GRAPEVINE TRUNK DISEASE IN MISSOURI VINEYARDS: PREVALENCE AND CAUSALITY OF FUNGAL SPECIES BY CULTIVAR

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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FUNGAL SPECIES BY CULTIVAR

Presented by Emily Serra,

a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Grapevine Trunk Disease (GTD) is an emerging fungal disease complex in vineyards worldwide. In 2019, pruning wood samples from a research vineyard in New Franklin, MO were collected from nine cultivars of *Vitis* interspecific hybrid cultivars, in which GTD pathogens were found at various frequencies depending on the cultivar. DNA was extracted from 48 individual isolates and submitted for Sanger Sequencing. White grape cultivars (63%) had significantly higher percentage of GTD than red grape cultivars (27%) ($p<0.001$). Cultivar Aromella had the highest percentage of GTD pathogens (88%), while Prophecy had the lowest percentage of GTD pathogens in its sample vines (12%).

The more prevalent GTD pathogens in the samples (*Diplodia seriata*, *Neofusicoccum parvum*, *Neofusicoccum ribis*, and *Pestalotiopsis uvicola)* were then subjected to Koch's Postulates, which is the plant pathology method for determining whether an organism causes a disease. Koch's Postulates were evaluated in a greenhouse by inoculating shoots of *Vitis* interspecific hybrid cultivar Vignoles, the most widely planted white grape cultivar in Missouri. Shoots were inoculated and placed in a greenhouse for ten days. All shoots inoculated with the fungal endophytes displayed symptoms of GTD, including brown tissue around the wound site, demonstrating that these regionally prevalent endophytes can rapidly cause symptoms consistent with GTD on young Vignoles vines. Examining the differing prevalence of these GTD-causing pathogens in various interspecific hybrid cultivars will aid in the development of cultivar-specific management strategies for GTD.

In a separate experiment, a survey of the incidence and severity of symptomatic vines was conducted at a commercial vineyard in Missouri. This site contained a block of *Vitis* interspecific hybrid cultivar Vidal blanc being removed, with much of the vines showing symptoms of GTD in the vascular tissue of the trunks. This research sought to evaluate the disease severity from the trunks in the vines being rogued. Although there was no statistical significance between the severity ratings and fungal species recovered from *Vitis* interspecific hybrid cultivar Vidal blanc samples ($p=0.7153$), 90% of the randomly sampled vines showed visual GTD symptoms, including cankers and brown discoloration of wood.

The experiments reveal that *Vitis* interspecific hybrid cultivars may have varying susceptibilities to GTD pathogens, with white cultivars having a higher incidence than the red cultivars studied. Additionally, young Vignoles vines can show symptoms of GTD in just 10 days after inoculation with four GTD pathogens. Both of these results have management implications, in that cultivars may need to be managed differently for GTD depending on whether they are a red or white grape cultivar, and sanitation practices may need to be initiated at the time of planting to prevent the development of GTD symptoms in young vines.

CHAPTER 1

Introduction

The research topics in this thesis pertain to Grapevine trunk disease (GTD) in Missouri vineyards. GTD is a fungal disease complex consisting of 133 fungal species from 34 genera (Gramaje et al. 2018). The disease complex is characterized by fungal pathogens colonizing the xylem tissue of the wood, which can spread from pruning cuttings, and environmental conditions such as wet weather and warm temperatures (Úrbez-Torres et al. 2010, Van Niekerk et al. 2011). The most common symptoms associated with GTD include cankers, brown streaking of wood, discoloration of wood, and general vine decline that leads to vine death (Mugnai et al. 1999).

Symptoms associated with the presence of GTD pathogens are cankers, lack of foliar growth, black streaking of wood, brown discoloration of wood, and soft-yellow wood (Urbez-Torres 2012). It is important to understand the correlation between fungal species associated with symptomatic vines in Missouri to help grape growers identify symptoms and to pave the way for more research into finding solutions to mitigate future infection.

Trunk disease fungal pathogens can infect young vines in nursery settings, as was demonstrated in the study by Fourie and Halleen (2004) that found GTD pathogens in two-year-old rootstock canes infected from mother plants and another by Giménez-Jaime (2006) that revealed all Spanish nurseries surveyed had infections caused by GTD fungal pathogens. Fungal pathogens are present during the propagation process and can impact young vineyards. However, there is a lack of research on defining disease symptoms in young vines, especially those of hybrid cultivars that are of economic importance to Missouri. It is important to establish a better understanding of the symptom expression in young hybrid cultivars so that management practices can be undertaken earlier by those managing young vine plantings. Additionally, while previous work has been done on completing Koch's Postulates on common GTD-associated fungal pathogens (Úrbez-Torres 2011, 2013), it has not been completed on young *Vitis* interspecific hybrid cultivars relevant to the Missouri wine industry. The cultivar Vignoles is the most widely grown white grape cultivar in Missouri, spanning 262.2 acres and encompassing 15.4% of the total acreage for grapevine growing (USDA National Agricultural Statistics Service Missouri 2016). By examining Koch's Postulates on young *Vitis* interspecific hybrid cv. Vignoles with common GTD-associated pathogens recovered from Missouri vineyards, growers can better manage newly planted Vignoles blocks and prevent GTD infection by understanding the causality of these common pathogens in young vines.

