R**esistance to ***P****hytophthora* ***s****ojae*) in commercial soybean cultivars which interact with the pathogens *Avir* gene products and confer resistance. This interaction, called gene-for-gene resistance, is a well-studied resistance mechanism within fungi, bacteria, and oomycetes (Petit-Houdenot, Y and Fudal, I., 2017; Anderson, R.G., et al 2015; Popa, C. M., Tabuchi, M., and Valls, M., 2016). *P. sojae Avir* genes contain an RXLR (Arginine-X-Leucine-Arginine) motif within their protein structure. The RXLR motif within these proteins is involved in secretion and host targeting, but is not required for protein activity (Bos JI, et al 2006). These *Avir* gene products enter plant cells and begin the process of inhibiting the plants immune response, increasing the plants susceptibility. The process by which these proteins are translocated into a host plants cells, as well as their activity once inside, is currently not well understood (Morgan and Kamoun 2007). Currently the most studied interaction of the *Phytophthora sojae Avir* proteins are their interaction with soybean Rps genes.

Genetic resistance to *P. sojae* is the most economic form of control for this pathogen. Single gene resistance is expressed and effective all season and can protect against any *P. sojae* pathotype that expresses the corresponding *Avir* protein. Currently there have been more than 30 Rps genes identified within various soybean germplasm (Sahoo et al 2017; Dorrance 2018). However, only a handful of these genes have been deployed within commercial soybean varieties, mainly 1k, 1c, 3a and to some extent 4 and 6 (Dorrance et al 2016). The other 30+ Rps

genes have not yet been incorporated into commercial cultivars as they have either yet to be tested against a variety of *P. sojae* populations or have been deemed already ineffective to populations in which they have already been tested. Generally, Rps genes are evaluated during pathotype surveys and Rps genes that confer resistance to more than 60% of the sampled population are deemed effective (Dorrance et al 2016). Soybean Rps genes produce a class of proteins termed NBS-LRR (Nucleotide Binding Site- Leucine Rich Repeat) proteins. These proteins are found within the cytoplasm of plant cells and are used to recognize *P. sojae Avir* genes and confer resistance. Individual R genes within soybeans confer resistance to any *P. sojae* individual that produces the corresponding *Avir* gene. Identification of effective Rps genes within state population is essential to determine effective Rps genes for deployment and evaluate currently used Rps genes for resistance management.

Phytophthora sojae pathotype surveys have been conducted over the past 60 years within soybean producing regions, both within the US and worldwide, documenting pathotype distribution and effective R-genes for management within these areas (Kaufmann Mj, Gerdemann JW, 1958; Miller et al 1997; Ryley et al 1998; Workneh et al 1999; Jackson et al 2004; Costamilan et al 2013; Dorrance et al 2016; McCoy et al 2022; Hebb et al 2021; Matthiesen et al 2021; Chowdhury et al 2020). These surveys are centered around statewide sampling of fields used in soybean production, either through plant samples or soil sampling. Isolation of *P. sojae* from plant samples is done via oomycete selective medium usually containing antibiotics and fungicides to deter unwanted microbial growth, while allowing oomycetes such as *P. sojae* to grow. Soil samples are processed using a soil bioassay utilizing conditions for optimal growth of oomycetes and "baiting" these organisms with a susceptible cultivar of soybean which are later isolated using selective media (Dorrance 2008). Soil samples

are more time intensive to process but preferred to plant samples as there is no selection bias for pathotypes. For instance, surveys conducted only using plant samples may only be identifying the pathotypes of *P. sojae* which can cause disease on the Rps gene which was planted that year. This is not ideal as not all pathotypes within a field are identical and there may be more isolate pathotypes within the soil that can cause disease on other Rps genes. Soil sampling can identify all pathotypes within a soil sample since a susceptible cultivar is used to bait for infection and isolation of *P. sojae*. Identifying all the pathotypes within a soil sample offers a more wholistic view of pathotype distribution within a survey as there is no Rps gene selection bias in sampling, allowing for more precise recommendations for effective Rps gene use. State surveys have observed that there is a high diversity in *P. sojae* pathotype structures between states, making deployment of new resistance genes cumbersome albeit necessary (Dorrance 2018). Over the years of repeated *P. sojae* pathotype sampling there has been an observed rise in pathotype complexity, or the number of soybean Rps genes an isolate can overcome. This reported rise in pathotype complexity has accompanied many states reporting that commonly used resistance genes (Rps 1c, 1k, 3a) are not as effective as they once were to the sampled *P. sojae* population (McCoy et al 2022; Hebb et al 2021; Matthiesen et al 2021; Chowdhury et al 2020).

