Phytophthora capsici in Tennessee: Fungicide resistance, population genetics and cultural control

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ABSTRACT

Phytophthora capsici, a plant pathogenic oomycete, is the causal agent of the vegetable disease Phytophthora blight of pepper and cucurbits. Since the identification of *P. capsici* in 1922, a significant amount of research has been conducted to understand its biology and disease management. Despite this, little research had been conducted on this species in the state of Tennessee. Three studies were done from 2018 to 2020, focusing on fungicide resistance, population genetics, and testing management strategies in the field. In 2018 and 2019 a total of 248 isolates of *P. capsici* were collected from five counties in Tennessee. These isolates were used in an *in vitro* fungicide sensitivity assay to test six fungicides labeled to control Phytophthora blight: mefenoxam, fluopicolide, oxathiapiprolin, mandipropamid, dimethomorph, and cyazofamid. Resistance to mefenoxam, cyazofamid, oxathiapiprolin, and fluopicolide was identified. The 248 isolates and an additional 74 isolates from 2004 and 2007 were genotyped using 39 single nucleotide polymorphism (SNP) markers. Genotypes were used to investigate the population genetics of *P. capsici* in Tennessee. Using various statistics and analyses such as analysis of molecular variance (AMOVA) and discriminant analysis of principal components (DAPC), it was discovered that populations of *P. capsici* are structed by geographic location. Each sampling location had high genotypic diversity, and there were no shared genotypes among sampling locations. Lastly, a field trial was conducted in 2019 and 2020 to test the effectiveness of ground cover combined with fungicides in reducing Phytophthora fruit rot while growing pumpkin. In 2019, the untreated control plots had significant loss of plants at 14 days after transplant compared to the plots treated with fungicides. Additionally, the untreated plots had significantly less fruit at harvest than plots treated with fungicides and ground cover. No other

significant differences among any treatments were observed in either year, most likely due to low disease incidence.

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INTRODUCTION

DISEASE INTRODUCTION

Phytophthora capsici (Leon.), the causal agent of the disease known as Phytophthora blight, has a very large host range of approximately 50 different host species (Tian and Babadoost 2004; Erwin and Ribeiro 1996). Among its many hosts, plants in the families Cucurbitaceae and Solanaceae are some of the most common. From Michigan to Georgia (Jackson et al. 2012) and New York to New Mexico (Dunn et al. 2010, Leonian 1922) there are growers across the country with crops suffering from Phytophthora blight. In Michigan, there are estimated 25% losses of pickling cucumber crops yearly, resulting in a potential loss of $33 million. These economic losses have made the profitable production of cucurbit crops very difficult, forcing many growers out of production entirely. Similarly, this same situation occurred in Illinois, the leading pumpkin producer in the United States (Hausbeck and Lamour 2004; Babadoost 2004). Currently in Tennessee, *P. capsici* is a known problem and many producers throughout the state are reporting losses to Phytophthora blight. Despite this, little is known about it within the state. It is important to conduct more research about *P. capsici* within the state of Tennessee to help provide clear management strategies in order to help reduce the potential for losses found in states like Michigan and Illinois.

Phytophthora capsici History

Our understanding of the disease known today as Phytophthora blight began early in the 20th century in the southwestern United States. New Mexico chili pepper growers began to see a new disease affect their crops. It was originally recognized under the name of "chili wilt," and it was later determined that this disease is caused by an infection of what was later identified as

Phytophthora capsici by L.H. Leonian of the New Mexico College of Agriculture in 1922 (Sanogo and Bosland 2013). As time passed, reports of this disease came from all reaches of the country, including California (Tompkins and Tucker 1937), Colorado (Bodine 1935), Florida (Weber 1932), New York (Wiant, and Tucker 1940), and many of the states in between, including Tennessee (Granke et al. 2012). Even though reports of *P. capsici* began in the early 20th century, it was not seen as a management problem until the 1980's and 1990's (Granke and Hausbeck 2013). Since the 1980's, researchers have spent considerable time and energy on trying to understand this organism better to provide more effective management strategies to farmers affected by Phytophthora blight.

Phytophthora capsici **Biology**

Phytophthora capsici is a alga-like organism within the kingdom Chromista, phylum Heterokontophyta, class Oomycota, order Peronosporales, and family Peronosporaceae (Babadoost 2004). Although taxonomically different from true fungi, the morphology and lifestyle of *P. capsici*, and many other oomycetes, are much like true fungi. Like most fungi, oomycetes are multicellular organisms that form filamentous structures called hyphae. Much like fungi, oomycetes use hyphae to proliferate, colonize substrates and obtain nutrients for development. Like fungi, many oomycetes are pathogens of plants with common symptoms of infection appearing as wilting, lesions of fruit, stems, crowns, roots and foliage, stunting, and plant death. To better control populations of either oomycetes or fungi, and the diseases they cause, it is important to understand key characteristics of their biology. Although there are similarities between fungi and oomycetes, they are very different in a few specific areas. Major differentiating characteristics between oomycetes and fungi are their sexual and asexual

structures, cell wall composition, life cycle and significant phylogenetic differences (Erwin and Ribeiro 1996).

Reproductive Structures

Oospores

Phytophthora capsici, like many other oomycetes, is heterothallic, which means it has two mating types called A1 and A2. When both mating types come together sexual reproduction is possible and starts via hormonal interactions. The two mating initials or gametangia are the oogonium (N) and antheridium (N) and the first step in producing oospores (2N, zygote) is gametangial contact. This is followed by the oogonium initiating penetration of the antheridium. It continues to push through the antheridium until it is surrounded by the antheridium, like a collar around the oogonium. The oogonium swells and becomes spherical, and the walls thicken. A fertilization tube forms from the antheridium and fuses with the oogonium. Meiosis occurs within both initials dividing from a diploid (2n) state to haploid (n). Then a haploid nucleus is exchanged from the antheridium, the oogonium is considered fertilized, and the 2N oospore is formed (Erwin and Ribeiro 1996; Hemmes and Bartnicki-Garcia 1975). The oospore develops further when the walls thicken to 2-6 µm and expand within the oogonium to a diameter of 22-35 µm. The entire structure, including the oogonium, which surrounds the oospore, is the product of sexual reproduction (Babadoost 2005).

Before germinating, oospores must go through a dormant stage and mature. Normally this dormant stage lasts about 30 days but has been observed to take upwards of 6 to 9 months (Hausbeck and Lamour 2004). If conditions are not right for germination, either due to lack of host, or environmental temperature or moisture, the oospores will remain dormant in soil.

Oospores are the survival structures of *P. capsici* and their persistence poses a key challenge to disease management. In one study by Babadoost and Pavon (2013), oospores were buried in soil under field and lab conditions, and rate of recovery and germination were measured at varying time intervals. After 48 months, oospores were still recoverable and able to germinate when conditions were favorable. When oospores are in a warm (22ºC to 33ºC), wet (≥ 60% relative humidity) environment, they will germinate to produce sporangia, which produce asexual zoospores that will search for a host, infect tissue, and continue their life cycle (Babadoost 2005; Hausbeck and Lamour 2004).

Sexual reproduction and oospore formation is one of the attributes of *P. capsici* that has contributed to its destructive nature because it enables the fungus to overwinter and build up inoculum from season to season. This persistence can be catastrophic for a field, as once the pathogen is introduced it will be there next season, and may still be viable for over three years, limiting crop rotation options at that location (Babadoost and Pavon 2013; Hausbeck and Lamour 2004). With sexual reproduction, alleles are recombined, and offspring are unique to their parents as well as each other. This process in *P. capsici* can be a factor in promoting fungicide resistance alleles that lead to increased fitness in a population by allowing genes for resistance to be passed to unique genetic individuals. The individuals with advantageous alleles survive selection and may recombine with other fit individuals, eventually establishing a larger population of resistant *P. capsici.* Oospores make management very difficult, but they also present a target that can be studied in order to improve disease management (Hausbeck and Lamour 2004; Erwin and Ribeiro 1996).

Sporangia and zoospores

Oospore germination is the beginning of the asexual stage of *P. capsici*. The germinated oospore produces a sporangium, which is an ovoid or elliptical sac that sits on top of a long hyphal-like structure called a pedicel. The lengths of sporangia can range from 35 to 138 µm and they are connected to the sporangiophore. The sporangium is papillate and has a tip that resembles the end of a lemon, which gives it a very distinct appearance. Zoospores form within and are eventually released from sporangia. Zoospores are single-celled, biflagellate, motile spores of *P. capsici*. They are clonally produced and are the main propagules of the asexual stage of its life cycle. A mature sporangium may contain anywhere from 20 to 40 zoospores, which are released from the tip of the sporangium. *P. capsici* also reproduces asexually through a different structure called a chlamydospore. A chlamydospore is a thick-walled single cell like an oospore, although they are rare in culture and in the field. The primary pathway for asexual reproduction in *P. capsici* is via zoospores, and the way they are dispersed is critical to their ability to cause disease (Erwin and Ribeiro 1996; Babadoost 2005).

Zoospores are unique compared to most fungal asexual spores because they are flagellated and motile. Zoospores are motile in water and able to move away from their release point and towards a host (Erwin and Ribeiro 1996). They are able to find a host through electrical and chemical signals given off by host roots, and the ability to travel through water allows the zoospores to get to these plants actively rather than by passive dispersal (van West et al. 2002; Granke et al. 2012). When zoospores find a host, they encyst into the tissue, germinate, begin growing hyphae, and eventually produce more sporangia. This continued cycle of asexual reproduction can rapidly increase disease within a field. One infected fruit can contain hundreds

of thousands of sporangia and all of those contain zoospores with the potential to infect more hosts. Another form of dispersal is simply by water moving the sporangia themselves, followed by release of zoospores (Granke et al. 2009). This factor can increase disease pressure very rapidly and severely when conditions are favorable, like during heavy rain or flood events, which should influence what approaches are taken in managing a field that is either infected with, or is still clean of *P. capsici*.

Management

Given the potential for such extreme economic loss when growing hosts of *P. capsici*, it is necessary to have management strategies in place to reduce the impact of this disease. Relying on one method of control, like fungicides, can lead to insufficient management of *P. capsici* because it often adapts to be resistant to treatments. What is required is an integrated approach that incorporates many forms of management to be most effective, profitable, and ecologically safe. A combination of chemical, cultural and biological controls, as well as host resistance can better serve the grower by potentially improving management (Sanogo and Ji 2012; Ristaino and Johnston 1999).

Cultural Control

In developing management strategies, incorporation of cultural controls is a key part of managing *P. capsici*. Cultural controls are defined as management through manipulation of interactions between the plant pathogen, the environment, and the host (Howard 1996). The primary methods of cultural control include crop rotation, environmental manipulation, and exclusion. Crop rotation is a common cultural practice in agriculture, and its usefulness in managing pests and diseases is particularly important. If a particular pathogen in a field is host-

specific, then rotating away from that host can reduce the pathogen population over time, providing an opportunity to reintroduce that host back into a field with lower disease pressure. Crop rotation in combination with tillage has been effective in managing some plant pathogens, including *Rhizoctonia solani*, *Fusarium* spp. *and Verticillium dahlia* (Peters et al. 2003; Xiao et al. 1998). Despite good results with other plant pathogens, crop rotation has been ineffective at reducing Phytophthora blight disease pressure due to the long-lived oospores that *P. capsici* produces (Lamour and Hausbeck 2003). Environmental manipulation is a form of cultural control where the growing environment is changed to reduce favorable disease conditions. Given that *P. capsici* favors moist soil conditions, requires water for zoospore movement, and infects plants through soil contact or splash, reducing standing water, improving drainage, and avoiding soil contact should reduce potential for development and spread of disease. In this case, management can include planting on slopes, avoiding low areas, planting on raised beds and covering with plastic and straw between rows (Ristaino and Johnston 1999; Granke et. al. 2012).

The last major cultural control practice that is commonly used is exclusion of the pathogen. Essentially this is the process of avoiding all possiblities of *P. capsici* entering the growing environment. Common ways in which *P. capsici* can enter the growing environment include through the movement of infested soil, equipment, transplants or fruit, and irrigation water (Granke et al. 2012). Managing irrigation water is especially important for avoiding the introduction of *P. capsici*. In previous studies the presence of *P. capsici* has been found in streams, ponds and other forms of surface water (Gevens et al. 2007; Hausbeck and Lamour 2004). If surface water is infested with *P. capsici* and is used as an irrigation source, disease is inevitable. Ensuring clean water is vitally important to avoid spreading *P. capsici* through

irrigation water. Another potential solution is the treatment of irrigation water with chemicals like chlorine, and or using a UV light treatment that can reduce or eliminate the potential of surviving *P. capsici* spores (Hong et al. 2003; Jones et al. 2014). This is just one strategey in avoiding disease, but sometimes the introduction of *P. capsici* is uncontrollable and the other strategies of chemical and biological control, and host resistance must be utilized (Barchenger et al. 2018).

Chemical Control

Chemical control is another strategy that is widely used in managing *P. capsici*. As with the management of diseases caused by true fungi, fungicides are implemented to help protect against, manage, and reduce populations of oomycetes like *P. capsici.* Fungicides can be applied in varying forms to improve treatment of specific symptoms of disease. Traditionally, products are applied as liquid sprays, either over the entire host or directed at specific plant parts. Common treatments are applied onto seeds, foliage, plant crowns, fruit, and soil (Granke et al. 2012). The target can be adapted to the need of the grower depending on site and severity of disease. Oomycetes require unique classes of fungicides to target their biological differences from true fungi. These are broken up into a few specific chemical classes that have unique molecular make-ups. Some of the major groups of chemicals currently being used to treat *P. capsici* are; phenylamide (PA), quinone inside inhibitors (QiI), carboxylic acid amides (CAAs), benzamides, and oxysterol binding protein homologue inhibition (OSBPI) (Kemble 2018). Each of these groups have their own advantages, disadvantages, modes of action, and risk of resistance. Given that, there are many potential products available for growers giving them options for developing more integrated disease management strategies.

For a long time, the primary chemical used to control *P. capsici* was mefenoxam, which is still widely used today. Mefenoxam is a PA group fungicide that acts on ribosomal RNA and disrupts RNA polymerization (Gisi and Sierotzki 2008). Soon after the introduction of metalaxyl (1977), and later the closely related mefenoxam (1997), cases of resistance were reported (Parra and Ristaino 1998) in states such as Michigan (Lamour and Hausbeck 2000), New York (Dunn et al. 2010), North Carolina (Cafe and Ristaino 2008), South Carolina (Keinath 2007), Georgia (Jackson et al. 2012), Florida (Ploetz and Haynes 2000), and more. Mefenoxam is often included in studies evaluating *P. capsici* fungicide sensitivity due to the prevalence of resistance. In many of these studies, there are varied percentages of resistant isolates, ranging from 7% up to approximately 60% (Keinath 2007; Dunn et al. 2010). These differences may be related to the varied selection pressures among these fields, which may be different for each population explaining the range in resistance. Consequentially, widespread resistance to mefenoxam presents a major problem in management. This has led to the use of newer products to be with mefenoxam to allow for rotation of fungicide active ingredients. This list includes, but is not limited to, the following active ingredients: cyazofamid (QiI); dimethomorph (CAA); fluopicolide (benzamide); mandipropamid (CAA); and oxathiapiprolin (OSBPI). The efficacy of these newer products has been tested in many studies through the last decade. All of these products reduce growth of *P. capsici in vitro* as well as in field studies, and can be recommended for use in place of, or alongside, mefenoxam in a management plan. (Gisi and Sierotzki 2008; Jackson et al. 2012; Jackson et al. 2010; Ji and Csinos 2015). However, the use of fungicides alone in managing *P. capsici* is likely to be unsuccessful without the integration of other strategies such as cultural practices, host resistance, and biological control.

Biological Control

Biological control is defined as a method of management that uses other organisms to antagonize, alter, or destroy a population of insect pests, weeds, or plant pathogens (Emmert and Handelsman 1999). Biological controls can be used as a tool to help reduce pathogen populations to limit disease. In the case of *P. capsici,* there are only a few biological controls that have been studied, including studies on biofumigation and the effect of antagonistic microbes (Wang et al. 2014). Biofumigation is a control method that uses antimicrobial chemicals that occur naturally in certain plants to reduce pathogen populations. Specifically, it has been seen that biofumigation using *Brassica* spp. can reduce the growth of fungi, increase the diversity of soil bacteria, and alter soil chemistry to be less favorable for fungal growth (Fan et al. 2008; Wang et al. 2014). In a study by Wang et al., a combination of biofumigation and antagonistic bacteria were used as a treatment in *P. capsici*-infected soil to see if rate of disease was reduced. In their results they saw a reduction in occurrence of disease compared to control treatments, suggesting that biofumigation can improve conditions for antagonists that suppress *P. capsici*, reducing disease (Wang et al. 2014). The area of biocontrol needs further study in general, but if more discoveries are made it could be a potential tool to improve management of *P. capsici*.

Host Resistance

Host resistance is considered to be one of the best disease management practices because it is simple to use, less impactful to the environment, and often can be more cost effective than relying of chemical control (Granke et al. 2012). Breeders have many challenges to face when developing new cultivars that may have resistance to *P. capsici*. The combination of pathogen traits like large host range, a large number of races, prevalent genetic recombination, random

mutations, and loss of heterozygosity make it very difficult for progress to be made in this field (Barchenger et al. 2018). There has been progress in breeding for Phytophthora blight resistance in pepper, with varieties now available on the market. In combination with chemical controls, these varieties such as 'Paladin' can be affective in lowering crop losses due to *P. capsici* (Foster and Hausbeck 2010a). Although, the potential resistance of pepper to *P. capsici* will depend of the virulence of each field's population, meaning that choice of variety may need to be specific for each field (Foster and Hausbeck 2010b). Breeding resistance into cucurbit crops has been more challenging, but there are some varieties with partial resistance, specifically within the squash and pumpkin species *Cucurbita pepo*, that have been seen to help reduce crown and root rot (Krasnow et al. 2017). As more resistant varieties of *Solanaceae* and *Cucurbitaceae* become available host resistance will become a more effective tool to manage *P. capsici*.

Population Biology in *Phytophthora capsici*

Population biology, as it relates to plant pathogens, is the study of epidemiology, genetics, ecology, and evolutionary characteristics of populations of plant pathogens. Understanding a population at its genetic level is especially important within the context of plant disease management because it can identify trends and differences among genes or alleles within a population that could be used in disease management. Population biology studies can reveal trends related to pathogen dispersal, host preference, and fungicide sensitivity. Population genetics can also be helpful in identifying differences among populations separated by geography to get a broader understanding of genetic variation over a geographical region, especially when considering how a pathogen disperses throughout a region (Milgroom 2015). Studying population genetics of *P. capsici* is important to managing populations because it will

provide a picture of the variation among isolates within a field, and from field to field within a region. Understanding the genetic variation among populations of *P. capsici* could shed light on evolutionary changes that happen over the region of study, and within each field, which could improve disease management by showing how *P. capsici* might have been dispersed through a region and whether separate populations are genetically similar.

A study of *P. capsici* investigating population structure in New York State revealed that genetic variation between populations in separate geographic locations was high (Dunn et al. 2010). They concluded that gene flow between populations was cut off, confirming that *P. capsici* within New York was not one large population but rather many highly differentiated groups. These observations were consistent with what is known about how *P. capsici* spreads, since it is mainly dispersed through surface water, soil, and infected plants, and it takes events such as floods or human transport to move large distances. This shows that management for every field must be looked at independently because the genetics of one population may be very different than another, making broad-spectrum management difficult (Dunn et al. 2010). For this reason, studying population genetics is especially important in areas with high reports of disease, as it could inform approaches to management. Populations of *P. capsici* in Tennessee have not been evaluated and are good candidates for genetic study.