While the susceptibility of *Vitis vinifera* cultivars to GTD in grape growing regions outside of Missouri has been reported, there has been limited research on the susceptibility of GTD fungal pathogens in *Vitis* interspecific hybrid cultivars commonly grown in Missouri. Economically important cultivars grown in Missouri vineyards include cultivars Norton (20.7% of the total grapevine acreage), Chambourcin (10.5% of the total acreage), Vidal blanc (8.2% of the total acreage), and Vignoles (15.4% of the total acreage) (USDA National Agricultural Statistics Service Missouri 2016).

The goal of this research is to: (1) understand the incidence and severity of GTD pathogens in a wide range of *Vitis* interspecific hybrid cultivars; (2) evaluate the incidence and severity of GTD pathogens in a symptomatic block of Vidal blanc; and (3), describe the symptom expression and determine causality of common GTD-associated fungal pathogens in young (<2 year old) *Vitis* interspecific hybrid cultivar Vignoles in a greenhouse study.

CHAPTER 2

Literature Review

History

Grapevine trunk disease (GTD) is an emerging fungal disease complex in vineyards around the world. It consists of Eutypa Dieback, Esca, and Botryosphaeria Dieback. The disease complex has been studied as early as the early-20th century in the United States with Reddick's research on the dead arm disease in 1914 (Reddick 1914). According to Mugnai et al. (1999), Esca may be as old as vine cultivation itself (Mugnai et al. 1999). GTD has increasingly gained attention of growers and researchers alike. However, GTD is not fully understood (Bertsch et al. 2013). Additionally, the increasing area of vineyards in the 1990s led to more prevalent symptoms and changes in production methods such as mechanized pruning, which can result in a greater number of pruning wounds (Gramaje et al. 2018).

A survey of fungal pathogens associated with GTD in Arkansas and Missouri showed that the most prevalent fungi were *Botryosphaeria* spp., *Diatrypella* spp., *Eutypa* spp., *Pestalotiopsis* spp., and *Plasmopara viticola* (Úrbez-Torres et al. 2012). These fungi inhabit the xylem of grapevines and result in general vine decline, decreased fruit yield, and eventual vine death (Larignon and Dubos 1997; Wicks and Davies 1999). In California, losses of $260 million per growing season were attributed to GTD (Siebert 2001). In South Australia, Eutypa dieback caused a loss of $2800 per hectare in a Shiraz vineyard during the growing season (Wicks and Davies 1999).

Epidemiology

One hundred and thirty-three species of fungi in 34 genera of ascomycetes and basidiomycetes are associated with GTD, making the fungal species of GTD the largest group of pathogens known to infect grapevines (Gramaje et al. 2018). Eutypa Dieback, Esca, and Botryosphaeria Dieback are the main diseases associated with the GTD complex (Mugnai et al. 1999). The fungal pathogens enter the plant through pruning wounds and are dispersed via ascospores or conidia onto the surface of the pruned vine during favorable environmental conditions such as rain, high humidity, and warmer temperatures (Úrbez-Torres et al. 2010). Spores from the fungi can spread via rain, wind, or arthropods and colonize the xylem after landing on the pruned wood (Moyo et al. 2014). In plants over eight years old, Esca symptoms consist of vascular and foliar symptoms. The vascular symptoms include white rot inside the trunk and branches of grapevines. Symptoms on leaves include chlorosis or spotting near the veins of the leaf or the margins (Mugnai et al. 1999). Because GTD cannot be completely eradicated, prevention and mitigation are the main methods of control (Úrbez-Torres and Gubler 2011). GTD pathogens are also known to cause cankers on the wood (Gramaje et al. 2016).

Common GTD-Associated Fungal Species

Some of the commonly known fungal pathogens associated with GTD are *Eutypa lata*, *Neofusicoccum parvum*, *Diplodia seriata*, *Phaeomoniella chlamydospora*, and *Phomopsis viticola*. *Eutypa lata*, which causes grapevine dieback known as Eutypa dieback, is an ascomycete fungal pathogen that affects perennial plants worldwide (Carter et al. 1983). *Neofusioccum parvum* is one of the more aggressive causal agents of GTD, as it forms a canker in the grapevine wood and can lead to wilted leaves and bud mortality but does not directly colonize leaves (Massonnet et al. 2017). *Neofusicoccum ribis* and *Diplodia seriata* are trunk disease pathogens of Botryosphaeriaceae species (Wunderlich et al. 2011). According to Kovács et al. (2017), *Diplodia seriata* affects GTD incidence in Hungary regardless of topography, soil type, and age of grapevines (Kovács et al. 2017). *Phaeomoniella chlamydospora* causes Esca and Petri Disease, which is the decline of young vines (Larignon and Dubos 1997). Pouzoulet (2017) reported that the resistance of *Phaeomoniella chlamydospora* was correlated with xylem vessel diameter, where larger diameter vessels had a higher incidence of the pathogen than smaller diameter vessels (Pouzoulet et al. 2017). *Phomopsis viticola* is another causal agent of grapevine trunk disease (Hewitt and Pearson 1988). This fungus colonizes mature grapevine tissue, resulting in the development of cankers (Úrbez-Torres et al. 2013).