Partial resistance

Single gene resistance provides season long protection against *P. sojae* isolates which produce the corresponding *Avir* gene but does not protect against those pathotypes which do not produce the *Avir* gene and can evade detection to cause disease. While Rps mediated resistance is qualitative (resistant or not resistant) and based on the presence or absence of a single gene, partial resistance is quantitative and likely relies on multiples genes. Partial resistance may be the culmination of different defense signal pathways, physiological or morphological changes or all

these examples combined (Wang et al 2012; Schneider et al 2016). Soybean lines with high levels of partial resistance allow for some growth of *P. sojae,* but the disease is not as severe as it is with low levels of partial resistance. Likewise, soybean varieties with high levels of partial resistance were found to have higher yields than those varieties with low levels of partial resistance (Dorrance A.E., McClure S.A. and Martin S.K. 2003). Partial resistance, unlike Rps mediated resistance, may not provide protection to seedlings until the seedlings reach the V1 (first trifoliate leaf emerges) growth point (Dorrance and McClure 2001). Disease management up until the V1 growth stage relies heavily on effective Rps gene use as well as utilizing seed treatments that contain a fungicide which is effective on oomycetes.

Chemical Management

Seedling diseases are exceptionally destructive when ample rain and poorly drained soils provide optimal conditions for infection over prolonged periods of time. Soybean seed treatments have been used for over 30 years to manage seedling diseases such as *Rhizoctonia* spp., *Fusarium* sp, *Pythium* sp, and *Phytophthora* sp. (Munkvold 2009). However, it wasn't until metalaxyl was released, in 1989, that an effective seed treatment for oomycetes specifically was available (RED for metalaxyl, EPA, 1989; Schmitthenner 1985). Ethaboxam, released in 2013, also has efficacy against most soilborne oomycetes however, some clades of *Pythium* are naturally less sensitive due to inherent mutations within the target site of ethaboxam (Noel et al 2019; Peng et al 2019). The most recent fungicide addition for *Phytophthora* management in soybeans is oxathiapiprolin. As of 2019, oxathiapiprolin is available as a soybean seed treatment and is labelled for *Phytophthora* sp in soybean but is not effective on most *Pythium* spp. (Miao et al 2016; Miao et al 2020). Of the three, metalaxyl has been studied the most intensively. These

three compounds represent the main seed treatments used to combat not only *Phytophthora sojae*, but all oomycete seedling diseases.

Metalaxyl is a member of FRAC code 4 (phenylamines) which are fungicides that act by inhibiting RNA synthesis in oomycetes, while being relatively inactive on true fungi (FRAC code list 2018). Metalaxyl provided better emergence and plant vigor compared to untreated controls in a greenhouse assay (Dorrance and McClure, 2001). However, in the field seed treatments containing metalaxyl had mixed results (Dorrance *et al*, 2009; Gaspar *et al*, 2014; Urrea, Rupe and Rothrock, 2013). Stand was significantly protected with treatments of metalaxyl but yield was unaffected, showing no economic advantage to using a seed treatment. This is likely not due to soybean seeding rates (number of seed planted per acre) as studies on seed treatment effects at reduced rates observed no increase in yield when using a metalaxyl seed treatment (Gaspar, Mitchell and Conley, 2015). These results, while discouraging, may not mean that seed treatments are ineffective or unwarranted. Seed treatments offer the best results in fields where poor drainage or high pathogen density cause disease year after year. Seed treatments may be beneficial in reducing these inoculum loads directly around the seed, thus allowing for better stand establishment. Reduction in stand, either through reduced planting or disease incidence, can be overcome by individual plant size compensation, thus leading to no difference in final yields (Gaspar, Mitchell and Conley, 2015). So, while initial stand within a field may be protected with metalaxyl seed treatments, allowing for reducing planting populations, plant size compensation in reduced stand areas may be responsible for yield recovery.