Purpose of study/ Research gap

Phytophthora capsici has been a well-known pathogen since its description by Leonian in 1922, with a myriad of primary research articles about all aspects of its biology from many regions in the U.S. and the world (Hausbeck and Lamour 2004; Granke et al. 2012; Barchenger et al. 2018). Despite this, primary research of *P. capsici* within the state of Tennessee is limited

to one paper from Donahoo and Lamour (2008) which looked to test whether interspecific hybridization could be achieved between *P. capsici* and *Phytophthora tropicalis*. In this study, the main report of *P. capsici* in Tennessee identified that there was disease found in pepper and cucurbit crops throughout the eastern part of the state. This study also reported that Tennessee populations were comprised of similar amounts of A1 and A2 mating types. This is a similar finding to other states' populations and indicates that *P. capsici* has been well established through oospore production (Donahoo and Lamour 2008). This leaves a large gap in the understanding of Tennessee populations of *P. capsici* and means that management recommendations are based on information gained through studies conducted on *P. capsici* in other states. Given the potential lack of gene flow between Tennessee populations and those from surrounding states, it cannot be assumed that they share genotypic or phenotypic characteristics. The purpose of this study is to investigate populations of *P. capsici* throughout the state of Tennessee. The intention is to investigate three specific questions; what are the fungicide sensitivity levels of *P. capsici* populations in Tennessee in relation to the major products used by Tennessee vegetable producers to control Phytophthora blight, what is the genetic structure of these populations and how may new cultural controls like ground cover reduce fruit rot caused by Phytophthora blight. These questions will help advise future management of *P. capsici* in Tennessee by showing which products are effective and whether each population is genetically distinct or like each other, which could shed light on how the pathogen spreads throughout the state.

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CHAPTER 1

Sensitivity of *Phytophthora capsici* from Tennessee to mefenoxam, fluopicolide, oxathiapiprolin, dimethomorph, mandipropamid, and cyazofamid

A version of this chapter was originally submitted for publication by Timothy B. Siegenthaler and Zachariah R. Hansen

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Abstract

Phytophthora blight is a destructive disease caused by the oomycete *Phytophthora capsici*, which affects vegetable production throughout the state of Tennessee and worldwide. Fungicides are a primary control method used in managing Phytophthora blight, but in some cases efficacy of these products has been reduced or lost in the field. In 2018 and 2019, efficacy of six fungicides was tested *in vitro* on 184 *P. capsici* isolates collected in Tennessee using radial growth assays. The fungicides included in the study were mefenoxam, fluopicolide, oxathiapiprolin, dimethomorph, mandipropamid, and cyazofamid. Seven isolates were resistant to mefenoxam, 86 were resistant to fluopicolide, one was resistant to oxathiapiprolin, and 13 were resistant to cyazofamid. None were resistant to dimethomorph or mandipropamid. Of the 86 isolates resistant to fluopicolide, five were also resistant to mefenoxam. Resistance to fluopicolide and cyazofamid was widespread in Tennessee, while it was more localized for mefenoxam and oxathiapiprolin. Based on the results of this study, fungicide resistance is

widespread in *P. capsici* in Tennessee, and implications for Phytophthora blight management are discussed.

Introduction

Phytophthora blight of cucurbits and peppers, caused by the oomycete *P. capsici*, is a destructive disease of a large number of vegetable crops and causes significant yield losses worldwide (Granke et al. 2012; Hu et al. 2013; Gobena et al. 2012). Phytophthora blight damages all parts of the plant and causes a variety of symptoms including crown rot, root rot, foliar spots, damping off, wilt, and fruit rot. These symptoms can kill plants or render fruit unmarketable, leading to large yield losses in fields with high disease incidence. *Phytophthora capsici* is well adapted to wet and warm conditions, can spread very quickly, and persist within a field for at least five years without a susceptible host (Granke et al. 2012). It accomplishes this through two modes of reproduction: the asexual stage where zoospores are produced, and the sexual stage where oospores are produced. Zoospores are single-celled biflagellate spores that spread through water and can quickly move throughout a field in rain events. Oospores are thick-walled overwintering structures that are the result of sexual reproduction. Oospores can survive extreme conditions, contribute to disease outbreaks in fields year after year, and lead to populations with high genetic diversity with the potential for rapid adaptation (Erwin and Ribeiro 1996).

These traits have made Phytophthora blight historically very difficult to manage. Proper management of this disease starts with avoidance by limiting entry of *P. capsici* into a field. Ensuring a pathogen-free water supply, washing equipment and boots between fields, and avoiding any transfer of infected soil or fruit into a clean field are important management

practices (Hausbeck and Lamour 2004; Barchenger et al. 2018). Unfortunately, once *P. capsici* has been introduced into a field, it is very difficult to manage and common cultural controls such as crop rotation do not ensure reduction of inoculum over time. Currently there is very little genetic resistance in commercially available pepper, and none for cucurbits. Chemical fungicides are the most effective and commonly used management tool for Phytophthora blight, but fungicide resistance has been reported for two commonly used products. Isolates resistant to mefenoxam and cyazofamid have been reported throughout the USA (Hausbeck and Lamour 2004; Kousik and Keinath 2008). This highlights the importance of rotating fungicide modes of action for fungicide resistance management. By avoiding the overuse of one chemical, growers can limit selecting for resistance in populations of *P. capsici*. Even with proper fungicide rotations there is still a possibility of resistance developing in the field. Ongoing monitoring and screening are needed to detect novel resistance to currently available products. It is important to screen samples collected throughout various growing regions because *P. capsici* populations can vary greatly from location to location (Granke et al. 2012; Dunn et al. 2010; Castro-Rocha et al. 2017; Parra and Ristaino 2001).

There have been many fungicides developed over the years to control oomycetes, but some of the most important have been the phenylamines: metalaxyl and metalaxyl-M (mefenoxam). Metalaxyl was first brought to market in 1977 and was commonly used to treat many oomycete pathogens (FRAC, 2019). This chemical maintained effective control for many years, but in 1991 resistance to metalaxyl was reported in *Phytophthora infestans* (Parra and Ristaino 2001; Deahl et.al. 1993). Despite this, metalaxyl was used for many more years until 1996 when it was replaced with metalaxyl-M, commonly known as mefenoxam. Mefenoxam

(FRAC 4) is the active enantiomer of metalaxyl and has high intrinsic activity and could be used at lower doses (Parra and Ristaino 2001). Like metalaxyl, resistance to mefenoxam was soon observed in many oomycete pathogens including *P. capsici* (Parra and Ristaino 2001). Mefenoxam is still commonly used to manage *P. capsici*, but since its release more fungicides have been developed with varying levels of efficacy and resistance risk. This list includes dimethomorph, mandipropamid, fluopicolide, cyazofamid, and oxathiapiprolin. The carboxylic acid amides dimethomorph (FRAC 40) and mandipropamid (FRAC 40) inhibit cellulose synthase. Resistance has been observed in the grape downy mildew pathogen *Plasmopara viticola*. However, these fungicides are considered to have low to medium resistance risk (FRAC, 2019: Gisi et al. 2007). The benzamide fluopicolide (FRAC 43), which delocalizes spectrin-like proteins in the cytoskeleton, is considered to have medium resistance risk (FRAC, 2019). The quinone inside inhibitor (QiI) cyazofamid (FRAC 21), which affects cytochrome bc1 at Qi sites during respiration, has known resistance in *P. capsici* (Jackson et al. 2012). The oxysterol binding protein homologue inhibitor (OSBPI) oxathiapiprolin (FRAC 49), which affects lipid transport and storage, has no known resistance in the field but is considered to have medium to high resistance risk. Given the documented resistance to some of these fungicides, and the potential for resistance development with others, it is important to screen populations of *P. capsici* for sensitivity to each of these fungicides.

There has been no work done on monitoring fungicide resistance of *P. capsici* in Tennessee. However, Phytophthora blight is known to be a serious problem for vegetable growers throughout the state and is routinely managed with various combinations of fungicides (personal observation). Despite efforts to follow current management recommendations such as

raised beds, plasticulture, crop rotation, and routine fungicide regimens, disease continually occurs and stands as a major threat to producers in the state. The goal of this study was to address this issue to improve management recommendations through two main objectives: (i) obtain a collection of *P. capsici* isolates from throughout the state of Tennessee; and (ii) determine the sensitivity of *P. capsici* isolates collected in Tennessee to mefenoxam, dimethomorph, mandipropamid, fluopicolide, cyazofamid and oxathiapiprolin. Ultimately, determining the levels of fungicide sensitivity and resistance in *P. capsici* populations in Tennessee will provide valuable data to inform vegetable disease management recommendations.

Materials and methods

P. capsici isolation

During the 2018 and 2019 growing seasons, samples of symptomatic crowns, stems, and fruit were taken from pepper, pumpkin, zucchini, yellow squash, acorn squash or butternut squash grown at five separate locations throughout middle and east Tennessee. Smaller pieces of each sample, approximately 30 x 30 mm, were taken from the margin of diseased and healthy tissue, surface sterilized using 0.5% sodium hypochlorite solution and rinsed in two baths of sterile deionized water (SDW) to remove excess sodium hypochlorite. Each surface-sterilized piece was then plated on PARP-H, a semi-selective media for *Phytophthora* (17 g cornmeal agar, 0.4 ml pimaricin solution (25 mg/ml), 250 mg ampicillin, 1 ml rifampicin solution (10 mg/ml), 5 ml PCNB solution (5 mg/ml), and 2 mg hymexazol per 1 L) (Ferguson and Jeffers 1999). Plated samples were incubated in the dark at room temperature for one to two days, or until visible

hyphal growth was observed. Hyphal tip transfers were taken from each sample and re-plated onto PARP-H to ensure clean colonies. Isolated colonies were labeled and stored on the bench at ~24° C until single zoospore isolation.

Single zoospore isolation and storage

In 2018, Colonies of suspected *P. capsici* were removed from storage and transferred to 5% unclarified V8 (UCV8) medium (50 ml V8 juice, 1 g $CaCO_3$, 32 g agar, and 950 ml DI water per 1 L) and stored in the dark for five to seven days (Jackson et. al. 2012). Isolates were then stored under continuous florescent light for two to three days to induce sporulation. Small squares, approximately 2-4 mm of sporulating media, were cut and placed into 1.5-ml microcentrifuge tubes filled with 1 ml SDW. The tubes were incubated at room temperature for 30 minutes, then vortexed to fully dislodge sporangia. Sporangia solution (100 µl) was pipetted and spread with a sterile glass spreader onto water agar (20 g agar per 1 L). Plates were stored at 25°C for 12 hours on the bench top with natural light. Using a compound microscope, individual germinated zoospores were identified, marked, and transferred to plates of P_5ARP (950 ml DI water, 50 ml of V8 juice, 1 g of $CaCO_3$, 5 mg pimaricin, 250 mg ampicillin, 10 mg rifampicin, and 100 mg of PCNB) (Ferguson and Jeffers 1999). After one to two days, single zoospore isolates were transferred to 10% UCV8 media (100 ml V8 juice, 1 g $CaCO_3$, 32 g agar, and 900 ml DI water per 1 L) for short-term storage. In 2019, isolates were obtained by hyphal tipping from initial culture. For long-term storage, 5-mm plugs of isolates were placed into sterile cryovials filled with SDW and approximately three sterile hemp seeds. Prior to storing, all colonies were ensured to be axenic by placing plugs of isolates into LB broth, incubating for 24 hours at 25°C with natural lighting, and then checking for turbidity indicating bacterial

contamination. In total, 87 isolates were collected in 2018 and 161 in 2019. All 2018 isolates and a subset of 97 isolates from 2019 were included in subsequent fungicide sensitivity experiments.

Mating Type

In square 90-mm plates, 5% clarified V8 medium (CV8) (50 ml clarified V8 juice, 1 g $CaCO_3$, 32 g agar, and 950 ml DI water per 1 L) was poured and allowed to cool, and then sliced into 30 x 30 mm squares using a sterile scalpel. From one plate of cut CV8, five squares of media were removed leaving only the corner pieces in the original plate. Four of the removed pieces were placed into the corners of another sterile 90-mm dish. This was repeated until enough plates were acquired to test all isolates. Each 90-mm plate was used to determine the mating type of two individual isolates, using two CV8 squares per isolate. On one square, a known A1 (isolate LT51) mating-type isolate was placed with a 5-mm plug of an unknown isolate. This was repeated on the second square with a known A2 (isolate LT263) mating-type isolate and a 5-mm plug of the same unknown isolate. Plates were incubated at 25° C in the dark for 5 to 7 days and then viewed under a compound light microscope. The mating type of an unknown isolate was determined by observing the presence of thick-walled oospores. When an isolate with an unknown mating type sexually reproduced and created oospores with a known A1, the unknown was considered an A2, or vice versa. This was done for all isolates collected in 2018 and 2019.

Isolate identification through PCR and sequencing

Isolates were stored on 10% UCV8 medium for 5-7 days. The Phire Plant Direct PCR Kit (Thermo Fisher, Waltham, MA) was used to conduct direct-colony PCR on each isolate to

amplify a portion of the internal transcribed spacer (ITS) region of ribosomal DNA using primers ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS-5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White et al. 1990). Each 20 µl reaction was comprised of 10 µl of 2X Phire plant PCR buffer, 1 µl each of 10 mM forward and reverse primers, 0.4 µl of Phire hot start II DNA polymerase, 7.6 µl of SDW, and a small amount of isolate tissue (enough to be barely observable on a pipette tip with the naked eye). Reactions were conducted using a Bio-Rad T100 thermal cycler (Bio-Rad, Hercules, CA) using the following program: 5 min at 98°C, then 40 cycles of 98°C for 5 s, 52°C for 5 s, and 72°C for 20 s, and a final phase of 72°C for 1 min. All PCR reactions included a negative control comprised of master mix only with no tissue added. Amplified products were expected to be approximately 800 bp to 850 bp and were visualized on a 1% agarose gel stained with ethidium bromide or Gel Red. All gels were run with negative controls (PCR reactions with no tissue added) and known positives of *P. capsici* ITS products. All PCR products were observed to be the same size, therefore the PCR products of a subset of approximately 30% of randomly selected isolates were sequenced to confirm their identity. PCR products were sequenced using Sanger Capillary Sequencing at the University of Tennessee Genomics Core. The sequences were then entered in the NCBI Basic Local Alignment Search Tool (BLAST) and samples were considered *P. capsici* when search queries matched with >95% coverage and identity.

Chemicals Tested

Technical-grade fungicides were used for *in vitro* fungicide sensitivity testing whenever possible, and formulated products were used when technical grade products were unavailable. Dimethomorph, fluopicolide and mandipropamid (technical grade) were purchased form Sigma-

Aldrich (St. Louis, MO). Ridomil Gold [45.3% active ingredient (a.i.) mefenoxam] and Orondis SC (18.7% a.i. oxathiapiprolin) were obtained as formulated products from Syngenta Crop Protection (Basel, Switzerland). Ranman (34.5% a.i. cyazofamid) was obtained as a formulated product from FMC Corporation (Philadelphia, PA).

Sensitivity of P. capsici to mefenoxam

To test the sensitivity of isolates collected in 2018 to mefenoxam, an *in vitro* radial growth assay was performed. Five-mm-diameter plugs of 1- to 2-week-old cultures were cut using a sterile cork borer. Plugs were then transferred to the centers of 100-mm Petri dishes containing 5% UCV8 amended with mefenoxam in the form of Ridomil Gold SL diluted in SDW to final concentrations of 0, 10, or 100 µg a.i./ml. In 2019, the 10 µg/ml dose was omitted and only the 100 µg/ml rate was tested as a discriminatory dose, which discriminated between sensitive and resistant isolates collected in 2018. All plates were incubated at room temperature for 3 to 4 days, or until the control plates containing no mefenoxam had grown to a diameter of 50 mm. Two perpendicular diameter measurements were recorded per plate, averaged, and the diameter of the plug was subtracted. The percent growth((total growth of treated plate/ total growth of control) x 100 = percent growth.)) of colonies treated with mefenoxam compared to the unamended controls was calculated and used to classify isolates into three categories of mefenoxam sensitivity. Two technical repetitions were included per isolate, and the experiment was conducted twice.

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Sensitivity of 2018 P. capsici isolates to dimethomorph, fluopicolide, mandipropamid, cyazofamid, and oxathiapiprolin using dilution series

All *P. capsici* isolates collected in 2018, were plated onto 5% UCV8 medium and incubated for 1 to 2 weeks at 25°C in the dark before being cut into 5-mm plugs and transferred onto the center of a 100-mm plate of 5% UCV8 amended with differing concentrations of each fungicide (Figure 1.1; All tables and figures are found in the appendix). The following final concentrations, represented as µg a.i./ml, were used for each compound: 0, 0.1, 0.25, 0.5, and 1 µg/ml dimethomorph; 0, 0.1, 0.25, 0.5, and 5 µg/ml fluopicolide; 0, 0.01, 0.025, 0.075, and 0.1 µg/ml mandipropamid; 0, 100, 500 and 1000 µg/ml cyazofamid; 0, 0.00025, 0.0005, 0.00075, and 0.001 µg/ml oxathiapiprolin (Table 1). These concentrations were developed using results from previous studies (Jackson et al. 2010; Jackson et al. 2012; Ji and Csinos 2015). Dimethomorph, fluopicolide, and mandipropamid were technical grade compounds dissolved and diluted in acetone. Cyazofamid and oxathiapiprolin were acquired as the formulated products Ranman and Orondis SC, respectively, and were diluted with SDW. All plates were incubated at room temperature for three to four days, or until the isolates on unamended control plates had grown to a 50-mm diameter. Two perpendicular diameter measurements were recorded per plate, averaged, and the diameter of the plug was subtracted. Two technical repetitions were included per isolate, and the experiment was conducted twice.

Sensitivity of 2019 P. capsici isolates to dimethomorph, fluopicolide, mandipropamid, cyazofamid, and oxathiapiprolin using discriminatory doses

All *P. capsici* isolates, collected in 2019, were plated onto 5% UCV8 medium and incubated for 1 to 2 weeks at 25°C in the dark before being cut into 5-mm plugs and transferred

onto the center of a 100-mm plate of 5% UCV8 medium amended with each fungicide. In 2019, discriminatory doses (DD) were developed and used instead of the previous series of concentrations. Discriminatory doses were based on the average EC_{75} (concentration necessary to reduce radial growth by 75%) for each chemical based on data collected in 2018, excluding cyazofamid (Lehner et al. 2015). For cyazofamid, the data collected in 2018 could not provide accurate calculations for EC_{75} values due to the prevalence of moderately sensitive and resistant phenotypes, so EC_{50} values were used. The discriminatory doses for each compound, represented as µg a.i./ml, were: 0.6 µg/ml dimethomorph, 0.35 µg/ml fluopicolide, 0.04 µg/ml mandipropamid, 500 µg/ml cyazofamid, and 0.0007 µg/ml oxathiapiprolin. All compounds were diluted with the same solvents as in 2018. All plates were incubated at room temperature for 3 to 4 days, or until the isolates on unamended control plates had grown to a 50-mm diameter. Two perpendicular diameter measurements were recorded per plate, averaged, and the diameter of the plug was subtracted. Two technical repetitions were included per isolate, and the entire experiment was done twice. In 2019, all sensitivity data were collected as percent growth on media amended at the DD relative to non-amended controls.