Toxins Causing Vine Decline Symptoms

Toxins of fungi *E. lata, Phaeoacremonium aleophilum*, and *Phaeomoniella chlamydospora* produce decline symptoms from GTD (Andolfi et al. 2011). The secondary metabolites that *E. lata* produces (acetylenic phenols and eutypine) are the most phytotoxic (Andolfi et al. 2011). According to Andolfi et al. (2011), the synthesis of eutypine occurs inside the trunk of the grapevine, which is then transported via the sap to leaves and flowers. Eutypine also modifies the rate of respiration in *Vitis vinifera* leaf cells by targeting the mitochondria. Because Eutypa toxins are non-specific, they appear to be important virulence factors, which cause symptoms of Eutypa Dieback (Andolfi et al. 2011).

Factors That Can Affect Pathogen Virulence

Pathogens in the vineyard are dependent on a variety of factors for survival, including environmental conditions, such as temperature and rainfall. Cultural practices such as pruning techniques, trellis systems, and the virulence of the pathogen also affect the prevalence of GTD (Rolshausen et al. 2010). Pathogen distribution seems to be influenced by climate conditions in various regions (Van Niekerk et al. 2011). A study conducted in various regions of South Africa listed common symptoms as: black streaking, brown streaking, chlorosis, brown soft wood rot, and yellow soft wood rot (Van Niekerk et al. 2010). In marginal rainfall regions, wedge-shaped necrosis occurred most frequently. Temperature and rainfall influence both the distribution of pathogens as well as the symptomology of the pathogens in each region (Van Niekerk et al. 2011). Understanding how climate affects GTD may be important for establishing future vineyard management practices with the changing climate.

GTD pathogens can also impact other crops. For instance, a study conducted by Cloete et al. (2011) revealed that fungi associated with GTD were also pathogenic to apple (*Malus domestica* cultivar ‘Granny Smith’) and pear (*Pyrus communis* cultivar ‘Packham’s Triumph’) trees with similar symptoms to that of GTD in grapevines. The symptoms were brown vascular streaking, wedge-shaped necrosis, brown internal necrosis, and soft rot. The fungal pathogens found with the pear and apple samples included *Botryosphaeria* spp., *Eutypa lata*, *Phaeoacremonium* spp., and *Diplodia seriata*. GTD pathogens in orchards are a possible threat of the disease finding its way to vineyards located within close proximity (Cloete et al. 2011).

Koch's Postulates with GTD-Associated Organisms

Although several fungal species have been associated with GTD, this does not determine whether or not an organism is a causal agent of the disease unless Koch's Postulates have been completed (Agrios 2005). To determine the causality of a pathogen by Koch's Postulates, the pathogen is sampled from the host for isolation in pure culture. Next, the presumed pathogen is inoculated onto a healthy host to show the same symptomology and then the pathogen is recovered from the inoculated host and re-isolated onto pure culture.

Koch's Postulates have been completed on some but not all of the growing list of fungal species associated with GTD. A study conducted by Úrbez-Torres and Gubler (2011) showed that Koch's Postulates were successfully completed on own-rooted cuttings of *Vitis* interspecific hybrids cultivars Chambourcin, Norton, Traminette, and Vignoles with 14 fungal species, including *Botryosphaeria dothidea*, *Diatrypella sp.*, *Diplodia seriata*, *Dothiorella americana*, *Eutypa lata*, *Eutypella vitis, Lasiodiplodia missouriana*, *Lasiodiplodia viticola*, *Neofusicoccum ribis*, *Neofusicoccum vitifusiforme*, *Pestalotiopsis sp.*, *Pestalotiopsis uvicola*, *Phaeomoniella chlamydospora*, *Phomopsis viticola*, and *Togninia minima*. The cuttings were placed in individual pots and maintained in a greenhouse for 24 weeks, which revealed development of necrotic tissue and vascular discoloration (Úrbez-Torres and Gubler 2011).

A later study conducted by Úrbez-Torres et al. (2013) showed that *Phomopsis viticola* was a causal agent of GTD in mature cordon tissue of 14-year-old *Vitis vinifera* cultivar Syrah vines in California two years after inoculation. Vascular discoloration from

Phomopsis viticola was found on inoculated cordon tissue at lengths of 28.4 to 35.9 mm with no significant differences between the discoloration caused by *Botryosphaeria dothidea* (Úrbez-Torres et al. 2013).

In China, Koch's Postulates were completed on 16 strains of *Diaporthe* (*Phomopsis*) on *Vitis* spp. (Dissanayake et al. 2015). In Iran, Koch's Postulates were completed on four species of *Phaeoacremonium spp*., *Phaemoniella chlamydospora*, *Cylindrocarpon liriodendra*, *Diplodia seriata*, and *Neofusicoccum parvum* on own-rooted vines Askari, Rishbaba, and Black cultivars ranging from four to 35 years-old over a 10-month period (Mohammadi et al. 2013). In Croatia, Koch's Postulates were satisfied by inoculating *Neofusicoccum parvum* into detached green shoots and lignified canes of the grapevine cultivar Skrlet for 10 days in the greenhouse and 30 days in a dark chamber (Kaliternam et al. 2013). In Spain, Koch's Postulates for *Pleurostoma richardsiae* were completed on *Vitis vinifera* cultivar Albariño. The completion of more Koch's Postulates studies on cultivars will help determine causality of organisms associated with GTD to better assist with management decisions within vineyards.