Ethaboxam is a member of FRAC code 22 (thiazole carboxamides) which act by inhibiting Beta-tubulin synthesis in oomycetes (Noel et al 2019; Peng et al 2019). Ethaboxam,

being a newer chemistry, has had significantly less research studies performed on it. Even so, recent studies have shown that it has exceptional management potential against a variety of oomycete pathogens, including *Phytophthora sojae* (Matthiesen, Ahmad and Robertson, 2016; Radmer et al 2017; Dorrance 2018). However, inherent resistance to ethaboxam has been describe in some *Pythium* spp. (Noel et al 2019). Due to this inherent resistance, ethaboxam is recommended to be used in conjunction with a broad spectrum oomicide such as metalaxyl or mefenoxam. As with metalaxyl, field studies have shown a protection of stand with an ethaboxam seed treatment, with variable effects on yield (McLachlan 2016; Cerritos-Garcia et al 2021; Garnica et al 2021).

Oxathiapiprolin is a member of FRAC code 49 (piperidinyl-thiazole-isoxazolines) whose mode of action is binding the oxysterol binding proteins (OSBP) of oomycetes. Little is known about the function of oxysterol binding proteins in oomycetes (Miao et al 2018). However, identification of single amino acid residue mutations within OSBPs oxysterol binding domain have been well categorized for *Phytophthora sojae, P. capsici* and *P. nicotianae* (Miao et al 2016; Bittner et al 2017; Miao et al 2018). While oxathiapiprolin is efficacious on *Phytophthora* and *Phytopythium* spp., it is not effective on most *Pythium* spp. (Miao et al 2016). This is due to *Pythium* spp. OSBP having significantly different amino acid residues and therefore likely a different protein structure (Miao et al 2020). Field trial evaluation of oxathiapiprolin as a seed treatment has found it be effective for managing early season oomycete diseases, protecting stand and occasionally yield (Hegstad et al 2021).

Cultural practices

Oomycetes such as *P. sojae* require free water for their motile zoospores to recognize root exudates, which attract the zoospores to the roots. Free water within the soil is either due to

the soil make up itself not allowing for the water to drain, or due to compaction from heavy machinery frequenting the field. To combat free water (also called pooling) in fields, drainage tiles can be implemented. Drainage tiles are a network of pipes laid under the field which are gravity fed towards an outlet, usually a waterway. These drainage tiles are interconnected tubes made of primarily polyethylene plastics. Compaction of soils can compound water pooling in the field, making it harder for the field to drain as well as allow plants to produce healthy root systems. Tillage has typically been used to counteract soil compactions. However, with recent conservation tillage practices used to promote healthy soils, tilling of fields has been reduced (Busari et al 2015). Conservation tillage has the potential to promote *P. sojae* inoculum accumulation within the soil and promote disease causing yield loss (Schmitthenner and Van Doren, 1985; Workneh et al 1998). Lastly, planting before severe weather events that could cause pooling in compacted or low areas within the field is not advised. Crop rotation is frequently used to disrupt disease cycles of foliar and soilborne phytopathogens. Unfortunately, due to the *P. sojae* oospore being able to survive for many years in the soil or plant debris, crop rotation is not a plausible method of control.

Future direction of management

Development of increasingly complex *P. sojae* pathotypes has increased the need for more resistance genes that can be incorporated into commercial varieties of soybean. The identification of new resistance genes does not necessarily mean they will be effective in production agriculture though. New Rps genes would need to be tested against various populations of *P. sojae* to determine if they are efficacious enough to widely deploy. Currently a set of standard soybean differentials is used to determine gene efficacy and new Rps genes are not included. This leads to an abundance of new and potentially effective genes that are never

incorporated into breeding lines of soybean while a handful of genes are repeatedly used (Dorrance 2018). Testing of novel Rps genes on a larger population of *P. sojae* isolates is needed to identify the next resistance genes to be deployed to manage *Phytophthora sojae.*