EC_{50} calculations

Mycelial growth data for dimethomorph, fluopicolide, mandipropamid, cyazofamid, and oxathiapiprolin were processed into percent growth of amended colonies relative to unamended controls. Effective concentration to reduce mycelial growth by 50% (EC_{50}) for each isolate-by-fungicide combination was calculated in Excel (Microsoft Co, Redmond, WA) by solving linear regression equations of the probit-transformed percent growth data versus the base-10 logarithm of each fungicide concentration. For every fungicide, the first and second experiment were

analyzed separately and subsequently averaged together to get one EC_{50} for each isolate-by-fungicide combination.

Qualifications for sensitive, moderately sensitive, and resistant

Thresholds for mefenoxam were based on slightly modified methods from Dunn et. al. (2010) and Jackson et. al. (2012). Isolates were considered sensitive if percent growth on 10 µg/ml was less than 40% growth of the unamended control; moderately sensitive if percent growth on 10 µg/ml was greater than 40% but on 100 µg/ml was less than 40%; and resistant if percent growth was greater than 40% on 100 µg/ml. For the remaining fungicides, thresholds were developed for EC_{50} values and relative percent growth when exposed to a discriminatory dose to categorize each isolate as sensitive, moderately sensitive, or resistant. For EC_{50} values, resistant isolates were defined as having an average EC_{50} that was higher than the highest concentration tested. Moderately sensitive isolates were defined as having an average EC_{50} between the highest and second highest concentration tested. Sensitive isolates were defined as having an average EC_{50} below the second highest concentration tested. For relative percent growth at each discriminatory dose, resistant isolates were defined as having an average value above 75% of the unamended-control, moderately sensitive between 50% and 75% of the unamended-control, and sensitive less than or equal to 50% of the unamended-control.

Results

Isolate collection

In 2018 and 2019, isolates of *P. capsici* were collected from various vegetable farms located across Tennessee. Counties sampled in 2018 and 2019 were Rhea, Putnam, and Bledsoe;

Lincoln County was sampled in 2019 only. The numbers of isolates per county in 2018 were 24 from Rhea, 43 from Putnam, and 20 from Bledsoe, for a total of 87 (Table S1). The number of isolates per county in 2019 were 87 from Rhea, 40 from Lincoln, 32 from Bledsoe, and two from Putnam for a total of 161 (Table S1). The number of isolates per host in 2018 were 14 from yellow squash, 10 from pepper, and 63 from pumpkin (Table S1). In 2019, the number of isolates per host were 54 from yellow squash, 5 from pepper, 20 from pumpkin, 41 from cucumber, 19 from zucchini, 20 from acorn squash, and 2 from butternut squash.

Morphological and molecular identification

For both collection years, all isolates were confirmed through morphological identification using a mating-type test, and a subset of isolate identities were confirmed molecularly. Isolates that formed thick-walled oospores when grown in the presence of the opposite mating type and were confirmed as *P. capsici*. Isolates that failed to produce oospores were excluded from further analysis. In 2018, 35 isolates were mating type A1 and 52 isolates were mating type A2. In 2019, the mating type ratio was 1:1 with 74 isolates of each mating type. Additionally, a subset of approximately 20% of isolates were identified through PCR and sequencing of a portion of the internal transcribed spacer (ITS) region of ribosomal DNA using primers ITS4 and ITS5 (White et. al. 1990). The isolates produced PCR products with a size of approximately 820 bp, confirmed by gel electrophoresis, and resulted in 95% query cover and 99.9% identity match with numerous *P. capsici* isolates based on NCBI BLAST.

Sensitivity to mefenoxam

In 2018, 87 isolates were tested at 10 µg/ml and 100 µg/ml (Table 1.1). Five isolates were resistant to mefenoxam, defined as relative growth above 40%, and grew an average of 74% of the unamended control at 100 µg/ml. The remaining 82 isolates were sensitive, defined as growing less than 40% relative to the unamended control, with an average of 7% ± 5% relative growth and a range of 0% to 25% (Figure 1.2). The average relative percent growth of all isolates was 11% ± 17%, with a range of 0% to 85% (Table 1.1, Table 1.S3). The resistant isolates were collected from one location in Rhea County and one location in Bledsoe County. In 2019, of the 97 isolates tested, two were resistant at the discriminatory dose of 100 µg/ml, defined as relative growth above 40%, and grew an average of 71% relative to the unamended control (Figure 1.3). The remaining 95 isolates were sensitive with an average relative percent growth of 11% ± 6% and a range of 0% to 32%. The average relative percent growth of all isolates was 12% ± 9%, with a range of 0% to 82% (Table 1.2, Table 1.S4). The resistant isolates were collected from one location in Rhea County.

Sensitivity to fluopicolide

Of the 87 isolates from 2018, 37 were resistant to fluopicolide, defined as having EC_{50} values above the highest concentration tested (Figure 1.4). Fifty isolates were sensitive to fluopicolide, with an average EC_{50} of 0.24 ± 0.06 µg/ml and a range of 0.11 µg/ml to 0.39 µg/ml. The average EC_{50} of all isolates was >5 µg/ml with a range of 0.11 µg/ml to >5 µg/ml (Table 1.1, Table 1.S3). The 37 resistant isolates were collected from each location in all three counties surveyed in 2018 (Table 1.S1). In 2019, 49 of 97 isolates were resistant when tested at the discriminatory dose of 0.35 µg/ml (Figure 1.5, Table 1.S4), defined as relative growth above

75%. Forty-eight isolates were sensitive, defined as relative growth less than or equal to 50%. The average relative percent growth of sensitive isolates was 29% ± 7%, with a range of 12% to 39%. The average relative percent growth of all isolates was 64% ± 36%, with a range of 12% to 96% (Table 1.2, Table 1.S4). All resistant isolates in 2019 were collected from one location in Rhea County (Table 1.S2).

Sensitivity to oxathiapiprolin

Of the 87 isolates from 2018, one isolate was moderately sensitive to oxathiapiprolin, defined as having an EC_{50} value between 0.00075 µg/ml and 0.001 µg/ml, with an EC_{50} of 0.00081 µg/ml. Eighty-six were sensitive, defined as having an EC_{50} value below 0.00075 µg/ml. The average EC_{50} of sensitive isolates was 0.00038 ± 0.00012 µg/ml with a range of 0.00017 µg/ml to 0.00074 µg/ml. The average EC_{50} of all isolates was 0.00038 ± 0.00013 µg/ml with a range of 0.00017 µg/ml to 0.00081 µg/ml (Table 1.1, Table 1.S3). One moderately sensitive isolate was collected from Putnam County in 2018 (Table 1.S1). In 2019, one of the 97 isolates was resistant at the discriminatory dose of 0.0007 µg/ml, defined as relative growth above 75%, and grew at 80% relative to the control (Figure 1.6). Fourteen isolates were moderately sensitive, defined as relative growth from 51% to 75%. These isolates grew an average of 55% ± 4% relative to the control with a range of 51% to 63%. Eighty-two isolates were sensitive, defined as relative growth less than or equal to 50%, and grew an average of 31% ± 4% relative to the control, with a range of 7% to 50%. The average relative percent growth of all isolates was 35% ± 15%, with a range of 7% to 80% (Table 1.2, Table 1.S4). All resistant and moderately sensitive isolates were collected from one location in Rhea County in 2019 (Table 1.S2).

Sensitivity to dimethomorph

Of the 87 isolates from 2018, four isolates were moderately sensitive to dimethomorph, defined as having an EC_{50} value between 0.5 µg/ml and 1 µg/ml, and an average EC_{50} of 0.55 ± 0.03 µg/ml, with a range of 0.51 µg/ml to 0.59 µg/ml. Eighty-six were sensitive, defined as having an EC_{50} value below 0.5 µg/ml, with an average EC_{50} of 0.40 ± 0.02 µg/ml and a range of 0.20 µg/ml to 0.50 µg/ml. The average EC_{50} of all isolates was 0.41 ± 0.06 µg/ml with a range of 0.2 µg/ml to 0.59 µg/ml (Table 1.1, Table 1.S3). Moderately sensitive isolates were collected from one location in Rhea County and one location in Putnam County (Table 1.S1). In 2019, all 97 isolates were sensitive, defined as relative growth less than or equal to 50% when tested at the discriminatory dose of 0.6 µg/ml. The average growth was 10% ± 11% of the non-amended control with a range of 0% to 50% (Table 1.2, Table 1.S4).

Sensitivity to mandipropamid

Of the 87 isolates from 2018, all were sensitive to mandipropamid at all concentrations tested. The average EC_{50} was 0.025 ± 0.0007 µg/ml with a range of 0.01 µg/ml to 0.065 µg/ml (Table 1.1, Table 1.S3). In 2019, all 97 isolates were sensitive when tested at the discriminatory dose of 0.04 µg/ml. The average growth was 17% ± 9% of the non-amended control with a range of 4% to 50% (Table 1.2, Table 1.S4).

Sensitivity to cyazofamid

Of the 87 isolates from 2018, 13 were resistant to cyazofamid, defined as having EC_{50} values above the highest concentration tested, therefore the EC_{50} could not be calculated (Figure 1.7). Twenty-nine isolates were moderately sensitive, defined as having an EC_{50} value between

500 µg/ml and 1000 µg/ml, and an average EC_{50} of 719 ± 154 µg/ml, with a range of 520 µg/ml to 978 µg/ml. Forty-five isolates were sensitive, defined as having an EC_{50} value below 500 µg/ml, with an average EC_{50} of 289 ± 115 µg/ml and a range of 35 µg/ml to 490 µg/ml. The average EC_{50} of all isolates for which an EC50a could be calculated was 596 ± 425 µg/ml with a range of 35 µg/ml to >1000 µg/ml (Table 1.1, Table 1.S3). Resistant and moderately sensitive isolates were collected from each location in 2018 (Table 1.S1). In 2019, 14 of the 97 isolates tested were moderately sensitive at the discriminatory dose of 500 µg/ml, defined as relative growth from 51% to 75%, with average growth of 54% ± 4% relative to the control and a range of 51% to 63% (Figure 1.8). Eighty-three isolates were sensitive, defined as relative growth less than or equal to 50%, and grew an average of 39% ± 8% relative to the control with a range of 13% to 50%. The average relative percent growth of all isolates was 41% ± 9%, with a range of 13% to 63% (Table 1.2, Table 1.S4). Moderately sensitive isolates were collected from one location in Rhea County and one location in Lincoln County (Table S2).

Discussion

In this study, resistance was found to mefenoxam, fluopicolide, cyazofamid and oxathiapiprolin, while all isolates remained sensitive to mandipropamid. Nearly all isolates were sensitive to dimethomorph also, with only two isolates showing moderate sensitivity. Resistance to mefenoxam has been reported in other states, such as Michigan, New York, and Georgia, but this is the first report of resistant to mefenoxam in Tennessee (Lamour and Hausbeck 2000; Dunn et al. 2010; Jackson 2012). Mefenoxam-resistant isolates were not widespread across sampling locations in this study but were found at two farms near each other in Rhea and Bledsoe counties. These locations are approximately 11 kilometers apart. One of these locations

is a large commercial vegetable farm with a history of high Phytophthora blight disease pressure. The farm implemented an aggressive fungicide-based disease management program that included Ridomil (mefenoxam), Orondis (oxathiapiprolin), Presidio (fluopicolide), and Ranman (cyazofamid). This farm also irrigated with untreated surface water that could promote the distribution of pathogen inoculum. Repeated plantings of susceptible crops year after year, including rotations of cucurbit and solanaceous crops, also probably enhanced disease pressure. These factors likely created favorable conditions for selection of resistant isolates. Only a small proportion of isolates were resistant to mefenoxam, which means that mefenoxam is still a viable option for managing Phytophthora blight, although it should be used in rotation with other fungicides and/or in mixtures. Resistance to mefenoxam should continue to be monitored in the future.

This study is among the first to report fluopicolide-resistant *P. capsici* isolates in the United States. In previous studies, fluopicolide has been observed to be effective in controlling mycelial growth of *P. capsici* (Jackson et al. 2010, 2012; Matheron and Porchas 2015). However, there have recently been two preliminary reports of resistance to fluopicolide in *P. capsici in vitro* (Siegenthaler and Hansen 2019; Wang and Ji 2020). In our study, 46% of isolates tested from Tennessee were resistant to fluopicolide. These isolates were collected from three separate counties: Rhea, Bledsoe, and Putnam. Rhea and Bledsoe are neighboring counties, but Putnam is geographically separated by approximately 120 kilometers. This suggests that fluopicolide resistance is geographically widespread in Tennessee and is potentially very common. With this is mind, fluopicolide should be limited in its use to control *P. capsici* in Tennessee. Currently in the southeastern US, it is recommended that fluopicolide be used in tank

mixes containing other fungicides with different modes of action (Kemble et al. 2020). This study supports that recommendation, but with such high prevalence of fluopicolide resistance in Tennessee even the use of fluopicolide in a tank mix could result in selecting for resistance to the other active ingredient in the tank mix. Therefore, rotating modes of action is still very important to mitigate risk of fungicide resistance development when fluopicolide is incorporated into a fungicide program. Of the 86 isolates resistant to fluopicolide, five were also resistant to mefenoxam, indicating the presence of isolates with resistance to multiple fungicide modes of action. Use of fluopicolide and mefenoxam together in a fungicide program should be considered carefully, and alternative modes of action should be included in the rotation to mitigate selection for isolates with multiple-resistance. Monitoring fluopicolide sensitivity should continue in the future to determine if this active ingredient should be recommended for Phytophthora blight management.

In this study, one isolate from 2019 was resistant to oxathiapiprolin. This is the first report of resistance to oxathiapiprolin found from field isolates. Along with this, fifteen isolates collected in 2018 and 2019 were moderately sensitive. In previous studies, resistance to oxathiapiprolin has been induced *in vitro* and mutations associated with oxathiapiprolin resistance have been identified in the *P. capsici* genome (Miao et al. 2016, 2018). Given this, the potential for resistance to occur is known and understood, which is why oxathiapiprolin is always applied as a tank mix, or not applied more than once (or twice) consecutively. However, the occurrence of resistance to oxathiapiprolin is increasingly likely as products containing this active ingredient become more commonly used to control Phytophthora blight. Despite this, oxathiapiprolin is still an effective product, but should be used in rotation with other products

labeled to control *P. capsici* to lessen the risk of resistance development. With only one resistant isolate found, it remains to be seen whether meaningful control failures will occur in the future, or if resistance will become widespread. This issue warrants further exploration.

In 2018, 13 isolates were resistant to cyazofamid. Resistance to cyazofamid has been reported in the US multiple times (Jackson et al. 2012; Kousik and Keinath 2008). In these cases, resistant isolates were identified in the southeastern US, making the occurrence of resistance in Tennessee less surprising. Resistant isolates were collected from every location sampled in 2018, indicating that resistance is widespread in Tennessee. However, a study by Jackson et al. (2012) used similar methods to the present study, and tested sporangia formation and zoospore germination, in addition to mycelial growth. Similar to our results when testing mycelial growth, they found many isolates were resistant to cyazofamid at similar concentrations to those tested here. Despite this, when testing sporangia formation and zoospore germination, the same isolates were sensitive and were controlled at much lower concentrations relative to the ones used in the mycelial growth test. Jackson et al. (2012) concluded that cyazofamid may not be effective at consistently controlling the growth of mycelia but may consistently reduce the production and germination of sporangia and zoospores, respectively. Results of our study are consistent with those found by Jackson et al. regarding mycelial growth. Future work should focus on determining if *in vitro* mycelial growth data for cyazofamid sensitivity corresponds to fungicide efficacy in the field, or if sporangia formation and zoospore germination are better measures of cyazofamid sensitivity from a practical disease management perspective.

For dimethomorph and mandipropamid, both of which have the same mode of action and belong to the same FRAC 40 group, no resistant isolates were identified in this study, and both

remain effective at controlling *P. capsici in vitro*. In 2019, four isolates were found to be moderately sensitive to dimethomorph. These finding are similar to others, and among the fungicides tested in this study, these represent good candidates for inclusion in spray rotations for managing Phytophthora blight (Jackson et al. 2012; Matheron and Porchas 2015). Dimethomorph sensitivity should continue to be monitored to determine if moderately sensitive isolates become more widespread throughout Tennessee, or if stronger resistance develops from repeated exposure and selection pressure.

This study identified widespread resistance to fluopicolide and cyazofamid throughout Tennessee. Additionally, localized low levels of resistance to mefenoxam and oxathiapiprolin were also found, and a small number of isolates were resistant to both fluopicolide and mefenoxam. There was no resistance found to dimethomorph and mandipropamid. These findings are important for informing recommendations to local vegetable growers but could also have national and international implications for the potential longevity of each of these active ingredients. Since fungicide resistance is intrinsically tied to selection pressure through fungicide applications, there will always be potential for the evolution of resistance to any of these single-site mode-of-action products if selection pressure is not mitigated by following labels and proper spray rotations. Given this, monitoring for resistance should continue to make sure disease management recommendations are up-to-date and accurate. Additionally, cultural practices aimed at reducing disease pressure may be equally important in preserving the efficacy of these fungicides as this can reduce the number of pathogen propagules exposed to selection pressure. Further study should be done to determine the relationship between *in vitro* fungicide resistance in *P. capsici* and loss of disease control in the field.

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Appendix

Table 1.1: 2018 *Phytophthora capsici* fungicide sensitivity data summary of 87 isolates tested in vitro.

Fungicides	Concentration (µg/ml)	AVG Growth (%)	SD (%)	Min Growth (%)	Max Growth (%)
Mefenoxam	10, 100	11	17	0	85
Fungicides	**Concentrations (µg/ml)**	**AVG EC50 (µg/ml)**	**SD (µg/ml)**	**Min EC50 (µg/ml)**	**Max EC50 (µg/ml)**
Fluopicolide	0.1, 0.25, 0.5, 5	> 5	> 5	0.11	>5
Dimethomorph	0.1, 0.25, 0.5, 1	0.41	0.06	0.2	0.59
Mandipropamid	0.01, 0.025, 0.075, 0.1	0.025	0.007	0.0098	0.064
Oxathiapiprolin	0.00025, 0.0005, 0.00075, 0.001	0.00038	0.00013	0.00017	0.00081
Cyazofamid	100, 500, 1000	596	425	35	>1000

Table 1.2: 2019 *Phytophthora capsici* discriminatory dose fungicide sensitivity data summary of 97 isolates tested *in vitro*.