Varietal Susceptibility

The susceptibility of 67 *Vitis vinifera* cultivars to Esca was evaluated by Murolo and Romanazzi (2014). The study revealed that the symptomatic plants among red and white cultivars were similar. Of the white grape cultivars tested, Passerina, Sauvignon blanc, Manzoni bianco, and Riesling cultivars had the highest incidence of symptomatic plants (35%), while the red grape cultivar with the highest incidence of symptomatic plants was Rebo. Rootstocks were also tested for susceptibility, with grapevines grafted onto SO4 having higher susceptibility than those grafted onto 1103P. This suggested that the disease resistance was likely due to the drought resistance of 1103P rootstock (Murolo and Romanazzi 2014). Understanding the susceptibility of rootstocks to GTD pathogens could influence the incidence of this disease in other cultivar/rootstock combinations.

In 2013, Travadon et al. conducted a study on the GTD susceptibility of seven *Vitis vinifera* cultivars (Cabernet Franc, Cabernet Sauvignon, Chardonnay, Merlot, Riesling, Petite Syrah, and Thompson Seedless) and four *Vitis* interspecific hybrid cultivars (Concord, Fennell 6, *V. arizonica* b42-26, and *V. rupestris* x *V. cinereal* Ill547-1). *Vitis vinifera* cultivars and Concord had similar susceptibilities to the pathogens *Neofusicoccum parvum*, *Phaeomoniella chlamydospora*, and *Tonginia minima* but cultivar Thompson Seedless was the most susceptible to *Phomopsis viticola*; whereas *V. arizonica* b52-26 was the most susceptible to *Eutypa lata* (Travadon et al. 2013). The study revealed that cultivars can have various susceptibilities to grapevine trunk disease pathogens (Travadon et al. 2013).

Susceptibility of table grapes to Botryosphaeriaceae species was studied in Chile in 2009, revealing that Flame Seedless was more susceptible to *Diplodia seriata* compared with Thompson Seedless and Redglobe (Morales et al. 2009). Redglobe was more susceptible to *Diplodia mutila* and *Botryosphaeria dothidea* in terms of the length of necrotic plant tissue (Morales et al. 2009).

Management Strategies

Sanitation practices, such as drenching rootstock cuttings in hot water treatments, biological treatments, or chemical treatments, have been used to prevent infection of rootstock cuttings in nurseries (Fourie and Halleen 2004). However, in Spain, a study revealed that GTD pathogens were found in all nurseries surveyed (Giménez-Jaime et al. 2006). Sanitation practices within the vineyard, such as removing pruning cuttings and diseased wood, can reduce the reservoir of inoculum in vineyards (Gramaje et al. 2018).

Spore dispersal from GTD pathogens are more frequent during and after significant wetness or rainfall (Lehoczky 1974). The spores from the fungi can be transferred by wind, water splashes, and insect vectors. Rainfall and high relative humidity (RH) levels approximately greater than 70% are important in counting spores of *Botryosphaeria* spp. which can lead to GTD infection (Van Niekerk et al. 2010). To prevent and control GTD pathogens, fungicides are applied after pruning dormant vines and immediately after rain events when infection is likely to occur (Van Niekerk et al. 2010). Additionally, GTD incidence may be reduced when appropriate fungicide applications are administered after periods of rainfall between budbreak and pre-bloom when conditions are more favorable for spores to infect pruned vines (Lehoczky 1974, Rolshausen et al. 2010, Klodd 2019).

For protection of pruning wounds, commercial products such as Biopaste (boron), Garrison (cyproconazole iodocarb), and Topsin M (thiophanate-methyl) provided control for *E. lata* in two California counties (Rolshausen et al. 2010). Although boron is known to inhibit glycolysis in fungi, it did not reduce *Botryosphaeriaceae* spp. infection in vines (Rolshausen et al. 2010). Garrison controlled more of the *Botryosphaeriaceae* spp., but Topsin M proved to be the most beneficial fungicide overall due to its ability to damage fungal cell division by targeting fungal mitosis. These products were directly applied on pruning cuts, which can be time consuming and costly in large vineyards (Rolshausen et al. 2010). Biopaste consists of Boron mixed with a commercial grafting seal and tree wound dressing (Doc Farewell's, Wenatchee, WA), while Garrison is a commercial pruning paint available in New Zealand (Chemcolour Industries, North Shore City, New Zealand). Topsin M is a broad-spectrum fungicide registered for use in all 50 states with the exception of Hawaii and Alaska.

Alternatively, natural antifungal compounds may be an option for GTD management. An in-vitro study showed that *D. seriata* and *P. chlamydospora* infections were reduced on pruning wounds using a mix of the natural antifungals chitosan oligosaccharide, garlic extract, and vanillin (Cobos et al. 2015).

A study conducted by Moyo et al. (2014) in South Africa revealed that arthropods can vector grapevine trunk disease pathogens. Millipedes (*Ommattoiulus moreleti*) and cocktail ants (*Crematogaster peringueyi*) were positive carriers of trunk disease pathogens in 33% of samples. Specifically, *Phaeomoniella chlamydospora* was isolated from pruning wounds exposed to arthropods, with 47% from millipedes and 27% from ants. The ants and millipedes transmitted the GTD pathogen via feeding on pruning wound sap. *Phaeomoniella chlamydospora* was discovered in the feces of millipedes, suggesting that they ingested the fungal pathogens. Based on the results of the study, useful management strategies for grapevine trunk diseases may include control of millipedes and cocktail ants due to their potential to vector pathogens (Moyo et al. 2014).