Conducting pathotype surveys is time consuming and potentially inaccurate if conditions during pathotyping are not correct (Dorrance 2008). Likewise, differentials representing the same resistance gene but of a different cultivar have been reported to have different reactions to the same isolate of *P. sojae* (Dorrance et al 2008). A more accurate way to determine pathotypes within a population would be to use molecular identification of an isolates *avr* gene and use that information to determine current population pathotypes. Current molecular technologies have enabled researchers to develop and test a set of markers for the identification of six avirulence genes within the *Phytophthora sojae* genome allowing for accurate predictions of pathotypes (Arsenault-Labrecque et al 2018). Correct phenotypes were predicted 99.5% of the time compared when compared to the hypocotyl inoculation method of pathotyping. This has since been developed into a commercial diagnostic assay that decreases the time needed to determined effective resistance genes to use against a *P. sojae* population (AYOS-diagnostics).

Conclusions

Phytophthora sojae can be effectively managed using genetic resistance, but the increase in pathotype complexity across soybean growing regions is leading to increasingly ineffective soybean resistance genes. Breeding for better partial resistance in commercial cultivars may be necessary both to increase resistance gene longevity as well as reduce yield losses in high performing cultivars lacking a resistance gene. This work emphasizes the need for a strong genetic background within commercial soybean varieties, as well as early season protection using oomycete specific fungicide seed treatments. In summation, no one manage practice can

completely control a population of *Phytophthora sojae* but used together yield losses can be minimalized.

Chapter 2: **Validation of a Preformulated, Field Deployable, Recombinase Polymerase Amplification Assay for *Phytophthora* Species**

Source:

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Summary

Diagnosticians and plant inspectors require rapid and accurate tests to diagnose causal agents of plant disease. Isothermal molecular tests, such as Recombinase polymerase amplification (RPA) assays can detect plant pathogens in the lab or field without time-consuming DNA extractions, offering results in less than an hour. However, there is currently no preformulated RPA assay for Phytophthora species commercially available to diagnosticians. This study investigates the effect of preformulation, lyophilization of primers and probe within a single pelleted tube, on a previously developed *Phytopthora* genus-specific RPA assays performance. Here, we observed that preformulated assays sensitivity and specificity were consistent and uniform in pathogen detection with preformulated RPA kits for *Phytophthora* detection, when conducted by different labs using different instruments for measuring results. Amplification of regulated and unregulated *Phytophthora* spp. target loci from crude diseased plant extracts required less than 30 minutes. This work herein represents an improvement in rapid molecular detection of *Phytophthora* spp. for diagnostic use.

Chapter 3: **'hagis', an R Package Resource for Pathotype Analysis of *Phytophthora sojae* Populations Causing Stem and Root Rot of Soybean**

Source:

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Summary

Single-gene resistance is an economic management strategy for many phytopathogens. Phytopathogen surveys to monitor resistance gene efficacy are conducted regularly to identify any breakdown of genetic resistance efficacy. To evaluate the data associated with these surveys, phytopathologists have used the Habgood-Gilmour Spreadsheet (HaGiS), written in Microsoft Excel, to describe pathogen virulence diversity, and determine the efficacy of tested resistance genes. However, the large datasets that are produced through genetic resistance survey work can make the use of excel based analysis cumbersome and limits reproducibility. The R programming language has become increasingly popular in plant pathology and our professions desire for reproducible research made HaGiS a prime candidate for conversion into a freely available R package. The R package hagis, described herein, can produce all outputs of the HaGiS Excel spreadsheet, including isolate virulence descriptions, resistance gene efficacy on the sampled population, and describe the diversity of virulence in the sampled population. In addition, further abilities (functions) were added that facilitate the analysis and comparison of resistance gene efficacy across multiple sampled populations. Allowing for investigative work on the temporal-efficacy of resistance genes, something that is difficult or impossible to test in Excel based programs. This work represents the first improvement on rapid, reproducible, phytopathogen virulence and resistance gene efficacy analysis in 20 years.