Fungicides	Concentration (µg/ml)	AVG Growth (%)	SD (%)	Min Growth (%)	Max Growth (%)
Mefenoxam	100	12	9	0	82
Fluopicolide	0.35	64	36	12	96
Dimethomorph	0.6	10	11	0	50
Mandipropamid	0.04	17	9	4	50
Oxathiapiprolin	0.0007	35	15	7	80
Cyazofamid	500	41	9	13	63

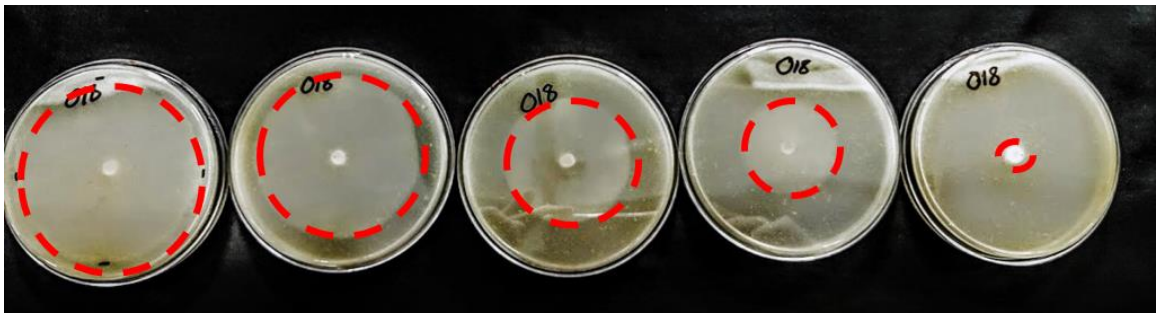

Figure 1.1. Range of growth of *P. capsici* on media amended with varying concentrations of dimethomorph, left to right 0, 0.1, 0.25, 0.5, 1 µg/ml.

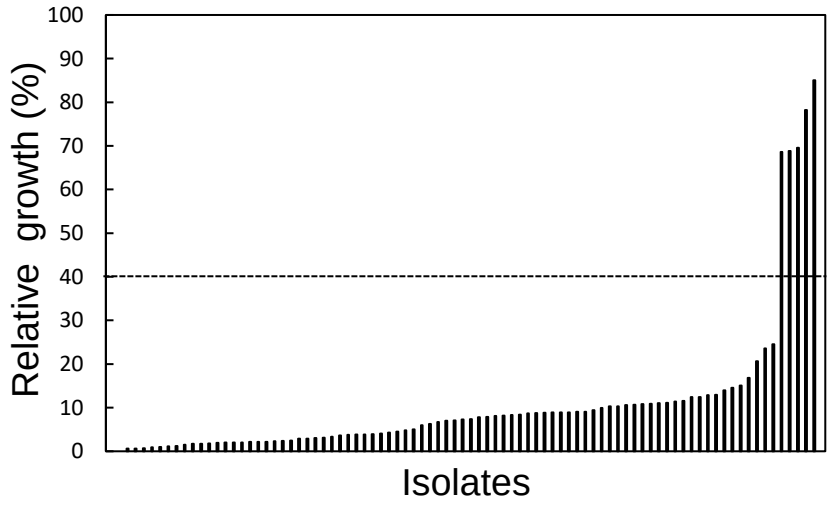

Figure 1.2. Relative percent growth of 87 *P. capsici* isolates collected in 2018 on medium amended with 100 µg/ml mefenoxam. Five isolates had relative growth above 40%, indicated by the dashed line, and were considered resistant.

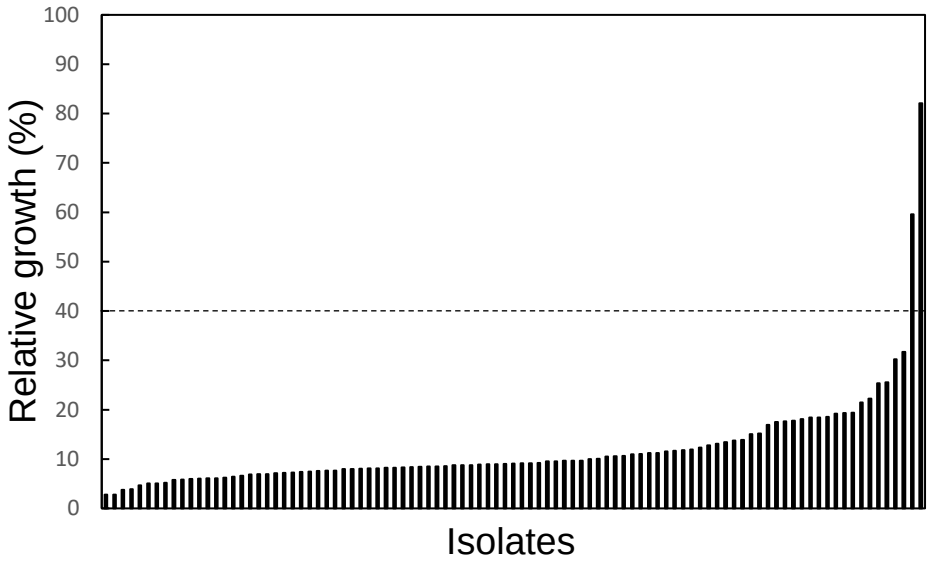

Figure 1.3. Relative percent growth of 97 *P. capsici* isolates collected in 2019 on media amended with 100 µg/ml mefenoxam. Two isolates had relative growth above 40%, indicated by the dashed line, and were considered resistant.

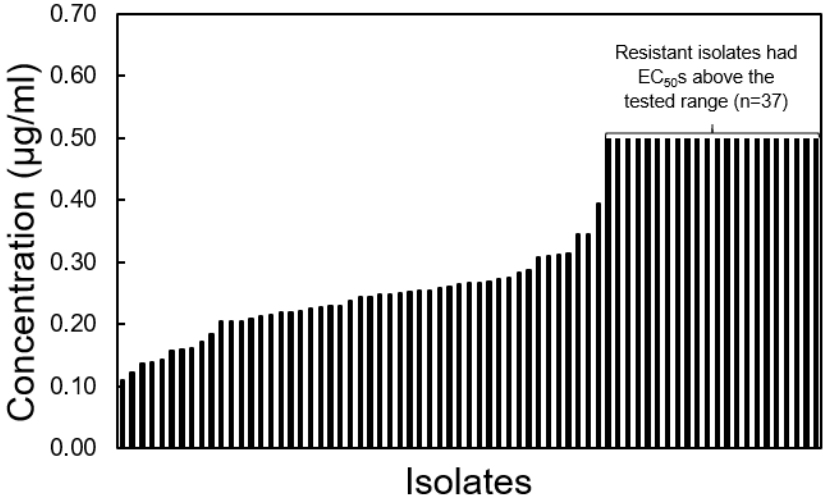

Figure 1.4. EC_{50} values of *P. capsici* isolates collected in 2018 on fluopicolide-amended media. All resistant isolates had EC_{50} values above the tested range (n=37).

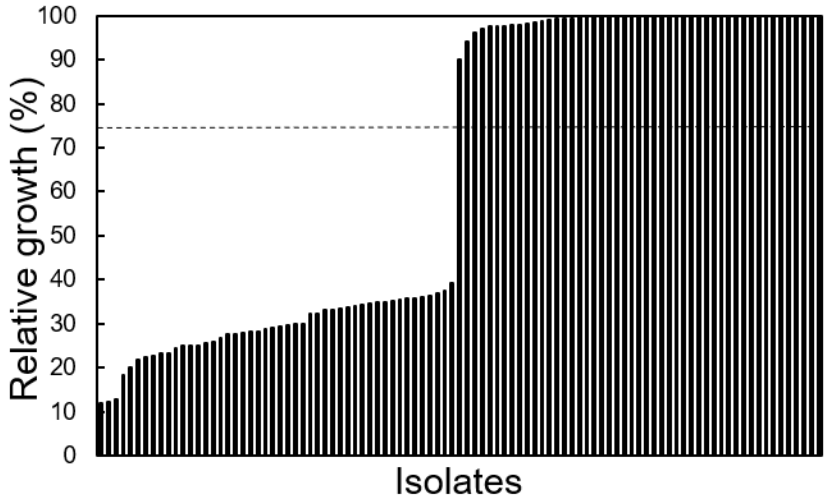

Figure 1.5. Relative percent growth of 2019 *P. capsici* isolates at the discriminatory dose of 0.35 µg/ml of fluopicolide in 2019. All isolates with relative growth above 75%, indicated by the dashed line, were considered resistant (n=49).

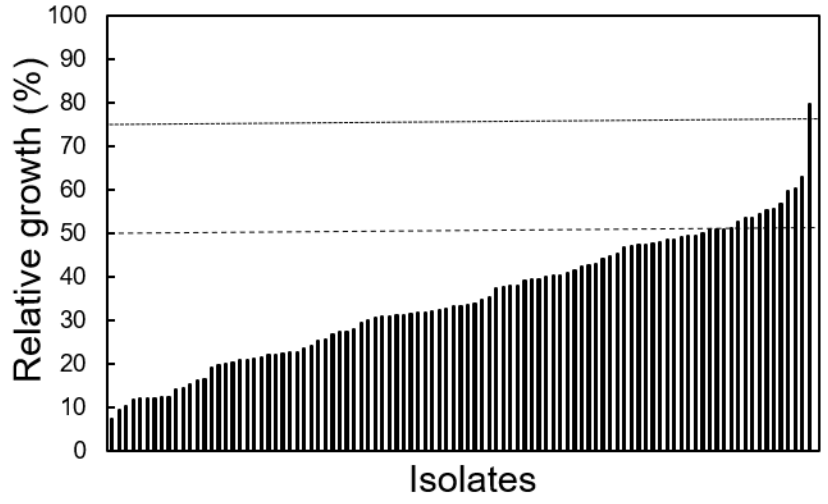

Figure 1.6. Relative percent growth of 2019 *P. capsici* isolates on oxathiapiprolin-amended media at the discriminatory dose of 0.0007 µg/ml. All isolates with relative growth above 50%, indicated by the dashed line, were considered moderately sensitive (n=14). One isolate with relative growth above 75%, indicated by the dotted line, was considered resistant.

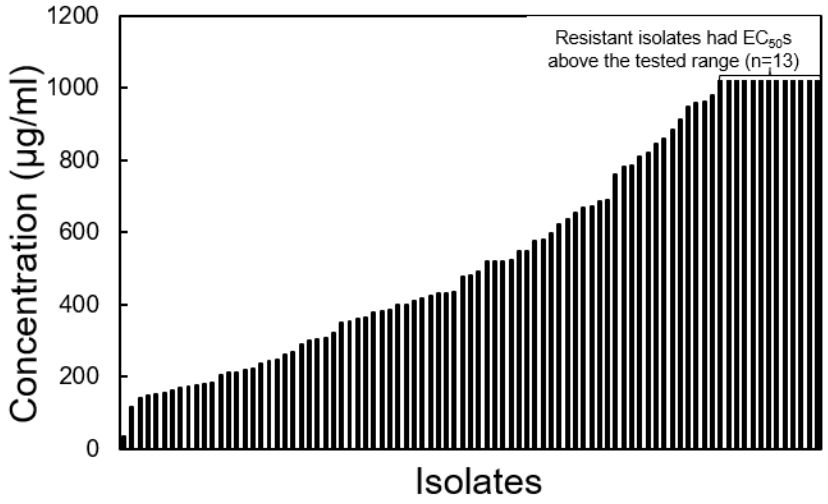

Figure 1.7. EC_{50} values of *P. capsici* isolates collected in 2018 on cyazofamid-amended media. All resistant isolates had EC_{50} values above the tested range (n=13). All isolates with relative growth above 50%, indicated by the dashed line, were considered moderately sensitive (n=29).

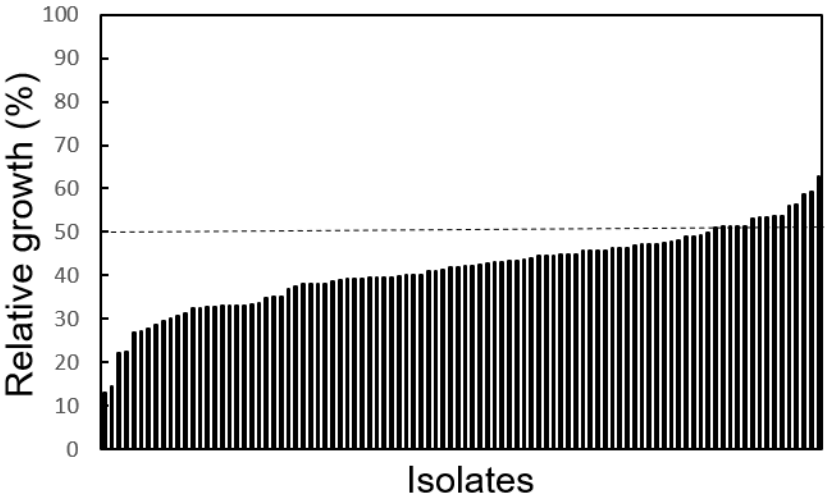

Figure 1.8. Relative percent growth of 2019 *P. capsici* isolates on cyazofamid-amended media at the discriminatory dose of 500 µg/ml. All isolates with relative growth above 50%, indicated by the dashed line, were considered moderately sensitive (n=15).

Supplementary Table 1.S1. 2018 *Phytophthora capsici* isolate data.

Isolate ID	Collection Date	Collection Location (County, Farm)[a]	Host	Mating Type
pctn18001	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18002	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18003	8/9/2018	Rhea 1	Yellow Squash	A2
pctn18004	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18005	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18007	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18008	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18009	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18011	8/9/2018	Rhea 1	Yellow Squash	A2
pctn18012	8/9/2018	Rhea 1	Yellow Squash	A2
pctn18013	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18015	8/9/2018	Rhea 1	Yellow Squash	A2
pctn18017	8/9/2018	Rhea 1	Yellow Squash	A1
pctn18018	8/9/2018	Rhea 1	Yellow Squash	A2
pctn18023	8/9/2018	Rhea 1	Pepper	A1
pctn18032	8/9/2018	Rhea 1	Pepper	A1
pctn18033	8/9/2018	Rhea 1	Pepper	A1
pctn18039	8/9/2018	Rhea 1	Pepper	A2
pctn18040	8/9/2018	Rhea 1	Pepper	A1
pctn18046	8/9/2018	Rhea 1	Pepper	A2
pctn18048	8/9/2018	Rhea 1	Pepper	A2
pctn18052	8/9/2018	Rhea 1	Pepper	A1
pctn18056	8/9/2018	Rhea 1	Pepper	A2
pctn18058	8/9/2018	Rhea 1	Pepper	A2
pctn18067	8/31/2018	Putnam 1	Pumpkin	A1
pctn18068	8/31/2018	Putnam 1	Pumpkin	A2
pctn18069	8/31/2018	Putnam 1	Pumpkin	A2
pctn18071	8/31/2018	Putnam 1	Pumpkin	A1
pctn18072	8/31/2018	Putnam 1	Pumpkin	A1
pctn18073	8/31/2018	Putnam 1	Pumpkin	A1
pctn18074	8/31/2018	Putnam 1	Pumpkin	A2
pctn18075	8/31/2018	Putnam 1	Pumpkin	A2
pctn18076	8/31/2018	Putnam 1	Pumpkin	A1
pctn18077	8/31/2018	Putnam 1	Pumpkin	A2
pctn18078	8/31/2018	Putnam 1	Pumpkin	A2
pctn18079	8/31/2018	Putnam 1	Pumpkin	A1
pctn18080	8/31/2018	Putnam 1	Pumpkin	A2
pctn18081	8/31/2018	Putnam 1	Pumpkin	A2
pctn18082	8/31/2018	Putnam 1	Pumpkin	A1
pctn18083	8/31/2018	Putnam 1	Pumpkin	A1
pctn18084	8/31/2018	Putnam 1	Pumpkin	A2

Supplementary Table 1.S1 Continued

Isolate ID	Collection Date	Collection Location (County, Farm)[a]	Host	Mating Type
pctn18085	8/31/2018	Putnam 1	Pumpkin	A2
pctn18086	8/31/2018	Putnam 1	Pumpkin	A2
pctn18087	8/31/2018	Putnam 1	Pumpkin	A2
pctn18088	8/31/2018	Putnam 1	Pumpkin	A2
pctn18089	8/31/2018	Putnam 1	Pumpkin	A1
`pctn18090	8/31/2018	Putnam 1	Pumpkin	A2
pctn18091	8/31/2018	Putnam 1	Pumpkin	A2
pctn18092	8/31/2018	Putnam 1	Pumpkin	A1
pctn18093	8/31/2018	Putnam 1	Pumpkin	A1
pctn18094	8/31/2018	Putnam 1	Pumpkin	A1
pctn18095	8/31/2018	Putnam 1	Pumpkin	A2
pctn18096	8/31/2018	Putnam 1	Pumpkin	A1
pctn18098	8/31/2018	Putnam 1	Pumpkin	A1
pctn18099	8/31/2018	Putnam 1	Pumpkin	A2
pctn18100	9/7/2018	Bledsoe 1	Pumpkin	A1
pctn18101	9/7/2018	Bledsoe 1	Pumpkin	A1
pctn18102	9/7/2018	Bledsoe 1	Pumpkin	A2
pctn18103	9/7/2018	Bledsoe 1	Pumpkin	A1
pctn18104	9/7/2018	Bledsoe 2	Pumpkin	A1
pctn18105	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18106	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18107	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18108	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18109	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18110	9/7/2018	Bledsoe 2	Pumpkin	A1
pctn18112	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18113	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18114	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18115	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18116	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18117	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18118	9/7/2018	Bledsoe 2	Pumpkin	A1
pctn18119	9/7/2018	Bledsoe 2	Pumpkin	A1
pctn18120	9/7/2018	Bledsoe 2	Pumpkin	A2
pctn18122	11/16/2018	Putnam 1	Pumpkin	A2
pctn18123	11/16/2018	Putnam 1	Pumpkin	A2
pctn18125	11/16/2018	Putnam 1	Pumpkin	A2
pctn18126	11/16/2018	Putnam 1	Pumpkin	A2
pctn18127	11/16/2018	Putnam 1	Pumpkin	A2
pctn18128	11/16/2018	Putnam 1	Pumpkin	A2
pctn18129	11/16/2018	Putnam 1	Pumpkin	A2
pctn18130	11/16/2018	Putnam 1	Pumpkin	A2

Supplementary Table 1.S1 Continued

Isolate ID	Collection Date	Collection Location (County, Farm)[a]	Host	Mating Type
pctn18131	11/16/2018	Putnam 1	Pumpkin	A2
pctn18132	11/16/2018	Putnam 1	Pumpkin	A2
pctn18133	11/16/2018	Putnam 1	Pumpkin	A2
pctn18134	11/16/2018	Putnam 1	Pumpkin	A2

[a] Each county and number combination (i.e. Rhea 1) is a specific location and is consistent between collections years.

Supplementary Table 1.S2. 2019 *Phytophthora capsici* isolate data.