The selection and planting of cultivars with low susceptibility to GTD-associated pathogens has been used as a management strategy for this disease complex. Susceptibility of cold climate *Vitis* cultivars to pests and diseases has been published (Smiley et al. 2016). Susceptibility of *Vitis* interspecific hybrid cultivars Aromella, Chardonel, Chambourcin, Noiret, Traminette, and Vidal blanc to Eutypa dieback and Phomopsis cane and leaf spot have been evaluated (Schloemann and Garofalo 2019).

CHAPTER 3

Survey of GTD pathogen incidence in grapevine cultivars

Introduction

Grapevine trunk disease (GTD) is a disease complex associated with a wide range of fungal species, including those that cause Black foot, Esca, and Eutypa, e.g. species of *Cylindrocarpon*, *Botryosphaeria*, *Eutypa*, and more (Bonfiglioli 2008). GTD is the largest complex of pathogens infecting grapevines, with 133 fungal species in 34 genera impacting grapevines worldwide (Gramaje et al. 2018). The pathogens associated with GTD grow in the xylem, entering through pruning wounds dispersed by ascospores or conidia released by wind, rain, or arthropods (Mugnai et al. 1999, Úrbez-Torres et al. 2010, Moyo et al. 2014). Weather conditions in Missouri, such as high humidity, rainfall, and favorable temperatures can increase the potential for diseases to develop and impact grapevine fruit and vegetation (Gu et al. 2000).

Vitis interspecific hybrid cultivars are grown for wine production in Missouri because of their adaptability to Missouri's climate and growing conditions (Gu et al. 2000). Among the popular cultivars are Vignoles, Chambourcin, Vidal blanc, Chardonel, and Norton. Studies have been conducted to identify GTD-resistant *Vitis vinifera* cultivars, but few have focused on *Vitis* interspecific hybrid cultivars grown in Missouri. It has been speculated that interspecific hybrid cultivars Chardonel, Chambourcin, Catawba, Traminette, and Niagara may be highly susceptible to Botryosphaeria dieback (Sosnowski et al. 2013). Further understanding of cultivar susceptibility to GTD could provide useful strategies for disease management within vineyards.

Vitis vinifera cultivars Aligote, Grolleau, Merlot, Semillon, and Sylvaner were deemed resistant to Eutypa dieback based on low incidence of foliar symptoms while Grenache, Cabernet Sauvignon, and Shiraz had high susceptibility (Carter 1991; Loschiavo et al. 2007). Cultivars Aligote, Semillon, and Sylvaner are white grape cultivars, while Grolleau, Merlot, Grenache, Cabernet Sauvignon, and Shiraz are red grape cultivars. This suggests that there may be susceptibility differences between white grape cultivars and red grape cultivars.

The purpose of this survey is to understand the incidence and severity of GTD in various *Vitis* interspecific hybrid cultivars in a Missouri vineyard and to determine which cultivars may be more or less susceptible to GTD. This research also seeks to identify if incidence of GTD affects pruning weight in each cultivar. With the information on cultivar susceptibility, growers in Missouri can better manage GTD in their vineyards through cultivar selection during vineyard planting or by employing different management strategies and sanitation practices with different cultivars.

Methods

Site and Sampling

Cuttings from eight *Vitis* interspecific hybrid cultivars at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, MO (Fig. 3.1) were collected during the winter months of January through March 2019. The two-acre vineyard at HARC was established in 2009. The block was laid out as a completely randomized design with three vine panels of replicated cultivars and sample vines were chosen based on the health and presence of all three replicates. Some cultivars contained dead or missing replicates within the block. *Vitis* interspecific hybrid cultivars sampled for this study were NY76 (released from Cornell in 2013 and now commercially known as Aromella) (n=8), Brianna (n=15), Chambourcin grafted to 101-14 rootstock (n=12), Chardonel (n=9), Noiret (n=12), Norton (n=15), Prophecy (n=17), Traminette (n=18), and Vidal blanc (n=18). The sample sized listed are for the number of vines in each cultivar. Each vine was pruned and then four samples from each cultivar were collected and processed in the lab. A total of 496 samples of approximately 1 cm-long were cut from the spur-pruned cuttings, which were taken from 124 vines trained on high wire trellis systems. The bark was not removed from the samples. At the time of sampling, no visual GTD symptoms (cankers, dark streaking of wood, brown discoloration of wood, white rot, or soft yellowish wood) were apparent in the cuttings. Pruning weights for each vine were obtained using a bungee cord to wrap the pruning wood and a hanging scale.