Chapter 4: ***Phytophthora sojae*** **pathotype distribution and fungicide sensitivity in Michigan**

Source:

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Summary

Surveys to monitor resistance genes and seed treatment as management options are needed for the successful management of Phytophthora stem and root rot (PRR), caused by *Phytophthora sojae*. However, the last survey conducted in Michigan to monitor *P. sojae* resistance gene efficacy was conducted 20 years ago (1993-1997). Likewise, there has not been a significant testing of the Michigan *P. sojae* population to commonly used seed treatments for management. Therefore, in 2017 soil samples from 69 field across Michigan were collected, and 83 isolates of *P. sojae* were isolated using a soil baiting process. These 83 isolates were used for phenotypic virulence characterization using a hypocotyl inoculation procedure to test 13 soybean resistance genes to the *P. sojae* population, and testing of the seed treatment compounds mefenoxam, ethaboxam, oxathiapiprolin, and pyraclostrobin. The *P. sojae* population was observed to be able to, on average, cause disease on 2 more soybean resistance genes than was observed in the 1993-1997 sampling. Likewise, the most widely used *P. sojae* resistance genes in soybean, Rps1c and Rps1k, are no longer effective at managing the *P. sojae* population in Michigan. The resistance genes Rps 3a, Rps3c, and Rps4 were found to have management efficacy, however, only Rps3a is available commercially in Michigan. There was no observed in vitro fungicide resistance in the sampled population to the chemical compound tested. This study identified a significant shift in *P. sojae* resistance gene efficacy, but that seed treatments are still a viable management option for managing PRR. This study and resulting work has improved management recommendations for PRR in Michigan.

Chapter 5: **Oomicide Treated Soybean Seeds Reduce Early Season Stand Loss to *Phytophthora sojae***

Source:

This chapter has been published in *Crop Protection*: McCoy, A.G., Byrne, A.M., Jacobs, J.L., Anderson, G., Kurle, J., Telenko, D.E.P., and Chilvers, M.I. (2022). Oomicide Treated Soybean Seeds Reduce Early Season Stand Loss to *Phytophthora sojae. Crop Protection*. Available at: https://doi.org/10.1016/j.cropro.2022.105984

Summary

Phytophthora stem and root rot (PRR), caused by *Phytophthora sojae* Gerdemann and Kaufman, is an important disease of soybean (*Glycine max* (L.) Merr.) in the Midwest United States. An integrated pest management approach including use of genetic resistance (single-gene and partial resistance) and seed treatments is needed to manage losses to this disease. To determine the effect of PRR partial resistance and oomicide seed treatments on soybean stand, plant vigor and yield, inoculated soybean plots were established in Indiana (2019), Michigan (2018/2019), and Minnesota (2019). *P. sojae* isolates were used which could overcome the planted Rps genes (Rps1c and Rps1k) to simulate the loss of single-gene resistance in a management setting. This study used nine commercial soybean varieties in Michigan, and a subset of 4 varieties were planted in Indiana and Minnesota which were treated with three different seed treatments: 1) non-treated, 2) base (ipconazole and clothianidin), and 3) Intego Suite (base + ethaboxam and mefenoxam) in Michigan, Indiana, and Minnesota. Results indicated that while soybean stand was significantly protected using Intego Suite as compared to the non-treated or base, there was only three varieties were yield differences based on seed treatment were significant. In this study, the partial resistance scores supplied by commercial soybean producing companies were not sufficient to identify differences in management efficacy.

Chapter 6: **Conclusions and Impact**

Conclusions

Phytophthora stem and root rot (PRR) continues to be a problematic and recurring disease in soybean production worldwide. Field populations of *Phytophthora sojae* are managed using seed treatments, and soybean varieties with high partial resistance as well as an effective resistance-gene. Therefore, the research in this dissertation has focused on updating and improving the detection and management of *Phytophthora sojae*.

Disease symptoms caused by *Phytophthora* spp., such as root and crown rot, fruit rot, and leaf blights can be hard to distinguish and differentiate from related organisms or fungi in the field. Plant materials that could harbor regulated *Phytophthora* spp. (i.e. *P. ramorum, P. kernoviae*) require timely screening and detection of *Phytophthora* spp. at ports of entry to limit their dispersal. A preformulated, lyophilized, field deployable recombinase polymerase amplification assay was validated on *Phytophthora* spp. including, *P. ramorum, P. kernoviae,* and *P. sojae*. This assay requires no refrigeration, DNA extraction, or thermal cycling (isothermal, 39-42°C amplification temperature) for use, and requires minimal equipment and reagents, making it suitable for on-site inspections and testing of plant materials at nurseries or ports of entry (McCoy et al 2020).