Isolate ID	Collection Date	Collection Location (County and Farm)[a]	Host	Mating Type
pctn19001	7/18/2019	Rhea 1	Zucchini	*
pctn19002	7/18/2019	Rhea 1	Zucchini	A2
pctn19003	7/18/2019	Putnam 1	Pumpkin	A1
pctn19004	7/18/2019	Putnam 1	Pumpkin	A2
pctn19005	7/18/2019	Rhea 1	Zucchini	*
pctn19006	7/18/2019	Rhea 1	Zucchini	A2
pctn19007	7/18/2019	Rhea 1	Zucchini	A1
pctn19008	7/18/2019	Rhea 1	Zucchini	A1
pctn19009	7/18/2019	Rhea 1	Zucchini	A1
pctn19010	7/18/2019	Rhea 1	Zucchini	A1
pctn19011	8/31/2019	Rhea 1	Zucchini	*
pctn19012	8/31/2019	Rhea 1	Zucchini	A1
pctn19013	8/31/2019	Rhea 1	Zucchini	A1
pctn19014	8/31/2019	Rhea 1	Zucchini	A2
pctn19015	8/31/2019	Rhea 1	Zucchini	A1
pctn19017	8/31/2019	Rhea 1	Zucchini	A2
pctn19018	8/31/2019	Rhea 1	Zucchini	A2
pctn19019	8/31/2019	Rhea 1	Zucchini	A1
pctn19021	8/31/2019	Rhea 1	Zucchini	A1
pctn19022	8/31/2019	Rhea 1	Zucchini	A1
pctn19023	8/31/2019	Rhea 1	Zucchini	A1
pctn19024	8/31/2019	Rhea 1	Zucchini	A2
pctn19025	8/31/2019	Rhea 1	Zucchini	A1
pctn19026	8/31/2019	Rhea 1	Zucchini	A2
pctn19028	8/31/2019	Rhea 1	Cucumber	A2
pctn19029	8/31/2019	Rhea 1	Cucumber	A2
pctn19031	8/31/2019	Rhea 1	Cucumber	A1
pctn19032	8/31/2019	Rhea 1	Cucumber	A2
pctn19037	8/31/2019	Rhea 1	Cucumber	A2
pctn19039	8/31/2019	Rhea 1	Cucumber	A2
pctn19040	8/31/2019	Rhea 1	Cucumber	A1
pctn19041	8/31/2019	Rhea 1	Cucumber	A1
pctn19042	8/31/2019	Rhea 1	Cucumber	A2
pctn19043	8/31/2019	Rhea 1	Cucumber	A2
pctn19045	8/31/2019	Rhea 1	Cucumber	A2
pctn19046	8/31/2019	Rhea 1	Cucumber	A2
pctn19048	8/31/2019	Rhea 1	Cucumber	A2
pctn19050	8/31/2019	Rhea 1	Cucumber	A1
pctn19051	8/31/2019	Rhea 1	Cucumber	A1
pctn19052	8/31/2019	Rhea 1	Cucumber	A2

Supplementary Table 1.S2 Continued

Isolate ID	Collection Date	Collection Location (County, Farm)[a]	Host	Mating Type
pctn19053	8/31/2019	Rhea 1	Cucumber	A2
pctn19055	8/31/2019	Rhea 1	Cucumber	A2
pctn19056	8/31/2019	Rhea 1	Cucumber	A1
pctn19058	8/31/2019	Rhea 1	Cucumber	A2
pctn19059	8/31/2019	Rhea 1	Cucumber	A2
pctn19062	8/31/2019	Rhea 1	Cucumber	A1
pctn19063	8/31/2019	Rhea 1	Cucumber	A2
pctn19064	8/31/2019	Rhea 1	Cucumber	A1
pctn19065	8/31/2019	Rhea 1	Cucumber	A2
pctn19067	8/31/2019	Rhea 1	Cucumber	A2
pctn19068	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19069	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19070	8/31/2019	Rhea 1	Yellow Squash	*
pctn19071	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19072	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19073	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19074	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19075	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19076	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19077	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19079	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19082	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19083	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19084	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19085	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19086	8/31/2019	Rhea 1	Yellow Squash	A2
pctn19089	8/31/2019	Rhea 1	Yellow Squash	A1
pctn19090	9/12/2019	Lincoln 1	Pumpkin	*
pctn19091	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19092	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19094	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19096	9/12/2019	Lincoln 1	Pumpkin	A1
pctn19097	9/12/2019	Lincoln 1	Pumpkin	A1
pctn19099	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19100	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19101	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19102	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19103	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19104	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19106	9/12/2019	Lincoln 1	Pumpkin	A2
pctn19107	9/12/2019	Lincoln 1	Pumpkin	A1
pctn19108	9/12/2019	Lincoln 1	Acorn Squash	*

Supplementary Table 1.S2 Continued

Isolate ID	Collection Date	Collection Location (County, Farm)[a]	Host	Mating Type
pctn19109	9/12/2019	Lincoln 1	Acorn Squash	A2
pctn19110	9/12/2019	Lincoln 1	Acorn Squash	A1
pctn19112	9/12/2019	Lincoln 1	Acorn Squash	A1
pctn19113	9/12/2019	Lincoln 1	Acorn Squash	A2
pctn19114	9/12/2019	Lincoln 1	Acorn Squash	A2
pctn19116	9/12/2019	Lincoln 1	Acorn Squash	A2
pctn19119	9/12/2019	Lincoln 1	Butternut Squash	A1
pctn19120	9/12/2019	Lincoln 1	Acorn Squash	A2
pctn19122	9/12/2019	Lincoln 1	Acorn Squash	A2
pctn19124	9/12/2019	Lincoln 1	Acorn Squash	A1
pctn19130	10/11/2019	Bledsoe 1	Yellow Squash	A1
pctn19138	10/11/2019	Bledsoe 1	Yellow Squash	A1
pctn19149	10/11/2019	Bledsoe 1	Yellow Squash	A1
pctn19152	10/11/2019	Bledsoe 1	Yellow Squash	A1
pctn19154	10/11/2019	Bledsoe 1	Yellow Squash	A1

[a] Each County and number combination (i.e. Rhea 1) is a specific location and is consistent between collections years.

[b] Missing mating type data.

Supplementary Table1.S3. 2018 *Phytophthora capsici* fungicide sensitivity data.

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	AVG Growth (%)	SD (%)	AVG EC_{50} (µg/ml)	SD (µg/ml)	AVG EC_{50} (µg/ml)	SD (µg/ml)	AVG EC_{50} (µg/ml)	SD (µg/ml)	AVG EC_{50} (µg/ml)	SD (µg/ml)	AVG EC_{50} (µg/ml)	SD (µg/ml)
pctn18001	17	3	0.16	0.02	0.38	0.04	0.021	0.001	0.00053	0.00036	263	6
pctn18002	13	2	0.27	0.04	0.37	0.14	0.024	0.001	0.00023	.[b]	35	39
pctn18003	11	7	0.31	0.07	0.35	0.03	0.009	0.012	0.00049	0.00031	672	.
pctn18004	24	10	N/A[a]	N/A	0.38	.	0.025	0.002	0.00032	0.00015	N/A	54
pctn18005	25	9	N/A	N/A	0.38	0.01	0.025	0.001	0.00030	0.00023	N/A	68
pctn18007	2	0	0.34	0.15	0.36	0.06	0.027	0.004	0.00031	0.00014	212	131
pctn18008	10	4	0.39	0.09	0.48	0.01	0.014	0.020	0.00042	0.00023	521	221
pctn18009	9	4	0.28	0.05	0.47	0.01	0.041	0.013	0.00066	0.00041	431	88
pctn18011	1	1	0.31	0.09	0.36	0.00	0.021	0.001	0.00028	0.00014	521	82
pctn18012	11	5	0.12	0.00	0.55	0.04	0.014	0.019	0.00036	0.00024	349	.
pctn18013	4	5	0.31	0.09	0.40	0.01	0.019	0.001	0.00037	0.00026	784	16
pctn18015	3	2	N/A	N/A	0.37	0.02	0.021	0.006	0.00031	0.00018	418	.
pctn18017	8	5	N/A	N/A	0.26	0.04	0.010	0.004	0.00036	0.00025	171	.
pctn18018	3	4	N/A	N/A	0.40	0.00	0.025	0.005	0.00028	0.00017	268	96
pctn18023	70	13	N/A	N/A	0.42	0.03	0.024	0.003	0.00040	0.00023	410	488
pctn18032	69	23	N/A	N/A	0.42	0.04	0.025	0.002	0.00039	0.00037	520	305
pctn18033	69	21	N/A	N/A	0.43	0.00	0.025	0.001	0.00041	0.00024	306	.
pctn18039	6	5	N/A	N/A	0.47	0.03	0.027	0.003	0.00042	0.00024	248	.
pctn18040	85	13	N/A	N/A	0.37	0.02	0.012	0.017	0.00043	0.00021	169	25
pctn18046	4	2	0.31	0.08	0.39	0.02	0.011	0.016	0.00037	0.00013	116	3
pctn18048	10	10	0.14	0.03	0.46	0.05	0.014	0.020	0.00030	0.00025	353	54
pctn18052	7	5	0.34	0.06	0.55	0.04	0.019	0.027	0.00046	0.00032	668	1220
pctn18056	2	2	0.29	0.03	0.45	0.01	0.028	0.000	0.00042	0.00032	151	174

Supplementary Table1.S3 Continued

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	**AVG Growth (%)**	**SD (%)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**
pctn18058	1	2	N/A	N/A	0.47	0.01	0.018	0.026	0.00046	0.00018	400	51
pctn18067	7	1	N/A	N/A	0.49	0.01	0.030	0.006	0.00036	0.00028	238	3
pctn18068	4	0	N/A	N/A	0.45	0.01	0.027	0.002	0.00031	0.00021	243	11
pctn18069	8	2	N/A	N/A	0.38	0.05	0.019	0.002	0.00039	0.00031	424	.
pctn18071	2	3	N/A	N/A	0.47	0.03	0.023	0.002	0.00029	0.00034	182	83
pctn18072	2	3	N/A	N/A	0.59	0.15	0.015	0.021	0.00032	0.00015	211	.
pctn18073	5	6	N/A	N/A	0.49	0.00	0.032	0.003	0.00027	0.00017	223	52
pctn18074	1	1	N/A	N/A	0.29	0.05	0.019	0.000	0.00040	0.00018	N/A	.
pctn18075	2	3	0.26	0.02	0.39	0.01	0.024	0.003	0.00036	0.00028	N/A	.
pctn18076	2	3	N/A	N/A	0.42	0.05	0.026	0.003	0.00040	0.00012	302	102
pctn18077	12	6	0.20	0.10	0.38	0.01	0.022	0.001	0.00043	0.00026	687	213
pctn18078	3	4	0.25	0.00	0.38	0.00	0.023	0.001	0.00041	0.00027	859	49
pctn18079	6	6	N/A	N/A	0.46	0.00	0.029	0.009	0.00027	0.00036	217	408
pctn18080	8	0	0.22	0.17	0.42	0.03	0.026	0.000	0.00034	0.00030	948	50
pctn18081	7	1	0.14	0.04	0.44	0.01	0.025	0.003	0.00036	0.00034	978	116
pctn18082	2	3	N/A	N/A	0.45	0.00	0.025	0.001	0.00029	0.00015	154	25
pctn18083	3	5	N/A	N/A	0.50	0.02	0.032	0.009	0.00034	0.00016	364	469
pctn18084	12	6	0.17	0.08	0.43	0.02	0.025	0.000	0.00037	0.00020	596	265
pctn18085	4	0	N/A	N/A	0.39	0.01	0.010	0.015	0.00072	0.00062	621	132
pctn18086	4	5	N/A	N/A	0.51	0.03	0.037	0.008	0.00039	0.00002	205	78
pctn18087	1	1	2.946	0.329	0.26	0.03	0.014	0.001	0.00008	0.00007	490	72
pctn18088	2	3	N/A	N/A	0.44	0.04	0.033	0.007	0.00038	0.00011	179	80
pctn18089	8	4	N/A	N/A	0.38	0.05	0.022	0.001	0.00081	0.00039	N/A	.
pctn18090	7	4	0.21	0.13	0.36	0.00	0.019	0.001	0.00040	0.00030	809	118
pctn18091	12	5	0.22	0.15	0.36	0.02	0.023	0.001	0.00039	0.00029	654	761

Supplementary Table1.S3 Continued

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	**AVG Growth (%)**	**SD (%)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**Isolate ID**	**AVG Growth (%)**	**SD (%)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**
pctn18092	2	3	N/A	N/A	0.48	0.01	0.035	0.003	0.00025	0.00022	148	386
pctn18093	2	3	N/A	N/A	0.49	0.02	0.038	0.006	0.00023	0.00022	178	60
pctn18094	5	7	N/A	N/A	0.45	0.02	0.026	0.000	0.00036	0.00031	377	248
pctn18095	9	0	N/A	N/A	0.44	0.02	0.065	0.043	0.00025	0.00015	361	66
pctn18096	2	3	0.27	0.02	0.33	0.03	0.014	0.002	0.00031	.	162	250
pctn18098	9	0	0.22	0.20	0.41	.	0.024	0.001	0.00038	0.00022	961	.
pctn18099	11	10	0.27	0.05	0.41	0.02	0.024	0.000	0.00035	0.00023	688	.
pctn18100	4	0	0.28	0.19	0.44	0.01	0.035	0.007	0.00040	0.00007	577	154
pctn18101	78	20	0.23	0.00	0.33	0.01	0.019	0.002	0.00023	0.00004	N/A	.
pctn18102	21	19	0.11	0.15	0.33	0.08	0.016	0.002	0.00026	0.00017	N/A	283
pctn18103	9	2	N/A	N/A	0.38	0.03	0.024	0.000	0.00074	0.00049	N/A	12
pctn18104	3	4	N/A	N/A	0.40	0.02	0.024	0.002	0.00038	0.00014	761	163
pctn18105	9	2	0.25	0.00	0.40	0.00	0.025	0.005	0.00022	0.00006	289	26
pctn18106	8	2	0.25	0.02	0.38	0.01	0.026	0.003	0.00019	0.00015	387	46
pctn18107	8	3	0.26	0.04	0.38	0.02	0.022	0.002	0.00017	0.00014	401	57
pctn18108	4	6	0.26	0.07	0.37	0.00	0.019	0.002	0.00019	0.00016	321	94
pctn18109	2	3	0.27	0.05	0.38	0.00	0.025	0.002	0.00037	0.00048	435	322
pctn18110	1	2	N/A	N/A	0.40	0.02	0.025	0.007	0.00041	0.00017	383	208
pctn18112	0	0	0.24	0.03	0.39	0.04	0.030	0.004	0.00054	0.00022	549	120
pctn18113	9	4	0.25	0.03	0.41	0.01	0.023	0.002	0.00021	0.00014	429	78
pctn18114	0	0	0.25	0.01	0.42	0.01	0.031	0.000	0.00060	0.00049	524	7
pctn18115	10	0	0.24	0.03	0.37	0.01	0.024	0.009	0.00018	0.00013	302	47
pctn18116	1	2	N/A	N/A	0.36	0.01	0.026	0.007	0.00043	0.00018	476	62
pctn18117	1	1	0.23	0.04	0.32	0.04	0.007	0.009	0.00050	0.00017	579	53
pctn18118	11	8	N/A	N/A	0.20	0.01	0.007	0.009	0.00051	0.00009	140	.

Supplementary Table1.S3 Continued

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	**AVG Growth (%)**	**SD (%)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**Isolate ID**	**AVG Growth (%)**	**SD (%)**	**AVG EC_{50} (µg/ml)**	**SD (µg/ml)**	**AVG EC_{50} (µg/ml)**
pctn18119	2	2	N/A	N/A	0.37	0.03	0.023	0.001	0.00050	0.00013	482	387
pctn18120	1	1	0.24	0.01	0.42	0.02	0.027	0.003	0.00064	0.00034	547	.
pctn18122	9	1	0.14	0.04	0.41	0.03	0.022	0.001	0.00039	0.00026	820	.
pctn18123	7	1	0.21	0.13	0.39	0.02	0.023	0.000	0.00039	0.00028	886	.
pctn18125	15	10	0.21	0.09	0.41	0.04	0.021	0.002	0.00038	0.00029	957	69
pctn18126	14	6	0.16	0.05	0.46	0.03	0.025	0.003	0.00043	0.00030	635	54
pctn18127	13	10	N/A	N/A	0.45	0.03	0.025	0.000	0.00068	0.00044	N/A	.
pctn18128	11	1	0.21	0.11	0.45	0.02	0.023	0.003	0.00044	0.00030	782	188
pctn18129	15	4	0.22	0.15	0.39	0.01	0.024	0.002	0.00041	0.00026	N/A	79
pctn18130	5	7	0.18	0.10	0.38	0.00	0.023	0.002	0.00040	0.00026	845	236
pctn18131	9	5	0.23	0.15	0.40	0.00	0.023	0.004	0.00040	0.00025	N/A	196
pctn18132	11	2	0.20	0.14	0.39	0.01	0.023	0.001	0.00037	0.00029	N/A	.
pctn18133	9	4	0.25	0.02	0.45	0.00	0.025	0.001	0.00043	0.00032	N/A	176
pctn18134	11	2	0.16	0.04	0.41	0.04	0.024	0.003	0.00044	0.00035	911	59

[a] EC_{50} values were greater than highest concentrations tested and were unable to be calculated.
[b] Periods indicate calculations were not done due to loss of one or more replicate data.

Supplementary Table 1.S4. 2019 *Phytophthora capsici* fungicide sensitivity data.