PCR and Identification

The samples were surface sterilized in a 10% bleach solution for 10 minutes and air dried at 24°C on Kimwipes (Kimtech, Roswell, GA). The samples were then plated onto potato dextrose agar (PDA) (DIFCO, Detroit, MI) amended with tetracycline hydrochloride (0.01%) (Sigma-Aldrich, St. Louis, MO) in 30 cm-diameter petri plates for isolation of pure cultures (Úrbez-Torres et al. 2006). Pure fungal colonies were incubated under fluorescent light for 12 hours per day at 24°C. DNA extractions were conducted using QIAGEN DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). DNA was stored in a -22°C freezer. PCR reactions were conducted to amplify the isolated DNA segment for sequencing. PCR reactions were completed using Promega GoTaq® Green Master Mix Protocol for a 25 µl reaction volume (Promega, Madison, WI). Oligonucleotide primers ITS1 (5′- TCCGTA GGTGAACCTGCG G -3′) and ITS4 (5′-

TCCTCCGCTTATTGATATGC -3′) were used to amplify the noncoding regions between regions of 18S, 5.8S, and 28S rRNA genes (White et al. 1990). The denaturation step was 2 minutes at 95°C and subsequent denaturation steps for 45 seconds. The annealing step was 56°C for 45 seconds. The extension step was 72°C for 1 minute followed by refrigeration at 12°C until samples could be transferred to the QIAxcel Advanced (Qiagen, Hilden, Germany), with 35 cycles.

The samples were then processed using QIAxcel to automate electrophoresis analysis of the DNA samples (QIAGEN, Hilden, Germany). If samples contained only one band, they were prepared for sequencing using QIAquick PCR purification kit (QIAGEN, Hilden, Germany). If samples contained more than one band, they were run on a gel and then prepared for sequencing using QIAquick Gel Extraction Kit. All sequencing was processed by the University of Missouri DNA Core Facility via their Sanger Sequencing Services. All sequencing data was processed using the National Center for Biotechnology Information (NCBI) Nucleotide BLAST at or above a 97% rate to establish each fungal species identity. These PCR and identification steps were used in the experiments in Chapters Four and Five.

The following sources were used to determine whether organisms identified in this experiment were associated with GTD: Mugnai et al. (n.d. and 1999), Larignon et al. 1997, Úrbez-Torres et al. (2006, 2012 and 2013), Rolshausen et al. 2010 and Bertsch et al. 2013.

Statistics

The pruning weight data was analyzed using JMP Student Edition (*Version 14*. SAS Institute Inc., Cary, NC, 1989-2019), and analyzed using analysis of variance (ANOVA). Means were separated by the Tukey-Kramer HSD test. Logistic regression was used for GTD pathogen percentage data using the GLIMMIX procedures of SAS 9.4 (SAS Institute, Cary, NC) to model the probability of each individual species containing GTD pathogens on the basis of the wine grape color of each cultivar. Another logistic regression analysis was performed to determine if any of the cultivars differed in their likelihood of having one or more GTD pathogen. The GLIMMIX procedure provides estimates and statistical inferences for linear mixed models. The percentages of fungal species per cultivar were calculated by dividing the number of each fungal species identified by the total number of fungal species identified for each cultivar. This was completed for all nine cultivars.

Results

Pruning Weight

The relationship between pruning weights collected from each sample vine and presence of GTD was not statistically significant ($p=0.8247$).

GTD-Associated Pathogens by Cultivar

The GTD pathogens identified from the nine cultivars were *Phomopsis viticola*, *Diplodia seriata*, *Neofusicoccum ribis*, *Neofusicoccum parvum*, *Pestalotiopsis uvicola*, *Aspergillus heteromorphus*, and *Basidiomycota sp.* (Fig. 3.2). Although data were statistically analyzed, *Phomopsis viticola* was the most common GTD fungal pathogen isolated from all nine *Vitis* interspecific hybrid cultivars surveyed. The percentage of *Phomopsis viticola* isolated from Vidal blanc, Noiret, Aromella, Chambourcin, and Brianna were 82, 80, 78, 67, and 60%, respectively. For Chardonel, Norton, and Prophecy, 50% of their GTD pathogens were *Phomopsis viticola*, followed by Traminette at 46%. *Neofusicoccum ribis* was isolated from five cultivars: Aromella (11%), Brianna (10%), Norton (25%), Traminette (23%), and Vidal blanc (18%). *Diplodia seriata* was isolated from four cultivars: Aromella (11%), Noiret (20%), Norton (25%), and Traminette (8%). *Neofusicoccum parvum* was identified in three cultivars, Chambourcin (33%), Chardonel (50%) and Traminette (15%). *Pestalotiopsis uvicola* was isolated from two cultivars, Prophecy (50%) and Traminette (8%), while *Aspergillus heteromorphus* and *Basidiomycota sp.* were isolated from only Brianna at 20% and 10%, respectively (Fig 3.2).

There is no significant difference between the amount of GTD identified in each of the nine cultivars surveyed at HARC (p=0.1701). The percentage of GTD associated pathogens in Aromella, Brianna, Chambourcin, Chardonel, Noiret, Norton, Prophecy, Traminette, and Vidal blanc were 88, 60, 25, 56, 42, 33, 12, 61, and 61%, respectively. Aromella was associated with the highest percentage of GTD fungal pathogens while Phophecy was associated with the lowest percentage of GTD fungal pathogens. Brianna, Chardonel, Traminette, and Vidal blanc all maintained higher than 50% of fungal species associated with GTD (Fig. 3.3).

Non GTD-Associated Fungal Species by Cultivar

The most common non-GTD fungal species found in cultivars were *Alternaria alternata* and *Fusarium venenatum*. *Alternaria alternata* was isolated from all nine cultivars surveyed while *Fusarium venenatum* was isolated from all cultivars with the exception of Brianna (Figs. 3.4- 3.12).