Effective resistance genes and seed treatments are integral part of the integrated pest management program used for *P. sojae* management. Consistent, widespread, repeated use of a single-dominant resistance gene (Rps-gene) or chemical compound for management can select for virulent or resistant pathogen genotypes and lead to a loss of management with these options. Surveys for *P. sojae* virulence (also called pathotype surveys) create large amounts of phenotypic data that has typically been input into the Excel based program “HaGiS”, the Habgood-Gilmour Spreadsheet, to perform pathotype analyses, identify effective resistance

genes, and describe pathotype complexity. While effective, Excel based programs can make it difficult to identify data entry errors and limit reproducibility of analyses used for research. Likewise, as datasets get larger Excel based programs can quickly become cumbersome to use. To promote reproducible research and facilitate efficient analysis of pathotype data, the 'hagis' R package was developed. The 'hagis' package performs the same analyses that the HaGiS Excel program can, while also incorporating the ability to make direct comparisons of pathotype diversity between sampled populations, spatially or temporally, an improvement on the HaGiS Excel spreadsheet, and for pathotype study data analysis methods (McCoy et al 2019).

The Michigan *Phytophthora sojae* population was sampled in 2017 to update management recommendations for resistance genes and seed treatment use. The Michigan *P. sojae* population had not been extensively surveyed since the mid 1990's, thus the efficacy of currently used resistance genes was unknown. This survey found that the vast majority of *P. sojae* isolates recovered were virulent on the Rps1 locus (Rps1a, 1b, 1c, 1d, 1k), which encompasses the two primary resistance genes used for management in North America: Rps1c and Rps1k. The genes Rps3a, Rps3c, and Rps4 were found to be effective for management of *P. sojae* in Michigan, however, only Rps3a is available in commercial varieties. Fungicide testing to four common seed treatment compounds (ethaboxam, mefenoxam, oxathiapiprolin and pyraclostrobin) found no evidence of insensitivity, evidence that seed treatments are still a viable option for early season management of PRR (McCoy et al 2022a). The loss of the Rps1c and Rps1k genes as viable management options in Michigan is concerning and raised questions as to how effective partial resistance and seed treatments would be for management of PRR on their own. In 2018, Michigan only, and 2019 field trials were established in Michigan, Minnesota, and Indiana to quantify the effects of seed treatments and partial resistance under PRR disease

pressure using isolates of *P. sojae* that could overcome the Rps1c and Rps1k resistance genes. Seed treatments significantly protected stand in most environments, but differences in stand only translated to differences in yield in the Michigan 2018 field trial location. The effect of seed treatments on plant vigor, as measured by plant dry weights, was only apparent early in the season and were not apparent after the V2 soybean growth stage. Effects of varietal partial resistance on plant health or yield was inconclusive and highlights the need for a more informative partial resistance rating that companies provide for their varieties (McCoy et al 2022b).

In conclusion, these works provide improved detection of *Phytophthora* spp. and evidence that the major resistance genes to *Phytophthora sojae* used in North America are no longer effective on the sampled *P. sojae* population in Michigan (McCoy et al 2022a). These findings are in agreement with reports from other states in the United States. Testing and deployment of novel resistance genes for management is needed. Seed treatments continue to be an effective early season management option but will not provide season long protection from *P. sojae* (McCoy et al 2022a; McCoy et al 2022b). This data will be useful in producing new management recommendations for PRR on soybeans.

Broader Impacts

The studies presented herein have immediate impacts on the management recommendations for farmers in the Midwest, and detection of *Phytophthora* spp. In chapter 2, I validate a preformulated, field deployable, recombinase polymerase amplification assay that can be used to detect regulated and non-regulated *Phytophthora* spp. in diagnostic labs, nurseries, or ports of entry on diseased plant material. In chapter 3 a new R package ‘hagis’ is developed and validated