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)
pctn19001	6	0	100	3	0	0	14	5	34	2	40	2
pctn19002	30	30	100	2	0	0	6	2	32	1	38	1
pctn19003	10	2	26	3	40	4	44	0	34	1	43	1
pctn19004	15	2	106	2	3	4	5	3	49	0	27	4
pctn19005	18	4	36	3	0	0	8	4	24	9	45	2
pctn19006	3	4	99	2	1	2	9	4	33	4	40	2
pctn19007	18	5	36	2	15	0	19	1	42	2	38	1
pctn19008	10	1	28	6	10	2	21	8	23	6	33	2
pctn19009	7	1	98	1	2	0	22	0	44	1	46	4
pctn19010	18	2	29	2	0	0	13	5	35	2	47	4
pctn19011	82	10	32	0	36	27	5	4	31	8	33	21
pctn19012	12	1	102	5	18	7	23	1	33	7	43	8
pctn19013	19	9	35	2	16	1	17	2	48	8	48	6
pctn19014	12	5	100	1	15	2	18	9	53	2	38	1
pctn19015	10	1	97	8	0	0	19	3	60	6	45	1
pctn19017	22	6	33	5	10	0	50	2	47	2	35	22
pctn19018	11	10	108	8	8	12	9	2	47	16	37	6
pctn19019	12	8	32	1	13	4	15	0	27	4	63	0
pctn19021	12	0	34	4	1	2	23	9	43	6	39	5
pctn19022	10	4	33	4	18	9	6	2	38	4	33	5
pctn19023	13	0	100	1	10	0	19	7	53	3	39	5
pctn19024	7	3	99	1	15	3	14	4	26	2	49	1
pctn19025	7	0	101	2	5	8	10	5	63	7	46	4
pctn19026	9	1	99	1	0	0	22	14	51	5	44	0
pctn19028	14	3	30	1	22	3	19	7	31	10	40	3
pctn19029	9	1	106	4	15	13	15	3	22	13	15	9
pctn19031	8	1	102	4	7	3	12	5	21	7	28	2
pctn19032	8	2	100	5	10	1	24	3	48	4	43	2

Supplementary Table 1.S4 Continued

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)
pctn19037	21	16	102	2	14	1	11	4	49	2	54	3
pctn19039	6	3	100	2	23	10	24	11	51	6	34	0
pctn19040	6	8	97	2	20	6	20	7	55	4	31	3
pctn19041	8	0	101	2	16	2	19	8	57	5	38	2
pctn19042	14	8	30	0	11	0	9	5	50	5	43	1
pctn19043	15	15	101	3	21	5	15	6	20	0	46	5
pctn19045	4	5	103	6	10	0	18	3	43	1	33	4
pctn19046	5	3	100	1	10	15	24	0	25	3	35	3
pctn19048	7	6	100	1	0	0	11	3	49	2	48	1
pctn19050	8	2	36	1	11	4	14	4	49	2	49	1
pctn19051	8	2	96	6	0	0	20	1	51	1	39	4
pctn19052	8	0	37	3	2	3	23	15	22	10	30	3
pctn19053	8	7	99	0	0	0	14	4	43	1	31	9
pctn19055	7	3	94	2	12	4	13	1	80	1	33	3
pctn19056	9	1	102	1	6	0	7	7	37	2	40	1
pctn19058	6	1	101	5	16	1	14	1	49	1	45	2
pctn19059	9	4	103	2	16	22	19	12	60	7	13	1
pctn19062	7	0	36	2	0	0	5	1	31	5	46	3
pctn19063	7	10	97	1	19	6	14	7	16	0	33	1
pctn19064	7	5	100	4	50	71	4	6	15	4	23	32
pctn19065	19	10	23	1	28	15	37	0	32	10	39	1
pctn19067	19	11	22	3	24	10	36	5	24	8	40	4
pctn19068	13	8	100	2	3	4	14	5	31	1	44	3
pctn19069	9	5	98	4	22	2	23	7	40	2	27	2
pctn19070	19	11	98	3	10	14	17	3	51	2	39	5
pctn19071	11	2	100	0	25	5	29	3	39	6	32	7
pctn19072	11	1	98	0	3	5	6	2	54	5	41	5
pctn19073	5	7	100	1	24	0	22	7	32	0	35	4

Supplementary Table 1.S4 Continued

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)	AVG Growth (%)	SD (%)
pctn19074	6	2	102	1	3	4	28	8	47	0	51	1
pctn19075	8	3	100	7	0	0	8	3	53	1	45	6
pctn19076	6	2	101	1	18	4	15	3	47	1	43	1
pctn19077	3	4	90	10	0	0	10	7	31	4	33	0
pctn19079	7	1	100	0	16	10	22	19	27	6	59	4
pctn19082	9	5	103	1	0	0	5	0	28	7	46	7
pctn19083	9	0	103	3	0	0	9	3	32	3	41	3
pctn19084	10	4	100	0	9	3	15	2	56	1	51	0
pctn19085	11	1	99	2	13	0	9	1	30	10	42	3
pctn19086	9	4	100	1	7	3	9	1	45	2	40	2
pctn19089	5	2	98	2	20	3	23	11	40	4	29	10
pctn19090	5	7	34	1	1	1	24	2	21	4	37	23
pctn19091	12	7	35	1	0	0	20	10	17	4	59	7
pctn19092	6	3	12	1	22	4	20	1	29	4	53	5
pctn19094	9	3	25	2	35	3	31	1	38	14	50	2
pctn19096	9	3	38	2	1	1	13	11	39	1	56	9
pctn19097	8	1	39	1	16	3	13	3	41	1	56	9
pctn19099	10	5	28	1	0	0	16	13	21	15	53	2
pctn19100	8	4	35	1	0	0	26	4	21	3	54	0
pctn19101	11	3	29	1	5	7	23	1	23	3	51	3
pctn19102	9	2	35	1	10	14	24	0	19	4	51	1
pctn19103	11	1	12	2	0	0	15	22	7	5	48	2
pctn19104	8	4	13	3	0	0	15	4	10	4	41	5
pctn19106	8	1	18	1	10	3	12	2	27	12	39	1
pctn19107	10	1	25	1	14	2	15	2	20	2	42	3
pctn19108	25	24	25	3	0	0	17	6	22	5	49	9
pctn19109	4	2	27	2	0	0	12	1	12	3	33	2
pctn19110	26	25	26	1	0	0	11	15	12	1	44	6

Supplementary Table 1.S4 Continued

	Mefenoxam		Fluopicolide		Dimethomorph		Mandipropamid		Oxathiapiprolin		Cyazofamid	
Isolate ID	**AVG Growth (%)**	**SD (%)**	**AVG Growth (%)**	**SD (%)**	**AVG Growth (%)**	**SD (%)**	**AVG Growth (%)**	**SD (%)**	**AVG Growth (%)**	**SD (%)**	**AVG Growth (%)**	**SD (%)**
pctn19112	17	17	20	0	3	4	10	6	15	5	46	4
pctn19113	11	7	28	0	0	0	11	11	14	2	47	10
pctn19114	18	14	25	1	28	40	14	4	12	3	30	4
pctn19116	60	4	23	1	27	38	4	6	12	7	51	42
pctn19119	18	26	28	0	6	9	20	3	12	2	46	2
pctn19120	18	6	22	1	0	0	10	2	32	2	45	0
pctn19122	32	2	28	3	0	0	10	3	12	2	22	6
pctn19124	9	3	23	2	17	11	21	5	20	0	42	3
pctn19130	13	9	29	2	0	0	20	18	38	3	40	2
pctn19138	8	7	34	2	3	5	39	15	39	4	47	4
pctn19149	9	6	33	1	0	0	29	18	40	4	42	1
pctn19152	8	0	35	2	0	0	14	20	45	4	47	3
pctn19154	6	9	30	3	0	0	44	31	35	8	42	2

CHAPTER 2

Population genetics of *Phytophthora capsici* in the State of Tennessee

This chapter was first authored by Timothy B. Siegenthaler with the editorial help of Dr. Zachariah Hansen (PI), Dr. Bonnie Ownley, Dr. Kurt Lamour, and Dr. Heather Kelly

Abstract

To understand the spread, survival, and evolution of the plant pathogen *Phytophthora capsici* a population genetics study was conducted using 296 isolates of *P. capsici* collected from five counties in Tennessee during 2004, 2007, 2018 and 2019. Using 39 single nucleotide polymorphisms as genetic markers, each isolate was genotyped. Using the R package Poppr, the population structure of *P. capsici* in Tennessee was determined to be isolated clusters structured by geographic sampling locations. Results suggest that each sampling location is genetically distinct from one another, and that there is no outcrossing among these either. This is like other studies done throughout the Midwest and Northeast United States. Ultimately this study adds to the general understanding of *P. capsici* genetics and more specific knowledge of the populations found in Tennessee.

Introduction

Phytophthora blight of cucurbits and pepper is caused by the oomycete *Phytophthora capsici* and was first reported on pepper in the state of New Mexico in 1922, it is now distributed across the United States and throughout the world (Leonian 1922; Gobena et al. 2012a; Hu et al. 2013). This disease can be devastating to production of many vegetables such as pumpkin, squash, cucumbers, and peppers (Hausbeck and Lamour 2004; Granke et al. 2012). Infection is characterized by plant wilt, crown rot, stem rot, fruit rot and leads to complete crop loss.

Phytophthora blight is a very challenging disease to manage primarily because of how it reproduces and is spread.

Infestation of a field may start with a very small number of flagellated asexual spores known as zoospores. Zoospores are produced within a sporangium, an asexual structure that is found on the outside of infected hosts. One infected fruit may contain millions of sporangia, all filled with 20 to 40 zoospores. In the presence of free water, a sporangium will release zoospores. Biflagellate zoospores are biflagellate and can move through the water in and on the surface of soil searching for a new host. Once infection of a new host occurs, the asexual cycle will be repeated. This cycle leads to fast and wide spread of inoculum throughout a site, especially when rain events or irrigation occur. This means that a few genetic individuals may dominate a field. If these individuals are fit and carry advantages such as fungicide resistance, an entire field of crops could be lost even with an organized plan of cultural and chemical control. In some parts of the world, such as China and Argentina, where large portions of farmland are dominated by a few clonal populations, due to mild or warm fallow seasons there is little pressure to kill *P. capsici.* Despite that, there is no evidence that these clonal populations survive from year to year through winter in the midwestern, northeastern and southeastern U.S. (Dunn et al. 2010; Granke et al. 2012). Persistence of *P. capsici* is due to the production of oospores. *Phytophthora capsici* is a heterothallic organism and has two mating types, A1 and A2. When two individuals with the opposite mating undergo gametangial contact, an oospore is formed. Oospores are thick-walled sexual spores that serve as overwintering structures, which can lead to reemergence of disease from year to year. Cultural controls such as host rotation could be implemented, but reemergence is common because oospores can remain viable in fallow soil for

more than 5 years (Lamour and Hausbeck 2003). Additionally, there is the potential for formation of asexual survival spores, known as chlamydospores. Although these are uncommon in nature, chlamydospores can enable clonal lines to persist from year to year (Islam et al. 2005). The spread of these three types of propagules from one field to another occurs primarily by two methods: surface waterways and human activities. In large rain events, runoff from infested fields may spread to nearby fields, either across the soil surface or through connecting a watershed, such as an irrigation stream. This can lead to introduction of zoospores into new fields downstream of the first. The main way that humans move these propagules is through transport of infested soil on farm equipment, tools, and boots. Additionally, the spread of infected fruit and potentially seeds can lead to introduction to new locations. These methods occur over relatively short distances and require a significant mechanical form to enable spread. These methods do not allow for even spread across very large areas of land, especially when compared with the wind transmission of other oomycetes such as *Phytophthora infestans*. These modes reproduction and spread of propagules influence the populations structure within and among populations in a region (Granke et al. 2009; Campbell and Ristaino 1999).

The population genetics of *P. capsici* has been a focus of many studies that began with collection of isolates from a specific location or region of interest. Isolates are genotyped using a series of genetic markers, such as microsatellites (SSR), amplified fragment length polymorphisms (AFLP), or single nucleotide polymorphisms (SNP). Genetic markers are a molecular tool that is used to genotype or fingerprint an individual. These markers are made up of a variable region that is not conserved within a species' genome and is flanked by highly conserved regions that are consistent from one individual to another. Variable regions can be

made up of many loci (SSR, AFLP) or one single locus or nucleotide (SNP), either way they serve the purpose of identifying differences between individuals within a species. These are identified through whole genome sequencing or sequencing large portions of a genome of the target species and then making comparisons with a reference genome of that same species (Milgroom 2015). Using computer language or software, these variable regions can be identified or called. Called variants with conserved flanking regions are used to develop primers that are then used in PCR reactions to construct genotypes. Genotypes constructed using these markers are then compiled and compared to one another to identify unique and shared genotypes and can used to produce many population statistics and structure visualizations. This data can provide more insight on how *P. capsici* is spread throughout a region, what degree of gene flow there may be between sampling locations, and whether populations are primarily reproducing clonally or sexually. From these studies, about three primary structures have been observed: widespread clonal populations, isolated sexual populations, and outcrossing sexual populations (Dunn et al. 2010; Hu et al. 2013; Gobena et al. 2012a; Castro-Rocha et al. 2017). These structures are greatly influenced by various factors such as weather, geography, agricultural practices as well as regional growing history. Given this, it is important to treat each region differently, and to avoid inferring the population structure of one location solely based on observations of another.

Little is known about the population genetics of *P. capsici* in Tennessee. It is hypothesized that given sampling locations are geographically distant, and because of the short distance spread of *P. capsici* that each sampling location would be highly diverse and significantly different from one another and there would be isolated mating populations. To investigate this hypothesis, isolates of *P. capsici* were collected in the years 2004, 2007, 2018

and 2019 from five counties in middle and east Tennessee (Figure 2.1; All tables and figures are found in the appendix). These isolates were genotyped using 39 SNP markers previously identified within the genome of *P. capsici* The three main objectives of the study were to: (i) Identify all mating types of each isolate and count the ratio of A1:A2; (ii) identify multilocus genotypes (MLG) and compare diversity of these between sampling locations; (iii) Identify significant differences between locations and among the populations within; and (iv) Visualize the structure of the populations to show if sampling locations are genetically similar or significantly different. By achieving these objectives, the study was designed to provide a significant amount of insight on the way that populations of *P. capsici* in Tennessee are structured, allowing for a much better understanding of the spread and reproduction trend of this pathogen across the state.

Materials and Methods

Sample collections

In 2018 and 2019, samples of infected plant material showing signs or symptoms of *P. capsici* were collected from farms in five counties throughout middle and eastern Tennessee. All samples were taken to the laboratory for sample preparation. In 2018, cuttings of lesions were surface sterilized using a 0.5% sodium hypochlorite solution, rinsed with sterile distilled water, and plated onto PARP-H medium (17 g cornmeal agar, 0.4 ml pimaricin solution (25 mg/ml), 250 mg ampicillin, 1 ml rifampicin solution (10 mg/ml), 5 ml PCNB solution (5 mg/ml), and 2 mg hymexazol per 1 L) (Ferguson and Jeffers 1999). Plated samples were stored at ~24°C in the dark for 1 to 2 days. From samples with mycelial growth, hyphal tip transfers were plated onto

additional PARP-H medium. All cultures were re-isolated as single-zoospore isolates and stored following the methods of Siegenthaler and Hansen, 2020. For DNA extraction a small amount of hyphal material was collected from each single-zoospore isolate and used to inoculate PARP-H broth aliquoted into a 24-well plate. Once inoculated, each plate was covered with a breathable sealing film and left to incubate at ~24°C, agitating the plate by shaking twice a day, every day for 5 to 7 days or until a mycelial mat formed on top of the broth within a well. Mycelia were then transferred into 96-well bead mill plates containing glass beads. Plates were placed in a -20°C freezer until further processing. In 2019, isolates were collected the same way as described for 2018, except that isolates were obtained by hyphal tip isolation rather than single zoospore isolation. For DNA extraction, instead of using mycelia, samples of infected plant tissue used for isolation were taken directly from lesions and placed into the same 96-well bead mill plates. Plates were stored in a -20°C freezer until further processing. Additionally, data from historical isolates, which were collected from one farm in Grainger County in 2004 and 2007, were provided by Dr. Kurt Lamour, University of Tennessee.

Mating Type

Mating types of all isolates were determined using the same methods as Siegenthaler and Hansen, 2020, where a 5-mm plug of each isolate with an unknown mating type was plated onto medium with another 5-mm plug of an isolate of a known mating type. Plates were incubated for 5 to 7 days and a compound microscope was used to look for the presence of oospores. The presence of oospores indicated that the unknown isolate's mating type was compatible with the known isolate's mating type.

DNA Extraction

Mycelium or plant tissue was freeze-dried in the 96-well bead plate and processed to a fine powder using a Mixer Mill (Qiagen, USA). The powder was processed using a MagMAX DNA Multi-sample ultra 2.0 kit according to the manufacturer's directions (ThermoFisher, USA) and the resulting DNA quantified using a Qubit device according to the manufacturer's directions (ThermoFisher, USA).

PCR and Sequencing

Approximately 1000 ng of DNA for each sample was submitted to Floodlight Genomics LLC (Knoxville, TN) for targeted sequencing. Thirty-nine SNP markers were amplified from the nuclear genome using different species specific primers for each site as described previously (Castro-Rocha et al. 2017). These were processed using an optimized Hi-Plex approach, which includes multiplex PCR amplification of each sample and sequencing of the targets using the Illumina HiSeq X platform. Floodlight Genomics delivered sample-specific raw sequence reads and all work was conducted at no cost as part of the FG-EROP (Educational and Research Outreach Program).

Sequence alignment and variant calling

The sample-specific reads were aligned to their target sequences using CLC Genomics Workbench version 9.5.3 using default settings. Variant sites were assessed using the Quality-based Variant Detection workflow with settings to require at least 40X coverage. Loci were considered heterozygous if the alternate allele had a frequency of at least 25%. Data were compiled from the resulting variant call format outputs and further processed in Excel.

Data analysis

Genetic data were analyzed using the excel plugin GENALEX 6.5, R version 3.6.1 and the R package, Poppr version 2.8.6 (Kamvar et al. 2014, 2015). GENALEX 6.5 was primarily used to format the data so it could be read and made an object within R and for calculating pairwise Fst population comparisons. Using Poppr, the number of multi-locus genotypes (MLG) per sampling location and year were identified. Isolates sharing MLGs were considered clones and data were clone corrected to account for these. To further understand the overall diversity of the samples collected in TN, Simpson's Index and Stoddart and Taylor's Index were calculated (Simpson, 1949; Stoddart and Taylor, 1988). Using discriminant analysis of principle component (DAPC), this genetic data was organized in a way which could visual potential structure between sampling population. In this analysis, populations were assigned by county and year, these were predefined as an input. Following the protocol contained in the Poppr primer, developed by Kamvar et al., first a DAPC cross validation was performed with 1000x replication and an optimal number of PCAs to be retained was 20, as well as retaining 500 DCs. The results from the DAPC cross validation were then plotted as a scatter plot. A minimum spanning network (MSN) was constructed to compliment the findings from the DAPC. This MSN was developed using the interactive tool provided through Poppr using Provesti's genetic distance. To detect population differentiation, analysis of molecular variance (AMOVA) was performed with additional significance testing through randomization tests (Excoffier et al. 1992)

Results

Isolate collection, genetic diversity, mating types, and Hardy-Weinberg equilibrium

In 2018 and 2019, 222 isolates of *Phytophthora capsici* were collected from symptomatic pumpkin, squash, zucchini, and pepper. An additional 74 isolates collected in 2004 and 2007 were provided by Dr. Kurt Lamour bringing the total number of isolates genotyped in this study to 296. Ninety-two isolates were from Rhea County, TN, 49 from Bledsoe County, 43 from Putnam County, 38 from Lincoln County, and 74 from Grainger County (Table 2.1) (Figure2.1). A total of 186 unique multi-locus genotypes (MLGs) and 109 repeated MLGs were identified (Table 2.1). One MLG was shared between two locations, B18 and P18. No MLGs that were found in any 2018 population were detected in 2019. Populations from Rhea 2018 (R18), Rhea 2019 (R19), Lincoln 2019 (L19), Grainger 2004 (G04), and Grainger 2007 (G07) contained the majority of these unique MLGs and were more diverse than Bledsoe 2018 (B18), Bledsoe 2019 (B19) and Putnam 2018 (P18). The disparity in genotypic diversity is reflected in the Stoddart and Taylor's Index or Simpson Index values calculated for each population, where R18, R19, L19, G04 and G07 all have indices that are much greater than that of B18, B19 and P18 (Table 2.1). In all populations excluding B19, P19 and G04, the A1:A2 ratios did not differ significantly from 1:1. B19 was the only population that did not have any isolates of A2 mating type. In a population-wise comparisons of Hardy-Weinberg equilibrium (HWE) statistics for all 39 loci and the eight populations, approximately 30% of loci significantly departed from the expectations HWE (Figure 2.2).

Population structure

Discriminant analysis of principal components (DAPC) grouped most isolates from the same county and the same year together. Isolates collected from the same county, but different year, were genetically similar as shown by the R19 and R18 or the G04 and G07 clusters (Figure 2.3). DAPC indicated that isolates that were geographically different are also genetically different. Similarly, geographically similar isolates have more genetic similarities. This suggest that the general population is structured by geographic location and that each sampling location is distinct from one another. The minimum spanning network (MSN) supports this result by illustrating that in general, isolates from different counties are genetically distant from one another (Figure 2.4). In a pairwise comparison of F_{ST} statistics all populations were statistically different at P value <0.05, even the isolates collected within the same field, but different year were distinct. These did have lower F_{ST} values such a 0.027 or 0.072, but these were still considered statistically different by this analysis (Table 2.2). The results of analysis of molecular variance (AMOVA) also support these results, where the amount of variation between populations is 17% and within populations is 83% (Table 2.3).

Discussion

Our analysis indicated that populations of *P. capsici* in Tennessee are structured as genetically isolated populations. These results are like the ones found in Dunn et al., 2012, where populations of *P. capsici* throughout New York were genetically diverse, and clustered by geographic location. This contrasts with studies where there was evidence of outcrossing populations with significant numbers of shared clonal genotypes across farms in Massachusetts and Long Island, New York (Gobena et al., 2012a; Castro-Rocha et al., 2012). Our study, like

the ones mentioned, identified isolates of both mating types A1 and A2, providing evidence of sexual reproduction within most Tennessee populations of *P. capsici*.