There were other fungal species not associated with GTD found in cultivars, but in smaller amounts: *Chaetomium rectangulare*, *Clonostachys rosea*, *Dichotomopilus funicola*, *Didymella pinodella*, *Didymella sp*., *Fusarium oxysporum*, *Fusarium proliferatum*, *Neosetophoma sp.*, *Nigrospora sphaerica*, *Paraconiothyrium brasiliense*, *Pestalotiopsis microspora*, *Rosellinia aquila*, *Seiridium cardinale*, *Seiridium sp.*, *Stagonospora foliicola*, and *Trichoderma sp*. (Figs. 3.4- 3.12)

GTD Susceptibility of White vs Red Grape Cultivars

White cultivars included in this study were associated with a higher incidence of GTD compared with red cultivars ($p<0.001$). White cultivars Aromella, Traminette, Brianna, and Chardonel were associated with higher percentages of GTD-associated fungal pathogens than the red grape cultivars Norton, Noiret, Chambourcin, and Prophecy. Sixty-three percent of white grape cultivars had GTD, while 27% of red grape cultivars had GTD (Fig. 3.13). The cultivar Aromella was associated with the highest percentage of vines containing GTD fungal pathogens (88%), indicating that this cultivar may be more susceptible to GTD than Prophecy, which had the lowest percentage of vines containing GTD fungal pathogens (12%).

Discussion

Although all sample cuttings were surface sterilized in 10% bleach prior to plating on PDA petri dishes, the bark was not removed from all samples. This could have had an effect on the fungal species identified from each sample. Because the bark was not intentionally removed from the HARC samples, some of the fungal species identified may not be fungal endophytes colonizing the inside the plant tissue but rather living outside the vascular system on the bark of the tissue. This may have impacted the number and types of fungal species identified from each sample vine.

Many of the non-GTD associated fungal species recovered from vines are found to be pathogenic to other plant species. *Alternaria alternata* causes leaf spot, rots, and blights on many plants such as pistachio, potato, tomato, and citrus (Pryor and Michailides 2002, Weir et al. 1998, Peever et al. 2002). *Fusarium venenatum* is closely related to *Fusarium graminearum*,which causes Fusarium head blight on wheat and barley (King et al. 2018). *Trichoderma sp.* is a commonly isolated soil microbe that can be used in biocontrol since it is parasitic to other fungi (Benítez et al. 2004). *Seiridium sp.* causes cankers in cypress (Barnes et al. 2001). *Didymella applanate* is a known causal agent of cane spur blight in red raspberry (Pepin and Williamson 1985). Since these fungal species were isolated from *Vitis* interspecific hybrid cultivars and are known to cause disease in other plant species, further research could look into the role these fungal species play in causing other diseases of grapevines.

Logistic regression was used in SAS 9.4 (SAS Institute, Cary, NC) to determine if GTD fungal pathogens were present or not in each cultivar while taking into account the non-normal distribution of the data and dissimilar sample sizes.

The cultivars Norton, Chambourcin, Traminette, and Vidal blanc are a few of the economically important cultivars of wine grapes grown in Missouri. The cultivar Norton was the most planted grapevine in all of Missouri at 351.4 acres (20.7% of the total grapevine acreage), while Chambourcin was planted in 177.7 acres (10.5% of the total acreage). Traminette was planted in 114.7 acres (6.7% of the total acreage) and Vidal blanc was planted in 139.4 acres (8.2% of the total acreage) (USDA National Agricultural Statistics Service Missouri 2016). Understanding the susceptibility of *Vitis* interspecific hybrid cultivars can help vineyard managers reduce incidence of GTD through cultivar selection. Cultivars Chambourcin and Norton may be less susceptible to GTD pathogens due to their lower percentages of GTD pathogens found in samples in comparison with cultivars Traminette and Vidal blanc, which had higher percentages of GTD pathogens. Further sampling of each cultivar from other vineyards in Missouri will help to confirm the susceptibility of each cultivar to GTD pathogens.

White grape cultivars sampled in this study (Aromella, Traminette, Brianna, Chardonel, and Vidal blanc) were associated with an increased incidence of GTD compared with red grape cultivars (Norton, Noiret, Chambourcin, and Prophecy). This suggests that white grape cultivars may have higher susceptibility to GTD pathogens. Although not statistically significant, the cultivar Prophecy had the lowest percentage of GTD, indicating that this cultivar may have lower susceptibility to GTD than the other eight cultivars surveyed. Future sampling from other vineyards across Missouri would confirm the susceptibility of these cultivars to GTD.

In this study, the higher incidence of GTD of white grape cultivars compared to red cultivars contradicted the results supported by the Sosnowski et al. (2013) study in South Australia that disease symptoms were greater in red grape cultivars than white grape cultivars. This could be due to a number of factors, including differences in *Vitis vinifera* cultivars versus *Vitis* interspecific hybrid cultivars tested as well as differences in climate and site. *Vitis vinifera* cultivars are generally less winter hardy and more susceptible to diseases (Khanizadeh et al. 2008, Reisch et al. 1993). For instance, *Vitis riparia* has transcriptional components that are involved in its resistance to Downy mildew caused by *Plasmopara viticola*. These resistance mechanisms are absent in *Vitis vinifera*, making it more susceptible to Downy mildew (Polesani et al. 2010). Additionally, *Vitis vinifera* cultivars cannot be successfully grown in soil where traditional American cultivars are grown due to low soil pH. *Vitis vinifera* cultivars Chardonnay, Pinot noir, and Riesling have high susceptibility to *Botrytis* bunch rot due to its compact clusters (Reisch et al. 1993). Vines of *Vitis vinifera* cultivars are often damaged by low winter temperatures, which can lead to Crown gall caused by *Agrobacterium vitis* (Creasap et al. 2006). *Vitis vinifera* cultivars are also highly susceptible to Black rot, a fungal disease caused by *Guignardia bidwellii*, whereas susceptibility is variable among hybrid cultivars (Wilcox 2015). *Vitis vinifera* and *Vitis* interspecific hybrid cultivars may have different susceptibilities to GTD.