for use on phytopathogen virulence data, allowing for reproducible and efficient analysis of pathogen virulence data. This package has already garnered use (16,070 downloads since June 2019 release) and has been cited in 8 pathogen virulence studies from North and South America. I then use the 'hagis' package in my own *P. sojae* pathotype study in Michigan, USA (chapter 4). This study revealed a loss of the two main resistance genes used for management in Michigan: Rps1c and Rps1k. Seed treatments remain effective for early season management. We then tested partial resistance and seed treatments as management options in Michigan, Indiana, and Minnesota with an inoculated field trial (chapter 5). We found that while seed treatments protect stand, the effect of seed treatments and partial resistance on final yield was variable and often inconclusive. This information has been distributed through extension, industry, and commodity board meetings throughout Michigan so farmers can make informed decisions about PRR management in their fields. Currently I am investigating pathotype complexity and resistance gene efficacy on a global spatial-temporal scale. This work is forthcoming and will clarify how *P. sojae* virulence has changed over time, as well as understanding how long resistance genes remain efficacious to various *P. sojae* populations around the globe.

List of Published Works and Contributions from the duration of my PhD

1. **McCoy, A.G.**, Byrne, A.M., Jacobs, J.L., Anderson, G., Kurle, J., Telenko, D.E.P., and Chilvers, M.I. (2022). Oomicide Treated Soybean Seeds Reduce Early Season Stand Loss to *Phytophthora sojae. Crop Protection*. https://doi.org/10.1016/j.cropro.2022.105984

AGM, AMB, MIC planned study; AGM acquired seed and treated seed for all locations; AGM, JLJ made inoculum for MI location; AGM performed field trial for MI location; GA, JK performed trial for MN location; DEPT performed field trial for IN location. AGM analyzed all data, generated figures, and wrote the original version of the manuscript. All authors revised the manuscript and contributed to the final manuscript.

2. **McCoy, A.G.**, Noel, Z.A., Jacobs, J.L., Clouse, K.M., Chilvers, M.I., (2021). *Phytophthora sojae* Pathotype Distribution and Fungicide Sensitivity in Michigan. *Plant Dis*. https://doi.org/10.1094/pdis-03-21-0443-re

AGM and MIC planned experiments. AGM and KMC collected data. ZAN provided insight and guidance on fungicide testing. AGM generated the figures, analyzed the data and wrote the manuscript. All authors revised the manuscript and contributed to the final manuscript.

3. Lin, F., Li, W., **Mccoy, A. G.**, Gao, X., Collins, P. J., Zhang, N., Wen Z., Sizhe, C., Wani, S.H., Gu, C., Chilvers, M.I., Wang, D. (2021). Molecular mapping of quantitative disease resistance loci for soybean partial resistance to *Phytophthora sansomeana*. *Theor. Appl. Genet*. 134:1977–1987 Available at: https://doi.org/10.1007/s00122-021-03799-x

DW, MIC, and FL designed the research. FL, WL, AGM, XG, PJC, NZ, ZW, SC, SHW, and CG carried out the experiments. FL, WL, and XG analyzed the data. FL, WL, and AGM developed the draft manuscript. DW and MIC supervised the manuscript. All authors revised the manuscript and contributed to the final manuscript.

4. Valle-Torres, J. T. J. Ross, D. Plewa, M. C. Avellaneda, J. Check, M. I. Chilvers, A. P. Cruz, F. Dalla Lana, C. Groves, C. Gongora-Canul, L. Henriquez-Dole, T. Jamann, N. Kleczewski, S. Lipps, D. Malvick, **A. G. McCoy**, D. S. Mueller, P. A. Paul, C. Puerto, C. Schloemer, R. N. Raid, A. Robertson, E. M. Roggenkamp, D. L. Smith, D. E. P. Telenko, and C. D. Cruz. (2020). Tar spot: An understudied disease threatening corn production in the Americas. *Plant Disease*. 104:2541–2550 Available at: https://doi.org/10.1094/PDIS-02-20-0449-FE

All authors contributed to a section of the manuscript. AGM contributed to “Molecular Diagnostics” and “Signs, Symptoms, Causal Agent(s), and Host Range” sections. JVT, TJR and DP wrote the original manuscript and are shared first authors.

5. **McCoy, A.G.**, Miles, T.D., Bilodeau, G.J., Woods, P., Blomquist, C., Martin, F.N., and Chilvers, M.I. (2020). Validation of a Preformulated, Field Deployable, Recombinase Polymerase Amplification Assay for Phytophthora Species. *MDPI-Plants*. 9:466 Available at: https://www.mdpi.com/2223-7747/9/4/466

Conceptualization, FNM and TDM; methodology, TDM; validation, AGM, GJB, PW and TDM; formal analysis, AGM; writing—original draft preparation, AGM; writing—review and editing, AGM, GJB, TDM, FNM, MIC, PW and CB.