Populations P18 and B19 had significantly lower MLG diversity than other tested regions and contained large numbers of clonal genotypes. This was reflected by the mating type ratios of these populations, which were highly skewed to A1 or A2, rather than the expected 1:1 ratio. The main explanation for these differing results is rooted in the seasonal cycle of *P. capsici* reproduction and spread. In a climate like Tennessee, where winter temperatures reach freezing, zoospores should not survive from one growing season to another, except when chlamydospores are produced, which are not common (Islam et al. 2005). Therefore, at the beginning of a growing season, new epidemics are started through the germination of oospores. Oospores germinate to form hyphae that find a host, infect, and then produce zoospores or directly produce sporangia filled with zoospores, which begin to spread through a field. Therefore, early in the season, the genetic diversity of the population nears its peak and begins to decrease as clonal zoospores spread, infect, and propagate more clonal zoospores. As the season progresses, fit genotypes begin to dominate a field and the representation of the potential genetic diversity decreases. This is reflected in the populations P18 and B19, where isolates were collected later in the growing season, after harvest, when only culls and late-ripening fruit were left in the field. This is the most likely explanation for the lack of diversity given there was no evidence of clonal genotypes persisting from 2018 to 2019. These results contrast from the ones found in places such as China and Argentina, where large clonal populations persist from year to year (Hu et al. 2013; Gobena et al. 2012). These locations have mild to warm fallow seasons in contrast to the

colder often below freezing temperatures of Tennessee winters, and these climate differences explain why there is no persistent clonal genotypes.

One unexpected result was that one MLG was found to be shared between two counties that were geographically distant. This MLG was found five times total across the entire state and four of these were from P18 with one from B18. Even if the two populations are descended from a single founder population, the possibility of finding the same genotype at both locations could only be explained through the movement of plant material, soil, or water. This seems unlikely given the large amount of geographic distance between locations, but this cannot be ruled out. Most likely, this event is explained by a labeling error, which placed the one isolate from B18 into the P18 population. Without concrete evidence of either situation it is difficult to make a conclusion of this finding.

Ultimately, this study serves to provide further data that supports the current knowledge about *P. capsici* population genetics. Like Dunn et al. (2010), our results suggest that spread is limited between locations and that population genetics within a field can be very diverse from one another. When relating this to disease management, our findings continue to support spread mitigation as the first strategy. It is imperative that humans take all precautions to limit behavior that may aide in the spread of this pathogen. Cleaning and washing equipment, tools, and boots worn by workers are important. As well as ensuring clean seed, transplants, and irrigation water is being used in the process of growing vulnerable vegetable crops (Granke et al. 2012; Jones et al. 2014). Additionally, spread mitigation also is very important in reducing the chance that fungicide resistant isolates are not shared from farm to farm.

Further study in Tennessee should be done to improve the resolution of this testing. One limiting factor of this study is the small number of collection sites, and in the future, this should be increased to provide more data, which can provide more significance to results found in Tennessee. This study has generated a good initial snapshot of the *P. capsici* population genetics in TN, but there are still many locations in the state that have not been included in this analysis. Additionally, it would be important to collect over more seasons, prioritizing collection from the same fields each year to ensure the evolutionary story can be told across more time. At this point, *P. capsici* is here to stay in the state of Tennessee, and it is imperative that monitoring this situation continues if it is the desire of researchers, extension specialists and growers to understand the spread, survival, and evolution of this pathogen.

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Appendix

Table 2.1. Collection information, genotypic diversity statistics, and mating type of *Phytophthora capsici* from Tennessee in 2004, 2007, 2018 and 2019.

Pop[a]	N[b]	MLG[c]	eMLG[d]	λ[e]	λC[f]	G[g]	Mating type		χ^{2h}	*P*[i]	Collection Date
							A1	A2			
R18	20	18	17	0.94	0.98	16.6	14	10	0.67	ns	8/9/2018
R19	72	56	17	0.97	0.90	43.9	30	46	3.37	ns	8/31/2019
B18	19	10	10	0.83	0.87	5.9	7	13	1.8	ns	9/7/2018
B19	30	3	2	0.32	0.33	1.4	32	0	32	0.001	10/11/2018
P18	43	10	6	0.73	0.75	3.7	14	29	5.23	0.05	8/31/2018 & 11/16/2018
L19	38	21	13	0.93	0.96	15.7	14	23	2.19	ns	9/12/2019
G04	23	19	16	0.93	0.97	15.1	17	6	5.26	0.05	2004[j]
G07	51	50	18	0.98	0.99	49.0	26	25	0.02	ns	2007[j]
Total	296	186	-	-	-	-	154[k]	152[k]	0.01	ns	-

[a] Populations are labeled with a single letter signifying the collection county followed by collection year: R = Rhea, B = Bledsoe, P = Putnam, L = Lincoln, and G = Grainger.
[b] Number of isolates collected from each county and year.
[c] Number of multi-locus genotypes (MLGs) per county and year.
[d] The number of expected MLGs at the smallest sample size ≥ 10 based on rarefaction.
[e] Simpson's Index (Simpson, 1949).
[f] Simpson's Index (Simpson, 1949), adjusted for sampling size.
[g] Stoddart and Taylor's Index of MLG diversity (Stoddart & Taylor, 1988).
[h] Chi-square statistic testing whether mating types at each site are 1:1.
[I] Significance of chi-square test: ns = indicating mating-type ratio does not significantly deviate from 1:1.
[j] Exact collection dates are unknown.
[k] Mating type totals include data from isolate which were not genotyped for this study.

Table 2.2. Pairwise comparison of F_{ST} values of *Phytophthora capsici* populations sampled from eight counties in Tennessee from 2004, 2007, 2018 and 2019.

	F_{ST} values						
Populations	**R19**	**L19**	**B19**	**R18**	**P18**	**B18**	**G04**
R19	0						
L19	0.24	0					
B19	0.17	0.46	0				
R18	0.03[b]	0.27	0.23	0			
P18	0.16	0.34	0.37	0.19	0		
B18	0.13	0.20	0.30	0.11	0.22	0	
G04	0.14	0.24	0.33	0.17	0.23	0.13	0
G07	0.20	0.23	0.37	0.21	0.27	0.16	0.07

[a] Populations are labeled with a single letter signifying the collection county followed by collection year: R = Rhea, B = Bledsoe, P = Putnam, L = Lincoln, and G = Grainger.

[b] F_{ST} values were calculated in GenAlex 6.5 with distances between populations based on AMOVA of each population. All values were significantly different from zero at $P < 0.001$, using 999 permutations, unless indicated otherwise.

[c] Value was significantly different from zero at $P < 0.05$, using 999 permutations.

Table 2.3. Analysis of molecular variance (AMOVA) for clone-corrected samples of Phytophthora capsici from Tennessee collected in years 2004, 2007, 2018 and 2019.

Source of Variation	**df**	**Sum of squares**	**Variance components**	**Percentage of variation**	**P-value[a]**
Between populations	7	905	2.71	17	0.001
Within populations	187	2490	13.31	83	0.001

[a] P values based on randomized significance testing performed to test the significance of the variance components.

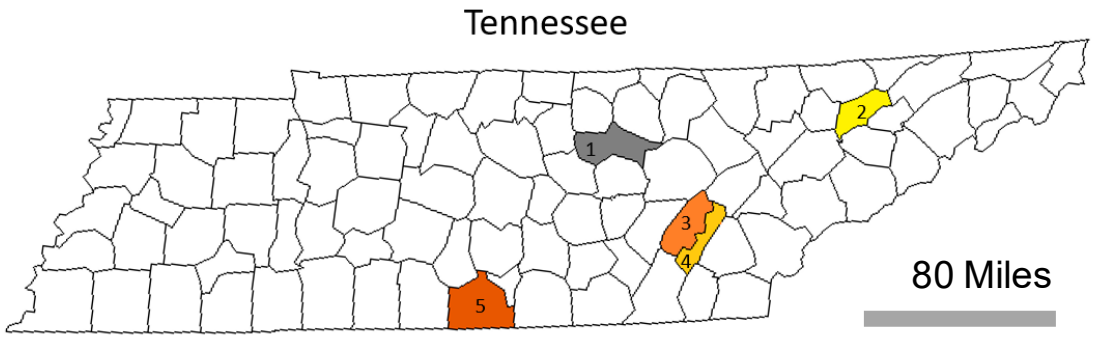

Figure 2.1. Map of Tennessee and the counties isolates were collected from in 2004, 2007, 2018 and 2019; 1 = Putnam County, 2 = Grainger County, 3 = Rhea County, 4 = Bledsoe County, and 5 = Lincoln County.

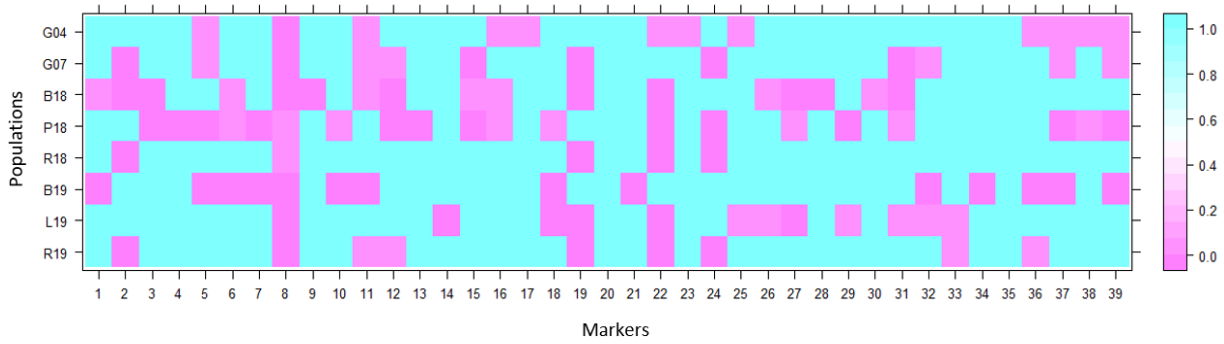

Figure 2.2. Heatmap of population-wise p-value comparisons of Hardy-Weinberg statistics between Tennessee populations and markers used in genotyping isolates collected in 2004, 2007, 2018, and 2019. All loci marked with pink are suspected of not being in HWE with $p \leq 0.05$.

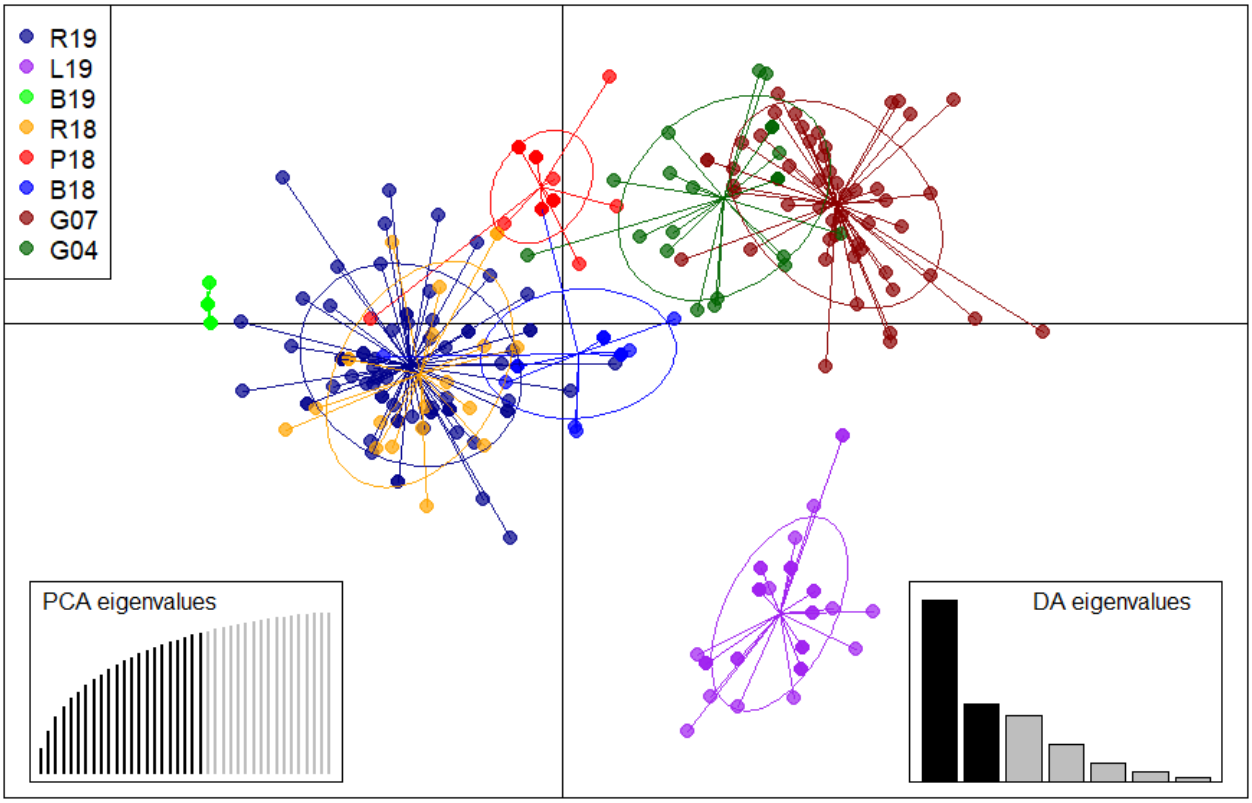

Figure 2.3. Discriminant analysis of principal components (DAPC) plot of all *P. capsici* isolates collected in 2004, 2007, 2018 and 2019.

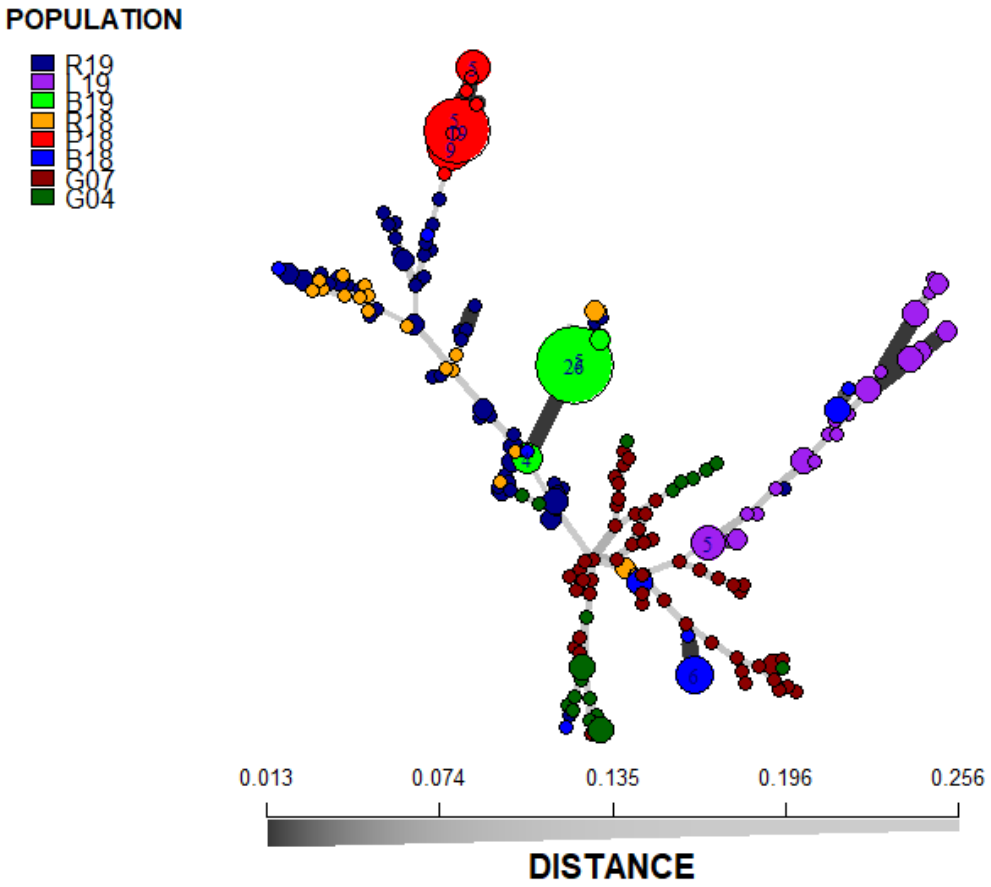

Figure 2.4. Minimum spanning network constructed using the Provesti's distance between isolates collected in Tennessee in 2004, 2007, 2018, and 2019.

CHAPTER 3

The effect of ground cover and fungicide on Phytophthora blight in Tennessee

This chapter was first authored by Timothy B. Siegenthaler with the editorial help of Dr. Zachariah Hansen (PI), Dr. Bonnie Ownley, Dr. Kurt Lamour, and Dr. Heather Kelly

Abstract

Cultural and chemical control strategies are very common in the management of plant disease. Managing fruit rot in vining crops such as pumpkin caused by *Phytophthora capsici* is challenging, and no current methods are effective. A study was conducted during 2019 and 2020 to test the potential efficacy of using ground cover and systemic fungicides to reduce disease incidence in pumpkin fruit. This study was conducted on a farmer's field that was naturally infested with *P. capsici.* Pumpkin seedlings were planted into raised beds covered with black plastic mulch with drip irrigation. There were four different treatments and six replications across the field set up in a randomized complete block design. Treatment one was a control with no ground cover or fungicide application, treatment two had only ground cover, treatment three had only fungicides applied, and treatment four had both ground cover and fungicides applied. This trial was conducted for approximately 14 weeks both years and at harvest data was collected. This resulted in finding that the early application of fungicide at transplant significantly reduced the loss of seedlings within the first 14 days after transplant. There were no significant results in any of the collected data such as, total yield, marketable yield, or unmarketable yield due to low disease incidence, which provided little data to determine the effects of treatment on fruit rot. Further study into cultural controls should be explored to help advise recommendations to growers.

Introduction

Phytophthora blight of cucurbits and pepper is caused by the oomycete *Phytophthora capsici* and was first reported on pepper in New Mexico, USA in 1922. The disease is now distributed across the United States and throughout the world (Leonian 1922; Gobena et al. 2012; Hu et al. 2013). Phytophthora blight can be devastating to production of many vegetables such as pumpkin, squash, cucumbers, and peppers (Hausbeck and Lamour 2004; Granke et al. 2012). Disease is characterized by plant wilt, crown rot, stem rot, fruit rot, and occasionally foliar symptoms, and can lead to complete crop loss (Figure 3.1) (All tables and figures are found in the appendix). Phytophthora blight is a very challenging disease to manage primarily because of how it reproduces and spreads.

Phytophthora capsici primarily spreads within a growing season through dissemination of asexual spores call zoospores. Zoospores are aquatic, motile, biflagellate spores which are produced within sporangia, which are structures that are located on the outer surface of infected hosts (Granke et al. 2012; Hausbeck and Lamour 2004). In the presence of water, the sporangia open and release zoospores into the surrounding liquid. These spores can quickly spread through water in or on the surface of soil within a field until a new host is located and a new infection begins. Since zoospores are spread through water, rainfall can influence the spread of *P. capsici* significantly leading to large outbreaks after high levels of rain. To manage disease caused by infections from zoospores, management strategies such as cultural and chemical controls must be implemented.

Cultural controls are methods of disease management that focus on manipulating the interactions of a plant pathogen with its host and the surrounding environment. For production of

vegetables, such as peppers and cucurbits, many cultural controls have been implemented to help manage disease. This begins by avoiding introduction of the pathogen initially. Introduction avoidance is primarily practiced by ensuring pathogen free water sources such as municipality, UV-treated, or chemical-treated water. Additionally, it is important to avoid bringing any potential contaminated soil, fruits, farm equipment or tools onto a clean farm. Growing recommendations include planting into raised beds covered with black plastic mulch and to establish plots in well drained soils on slopes and to avoid low areas within a field (Granke et al. 2012). These practices encourage water drainage and avoidance of water accumulation around the roots, crowns, and fruit. In theory, these cultural practices reduce disease pressure, but in practice they are almost always not enough to manage Phytophthora blight. In combination with chemical controls, the efficacy of cultural controls strategies can be increased, but disease is still often a major problem, especially in crop systems such as vining cucurbits where black plastic mulch offers no protection from soil contact and rain splash.

Chemical controls are a valuable tool in managing Phytophthora blight and are often the primary strategy used in vegetable production in the U.S. Fungicides are the main class of chemicals used to manage fungal or oomycete pathogens. In the case of Phytophthora blight, there are many different products labeled to control it with differing modes of action. Traditionally, the primary chemical used to manage Phytophthora blight has been mefenoxam. Over time, chemical resistance has been identified in many different states, including Michigan (Lamour and Hausbeck 2000), New York (Dunn et al. 2010), North Carolina (Cafe and Ristaino 2008), South Carolina (Keinath 2007), Georgia (Jackson et al.2012), Florida (Ploetz and Haynes 2000) and Tennessee (Siegenthaler and Hansen 2020). Other chemicals have been developed,

such as fluopicolide, cyazofamid, mandipropamid, dimethomorph and oxathiapiprolin. These have varying levels of efficacy, different modes of action and some populations of *P. capsici* have begun to develop resistance to a few of these chemicals. In a previous study (Siegenthaler and Hansen 2020), these chemicals were evaluated for efficacy against *P. capsici* from Tennessee. Dimethomorph, mandipropamid and oxathiapiprolin all had good efficacy and little to no isolate had any insensitivity to any of these chemicals. These characteristics made them good candidates to be used as the fungicides in this trial.

Managing fruit rot in vining crops, such as cucurbits, can be very difficult to manage because there are no cultivars available with resistance to *P. capsici* fruit rot, and the cultural or chemical controls mentioned previously are thought to do little to protect fruit. Therefore, it is important to explore other cultural options to potentially provide disease control, such as ground covers to help reduce fruit rot and improve yields in crops like pumpkins. In other crops, such as strawberry, the use of straw mulch was tested to reduce transmission of *Phytophthora cactorum* onto fruit through rain splash. These studies provided evidence that ground cover could reduce disease incidence within these systems from rain splash (Ntahimpera et al. 1998; Madden and Ellis 1990). In another study, no-till cover crops have been utilized as ground cover in managing *P. capsici* in pepper crops (Ristaino et al. 1997). Here, a cover crop such as winter wheat is planted before winter and is then rolled sown flat covering the surface of the soil. This method reduced disease as compared to bare soil as well as the traditional black plastic mulch.

In this study, the main hypothesis was that ground cover in the form of straw mulch would provide a barrier between the soil and pumpkin fruit, reducing the incidence of disease compared to bare soil. Additionally, this study evaluated whether the application of fungicides,

combined with straw mulch, is sufficient to manage Phytophthora blight under high disease pressure.

Materials and Methods

Field trial establishment

The trial was conducted during the summers of 2019 and 2020 at Little Creek Produce (LCP) in Putnam County, TN on silty clay loam soil. LCP is a vegetable farm with a history of growing cucurbits with moderate to severe incidence of Phytophthora blight. Two 100-cell flats were seeded with Thiram-treated 'Magic Lantern' pumpkin seeds (4.5oz/100lbs of seed, Bayer CropScience LP, Research Triangle Park, NC) into potting mix on June 14th, 2019 and June 4th, 2020. Seedlings were maintained under lab lighting with an 18-hour day/night cycle and then moved into a greenhouse approximately three weeks after seeding. Seedlings were transplanted into raised beds covered in black plastic mulch on July 12th, 2019 and June 22nd, 2020. Drip irrigation tape was laid on top in 2019 or under the plastic in 2020 and connected to a water source managed by the farm manager. Plant beds were spaced 25 ft apart and were approximately 100 ft long. Plots were 12 ft long with 20 ft spacing within rows and plants were spaced 4 ft apart with four plants per plot.

The experiment included four treatments and evaluated two variables: between row soil cover with straw mulch (M) and the efficacy of fungicides (F). The four treatments were: (1) -M-F, which included bare soil and no fungicides applied, (2) +M-F, which included straw mulch as a ground cover and no fungicides applied, (3) -M+F, which included bare soil and fungicides applied, and (4) +M+F, which included straw mulch as ground cover and fungicides applied

(Table 3.1) (Figure 3.2). Straw mulch was purchased in standard-size bales from Knoxville Seed and Greenhouse. Mulch was applied in a 12 ft × by 16 ft area on each side of the plant bed to cover the soil thoroughly. At the time of transplanting, all seedlings were treated for insect control with 150 ml of water containing Coragen (FMC Corp., Philadelphia, PA; 7.5 fl oz/acre) combined with MANA Alias (MANA Inc., Raleigh, NC; 12 fl oz/acre). An additional 150 ml of untreated water was applied to each seedling as a soil drench. For treatments receiving fungicides (+F), 150 ml of water containing Orondis Gold was applied at planting as a soil drench (9.6 fl oz/acre, Syngenta Crop Protection, Greensboro, NC) instead of the 150 ml of untreated water. Following planting, fungicides were applied on a 10-to-14-day spray interval. Orondis Ultra (8 fl oz/acre; Syngenta Crop Protection, Greensboro, NC) and Zampro (14 fl oz/ acre; FMC Corp., Philadelphia, PA) were alternated each spray to avoid consecutive applications and applied no more than three times during the growing season. These fungicides were applied using a pressurized CO_2 backpack sprayer with a two-nozzle boom. For all applications, the sprayer was pressurized to 30 psi and treatments were applied to thoroughly cover the plant canopy. Prior to vining of plants, Paraquat® was applied to control weeds between rows by the farm manager with a hooded sprayer. The trial was arranged in a randomized complete block design with six replicates.

Data collection and analysis

Harvest of each trial occurred on October 17th, 2019 and September 23rd, 2020. At the harvest of each trial, data collected included total number of fruits, number of diseased fruit total yield, marketable yield and unmarketable yield. Fruits were determined to be diseased when clear symptoms or signs of *Phytophthora capsici* infection were present. Fruit were considered

marketable if they remained free from rot, or major blemishes and had intact handles. All data was analyzed in R version 3.6.1 using core packages. Data was normalized using square root transformation and each treatment effect was analyzed separately using a linear mixed effect model with treatments as fixed effects and replications as random effects. Means were separated using Tukey's Honestly Significant Difference (HSD) at P=0.05.

Results

Plant Count Yield and Fruit Counts

In 2019 there was a significant loss of plants in treatment one (T1) plots within the first 14 days of transplant (Table 3.2). The average plant count of T1 was significantly less than treatment three (T3) and treatment four (T4), but not treatment two (T2) (Table 3.1). On average, T1 plots lost approximately one plant, whereas treatments 3 and 4 lost zero. In 2020, there were no significant differences in plant count between treatments. In 2019 and 2020 total yield, unmarketable yield, and marketable yield were not significantly affected by any treatment. In 2019, the average unmarketable weight per treatment was 3.4 kg compared to 9.65 kg in 2020 (Table 3.3). For fruit counts at harvest, the total count in 2019 was the only significant result. Treatment one had significantly less total fruit than T4, with an average for 2.8 fruits compared to 7.8. There were no significant differences in total fruit count in 2020. In 2019, marketable count and unmarketable counts were recorded, and no significant differences were found. In 2020 these data were not collected due to a recording error. Diseased fruit counts were recorded at harvest of both years, and there were no significant effects of treatment on diseased fruit counts.

Discussion

In this study it was demonstrated that, in general, the treatments of straw mulch ground cover and the application of fungicides did not reduce disease incidence or influence yield and fruit counts significantly from untreated controls. However, the effects of fungicides on plant count at 14 days after transplanting, and total fruit count at harvest, were significant in 2019. T1 plots, which did not receive fungicides or mulch at the time of transplant, suffered early plant loss in comparison to T3 and T4. T2 plots followed a similar trend as T1, but these results were not statistically different from T3 or T4. This early plant loss led to significant differences in the final fruit count at harvest in T1. The total fruit count of T1 was statistically lower than T4. This result makes sense given that T1 plots had a much lower number of plants that could bear fruit compared to T4 plots, which ultimately reduced the potential final count at harvest. In 2020, this result was not observed. One explanation could be that inoculum pressure may have been higher in 2019 than in 2020. The 2019 trial was planted into the field following a full season of pumpkin production that had high incidence of disease. In 2020, the field would had reduced inoculum pressure due to a small plot of pumpkins being grown the previous year that had little disease. This potentially led to lower rates of disease early in the 2020 growing season, which resulted in lower numbers of dead plants in untreated plots. These results indicate that early fungicide applications can reduce early plant loss, presumably due to seedling blight or crown rot, when conditions are favorable for disease. This is supported by other studies on the effectiveness of transplant soil drenches of both oxathiapiprolin and metalaxyl against *P. capsici* and *Pythium spp*. (M. F. Male and L. L. Vawdrey 2010; Ji and Csinos 2015; Matheron and Porchas 2015)

Beyond this exception, these results differ from past studies focused on field efficacy of fungicides against Phytophthora blight (Matheron and Porchas 2014, 2015; Ji and Csinos 2015). These three studies evaluated the effectiveness of fungicides such as oxathiapiprolin, dimethomorph or mandipropamid, to reduce symptoms of Phytophthora blight on plant material. They reported that these fungicides are effective in reducing disease or severity of disease. This difference is likely a reflection of the low disease incidence in both years of this study. In 2019, no fruit at harvest could be identified as having any signs or symptoms of rot cause by *P. capsici*. In the following 2020 season, disease incidence was significantly higher, on average, less than one fruit per plot had signs or symptoms of Phytophthora blight. Ultimately, disease incidence was too low in this study to determine if treatments had any effect on harvest metrics. Additionally, there were many other factors that affected plant survival and fruit quality. In 2019, there were serious issues with drought and lack of consistent irrigation. This trial was held on a local farmer's plot and management of standard production practices, such as irrigation, were left in their care. Unfortunately, in 2019 many plants died in the middle of the season leading to significant losses not attributed to disease. This lack of rain could be a primary explanation of why disease incidence was so low in 2019. *Phytophthora capsici* needs soil moisture to thrive and continue to spread throughout a field. One study done to test the effects of rainfall on *P. capsici* found that disease greatly increased with rainfall (Bowers et al. 1990). In 2020, weed and disease pressure were high with much of the unmarketable weight being attributed to fruit rot and plant wilt from other pathogens. High levels of *Fusarium* spp., and low levels of *Athelia* (*Sclerotium*) *rolfsii* (southern blight) led to an increase in unmarketable fruits. General plant decline was attributed to *Pseudoperonospora cubensis* (cucurbit downy mildew), and

Plectosporium tabacinum (Plectosporium blight). Downy mildew disproportionately affected plants in T1 and T2 plots because they were not treated with the treatment fungicides, which are also active against *Pseudoperonospora* spp. In general, there were many difficulties in managing all these additional factors that affected the outcome of this study. Weed management challenging in both years of the trial. Both years had two applications of an herbicide applied early in the season. This treatment was generally effective in between rows in 2019, but weeds were a problem still because the plastic mulch was very damaged and allowed growth around the pumpkin plants eventually killing them in the middle of the season. In 2020, the herbicides were much less effective and weed control was a complete failure. The main reason that the herbicides were less effective in 2020 was due to a change in procedure. In 2019, the soil was cultivated before transplanting, but in 2020 this was not done per the request of the grower. The lack of soil cultivation allowed for a much greater number of weeds to survive the herbicide application leading to a larger population by the middle of the pumpkin growing season. The challenges of on-farm studies coupled with limited resources, while working during a pandemic, led to failures in the studies' effectiveness to determine the efficacy of the treatments being tested.

Considering all the factors presented, this study produced inconclusive results. In the future there should be more research focused on developing and testing new cultural and chemical control methods for Phytophthora blight management. If possible, this study should be repeated in a more controlled environment where more of the outside variables could be controlled, especially irrigation and weed management. A university research station would be best for this kind of work. Many more variables could be controlled in this situation such as inoculum load, weed pressure, and consistent irrigation. Unfortunately, *P. capsici* research would

be very dangerous to the future sustainability of most field stations. Many precautions would need to be taken such as having dedicated farm equipment and tools to avoid spreading propagules to other parts of the station. Even then, it could be very difficult to manage *P. capsici* on a research station. Hopefully, in the future these potential studies could provide more conclusive data about whether ground cover combined with fungicides can improve the outcome of growing cucurbits under the pressure of *P. capsici.*

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Appendix

Table 3.1. Pumpkin field trial plot design

Variables	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Straw Mulch	No	Yes	No	Yes
Fungicide	No	No	Yes	Yes

Table 3.2. Average fruit counts, diseased count, plant counts at 14 days after transplant, and yield data at harvest of 2019 and 2020 trials.

Years	Treatments	Total Yield (kg)	M[a] Yield (kg)	UM[a] Yield (kg)	Total Count	M[a] Count	UM[a] Count	Disease Count	AVG Plant Count
2019	1	7.0	6.0	1.0	2.8A[b]	2.4	0.4	0	3.0A[b]
	2	19.4	16.0	3.3	8.4AB	3.6	3.4	0	3.3AB
	3	15.6	9.4	6.2	6.8AB	3.8	3.0	0	4.0B
	4	19.4	16.3	3.1	7.8B	3.8	3.2	0	4.0B
2020	1	18.8	12.4	6.4	6.0	.	.	0.5	3.5
	2	14.7	2.7	12.0	6.5	.	.	0.5	3.7
	3	22.0	9.4	12.6	7.3	.	.	1.5	3.8
	4	22.6	15.0	7.6	7.5	.	.	0.8	3.8

[a] M = Marketable, UM = Unmarketable.

[b] Values followed by different letters are significantly different, Tukey's HSD $P<0.1$.

[c] (.) represents unrecorded data due to procedural error.

Figure 3.1 Image of sporulating pumpkin fruit infected with *P. capsici* taken on August 31st, 2018 in Cookeville, Tennessee.

Figure 3.2. 2019 pumpkin field trial located in Cookeville, TN, with straw mulch vs. bare soil treatments visible. Photo taken on July 12th, 2019.

CONCLUSION

Fungicide Sensitivity

The purpose of this study was to determine if isolates of *Phytophthora capsici* in Tennessee are resistant to any of the fungicides marketed to control Phytophthora blight of cucurbits and pepper such as mefenoxam, fluopicolide, cyazofamid, oxathiapiprolin, mandipropamid, and dimethomorph. Based on previous studies done in other states, it was expected that there would be fungicide-resistant isolates in Tennessee (Dunn et al. 2010; Lamour and Hausbeck 2000; Jackson et al. 2012; Keinath 2007). This study found that isolates of *P. capsici* from Tennessee were resistant to four of the six fungicides tested. These four fungicides were mefenoxam, cyazofamid, fluopicolide, and oxathiapiprolin. These results are significant because they were the first report of fungicide-resistant isolates in Tennessee. Additionally, to our knowledge, this is the first report of fluopicolide and oxathiapiprolin resistance in *P. capsici*.

The implications of this study lie in how they can better inform the recommendations of the University of Tennessee Extension program. Plant pathogens such as *P. capsici* can be very diverse from state to state, and what may be true in past research may not apply in Tennessee. It is very important to have primary research that applies to each state in order to ensure that management recommendations can be tailored to the needs of local growers. Additionally, it is important for general *P. capsici* research to continue testing in every state to potentially find new mutations. This provides valuable information to other researchers and can inspire or inform future projects within this field of study.

Population Genetics

This experiment was designed to investigate the genetic diversity and population structure of *Phytophthora capsici* in Tennessee by using genetic markers, sequencing, and

genotype analysis. We hypothesized that the individual populations of *P. capsici* across Tennessee would be genetically isolated and structured by geographic location. We also hypothesized that these populations would be highly diverse and actively reproducing sexually. This was based on population genetic studies done previously in states like New York, where populations of *P. capsici* throughout the state are structured by their location and are genetically diverse (Dunn et al. 2010).

Populations of *P. capsici* in Tennessee were determined to be statistically differentiated, structured by geographic location, genetically diverse, and reproducing sexually. The statistics and analyses performed provided good evidence and support for the hypotheses of this study. This suggests that there is no spread of *P. capsici* from farm to farm, and that there has been no recent sharing of genotypes. In addition, there is currently no evidence of outcrossing, and each population, except one, had evidence of sexual reproduction. Overtime, these populations will probably continue to diverge genetically and become more and more diverse. Ultimately, this study provides new findings about populations of *P. capsici* in Tennessee, which helps us understand more about the spread, survival, and evolution of this pathogen in the state.

Field Study

`This trial was designed to test the efficacy of ground cover, such as straw mulch, combined with fungicides in reducing incidence of Phytophthora blight of pumpkins. We hypothesized that by using ground cover, which protects pumpkin fruit from contacting *P. capsici* infested soil, losses to fruit rot would be reduced. This was based on previous research that concluded ground cover protected plants from rain splash and transmission of other plant pathogens (Yang and Madden 1993; Madden and Ellis 1990). Additionally, we hypothesized that

application of fungicides would protect the pumpkin plants from other disease symptoms such as seedling death and crown rot. Ultimately this research concluded that the majority of the treatments had no significant effect on any disease incidence or harvest metrics, such as total yield. The main exception is that there was a significant loss of plants at 14 days in plots treated with no fungicide or ground cover compared to the ones with fungicides applied. Additionally, the same plots with no fungicide or ground cover treatments had significantly less fruit at harvest compared to plots treated with fungicides and ground cover. Ultimately this study was mostly inconclusive and provided little evidence to accept or reject the hypotheses. This is because disease incidence was very low both years of study, which did not allow for the effect of treatments to be detected. More research needs to be done in this area to fully determine if ground cover is effective in controlling fruit rot caused by Phytophthora blight.

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VITA

Originally from Ohio, Timothy Brent Siegenthaler grew up in Canton, Ohio. After high school, he attended Kent State University and received a Bachelor of Science degree in Biological Sciences. After taking courses taught by a plant pathologist and experiencing the world of plant disease, Timothy knew he wanted to pursue a career in research within this field. He chose to attend the University of Tennessee, Knoxville in 2018 to pursue a Master of Science degree in Plant Pathology. His research included testing *in vitro* sensitivity of plant pathogens to fungicide, population genetics and testing disease management strategies in a field setting. After graduation, Timothy is going to search for work in biological research and potentially move onto pursuing a Doctor of Philosophy in Plant Pathology or a related field. He is very grateful for the opportunity to attend the University of Tennessee, Knoxville and be a part of the Department of Entomology and Plant Pathology.