6. Lin, F., Wani, S.H. Collins, P.J., Wen, Z., Li, W., Zhang, Z., **McCoy, A.G.**, Bi, Y., Tan, R., Zhang, S., Gu, C., Chilvers, M.I., Wang, D. (2020). QTL mapping and GWAS for identification of loci conferring partial resistance to *Pythium sylvaticum* in soybean (*Glycine max* (L.) Merr). Available at: https://doi.org/10.1007/s11032-020-01133-9

DW, and FL designed the research. FL, SHW, PJC, ZW, WL, NZ, AGM, YB, RT, SZ, CG carried out the experiments. FL, and SHW analyzed the data and developed the draft manuscript. DW and MIC supervised the manuscript. All authors revised the manuscript and contributed to the final manuscript.

7. **McCoy, A. G**.*, Roth, M. G.*, Shay, R.*, Noel, Z. A.*, Jayawardana, M. A., Longley, R. W., Bonito, G., Chilvers, M. (2019*).* Identification of Fungal Communities Within the Tar Spot Complex of Corn in Michigan via Next Generation Sequencing. *Phytobiomes*. 3:235–243 Available at: https://doi.org/10.1094/PBIOMES-03-19-0017-R.

a. **Spotlight on the American Phytopathological Society's website**

AGM, MGR, RS, MAJ, GB, and MIC planned experiments. AGM, MGR, RS, and MAJ collected and processed samples. RWL performed PCR and constructed libraries. AGM submitted raw sequeces to Sequence Read Archives. MGR and ZAN analyzed data and generated figures. AGM, RS, MGR, ZAN, and MAJ wrote the manuscript. * indicates co-first authors.

8. **McCoy, A.G.,** Noel, Z., Sparks, A. H., and Chilvers, M. (2019). 'hagis', an R Package Resource for Pathotype Analysis of *Phytophthora sojae* Populations Causing Stem and Root Rot of Soybean. *Molecular Plant-Microbe Interactions.* 32:1574–1576 Available at: https://doi.org/10.1094/MPMI-07-19-0180-A.

a. **One of MPMI's top 10 most downloaded articles for 2019**

AGM and Zan wrote R code with contributions from AS. AGM wrote the manuscript. AGM, ZAN, AS and MIC edited the manuscript.

9. Chilvers, M.I., **McCoy, A.G.,** Byrne, A.M., Cornett, A.J., Chang, H.-X., Noel, Z.A., Koeman, S. (2019). Effects of fungicides on the management of tar spot of corn in Michigan, 2018. *Plant Disease Management Report*. http://www.plantmanagementnetwork.org/pub/trial/PDMR/volume13/abstracts/CF016.asp

MIC, AMB, AJC, HXC, and SK collected data. ZAN and AGM analyzed data. MIC wrote the manuscript. MIC, AGM, AMB, AJC, HXC, and ZAN edited manuscript.

10. **McCoy, A. G.**, Romberg, M. K., Zaworski, E. R., Robertson, A. E., Phibbs, A., Hudelson, B. D., … Chilvers, M. I. (2018). First Report of Tar Spot on Corn (*Zea mays*) Caused by *Phyllachora maydis* in Florida, Iowa, Michigan, and Wisconsin. *Plant Disease*, PDIS-02-18-0271. Available at: https://doi.org/10.1094/PDIS-02-18-0271-PDN

AGM, ERZ, AER, AP, BDH, DLS, RLB, RNR collected samples. MKR and JMB measured spores. AGM performed DNA analyses. AGM and MIC wrote the manuscript. AGM, ERZ, AER, AP, BDH, DLS, RLB, RNR, MKR and JMB edited manuscript.

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RAK and MCA designed study. RAK, AGM, JDL, SS, and MCA collected samples. KN provided nomenclature expertise and comparative sequences. RAK analyzed data and generated figures. RAK and MCA wrote the manuscript. RAK, DJL, SS, KN, AGM and MCA edited manuscript.

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