For the *Vitis* interspecific hybrid cultivars sampled in this study, the white varieties may have higher susceptibility to other pathogen groups than red varieties based on individual differences in each cultivar. According to an extension bulletin released by Reisch et al. (2014) regarding the *Vitis* interspecific hybrid cultivar Aromella, the cultivar is winter hardy and has medium susceptibility to downy mildew, powdery mildew, and *Botrytis* bunch rot. However, trunk damage was not evaluated for this variety. The cultivar Brianna is cold hardy but highly susceptible to crown gall and moderately susceptible to downy mildew and powdery mildew (Smiley et al. 2016). Chardonel may be susceptible to trunk damage caused by low temperatures and can also be susceptible to crown gall (Reisch et al. 1990). The cultivar Traminette is listed as partially resistant to several fungal diseases but can experience trunk damage in heavier soils (Reisch et al. 1996). Trunk damage could lead to grapevine trunk disease fungal spores infecting the wound site. For the red cultivars, Noiret is moderately susceptible to downy mildew, black rot, *Botrytis* bunch rot and can be susceptible to trunk damage due to winter injury at the graft union (Reisch et al. 2006). In a study conducted by Gao et al. in 2014, hybrid variety Norton is less susceptible to pathogens than *Vitis vinifera* cultivar Cabernet Sauvignon based on gene expressions that regulate defense mechanisms (Gao et al. 2014). Chambourcin has a higher disease resistance than *Vitis vinifera* cultivars and is therefore better suited for growing in the midwestern United States (Dami et al. 2005).

According to Schloemann and Garofalo (2019), the cultivars Aromella, Chardonel, and Traminette have unknown susceptibility to Phomopsis cane and leaf spot and Eutypa dieback. Vidal blanc is slightly susceptible to both Phomopsis cane and leaf spot as well as Eutypa dieback. Chambourcin has moderate susceptibility to Phomopsis cane and leaf spot but unknown susceptibility to Eutypa dieback while Noiret has unknown susceptibility to both Phomopsis cane and leaf spot and Eutypa dieback. Based on this information, hybrid cultivars have varying degrees of susceptibility to trunk diseases (Schloemann and Garofalo 2019).

Phomopsis viticola was recovered from all cultivars, revealing that this particular pathogen may be one of the main causal agents of GTD for hybrid cultivars in Missouri vineyards. Management strategies to control *Phomopsis viticola* causing Phomopsis cane and leaf spot in the vineyard include spray treatments between bud break and pre-bloom after wet weather since they can overwinter on the previous year’s canes and release spores when wet. Applications of Captan (*N*-(trichloromethylthio) cyclohex-4-ene-1,2-

dicarboximide), Mancozeb (zinc ion and manganese ethylene bisdithiocarbamate), Ziram (zinc dimethyldithiocarbamate), and Pristine (pyraclostrobin and boscalid) are effective in controlling *Phomopsis viticola* (Klodd 2019). These control measures may not have been effective in the samples collected depending on the weather and when the spray applications were administered. Since *Phomopsis viticola* overwinters on the previous year's wood and releases its spores after a period of rainfall, sufficient rainfall coupled with spray applications not completed in a timely manner may have caused the samples to be infected. Additionally, it is known that symptoms of *Phomopsis viticola* infection may not appear on woody tissue until three to four weeks after the pathogen infects the tissue (Klodd 2019). Since all the samples in this study were asymptomatic at the time of collection, the *Phomopsis viticola* infection may have taken place three to four weeks prior to sample collection.

Alternaria alternata, a pathogen not associated with GTD, was also found in every cultivar surveyed. Future research from other Missouri vineyards may determine if *Alternaria alternata* is prevalent on other hybrid grape cultivars.

White grape cultivars in this study are associated with a higher incidence of GTD than red grape cultivars. This opens up further research in sampling *Vitis* interspecific hybrid cultivars throughout Missouri vineyards to confirm the susceptibilities and identify susceptibility to other cultivars that are economically important to the Missouri wine industry. With this information, vineyard managers may reduce incidence of GTD in their vineyards with cultivar selection during planting and by choosing management practices that take into account variation in cultivar-susceptibility.

Fig. 3.1. Satellite image of Horticulture and Agroforestry Research Center (HARC) vineyard plot. Survey of cutivars' incidence of Grapevine trunk disease (GTD) was completed for these cultivars: Aromella, Brianna, Chardonel, Chambourcin, Noiret, Norton, Prophecy, Traminette, and Vidal blanc. Image provided by Google Maps 2020 satellite data, Maxar Technologies.

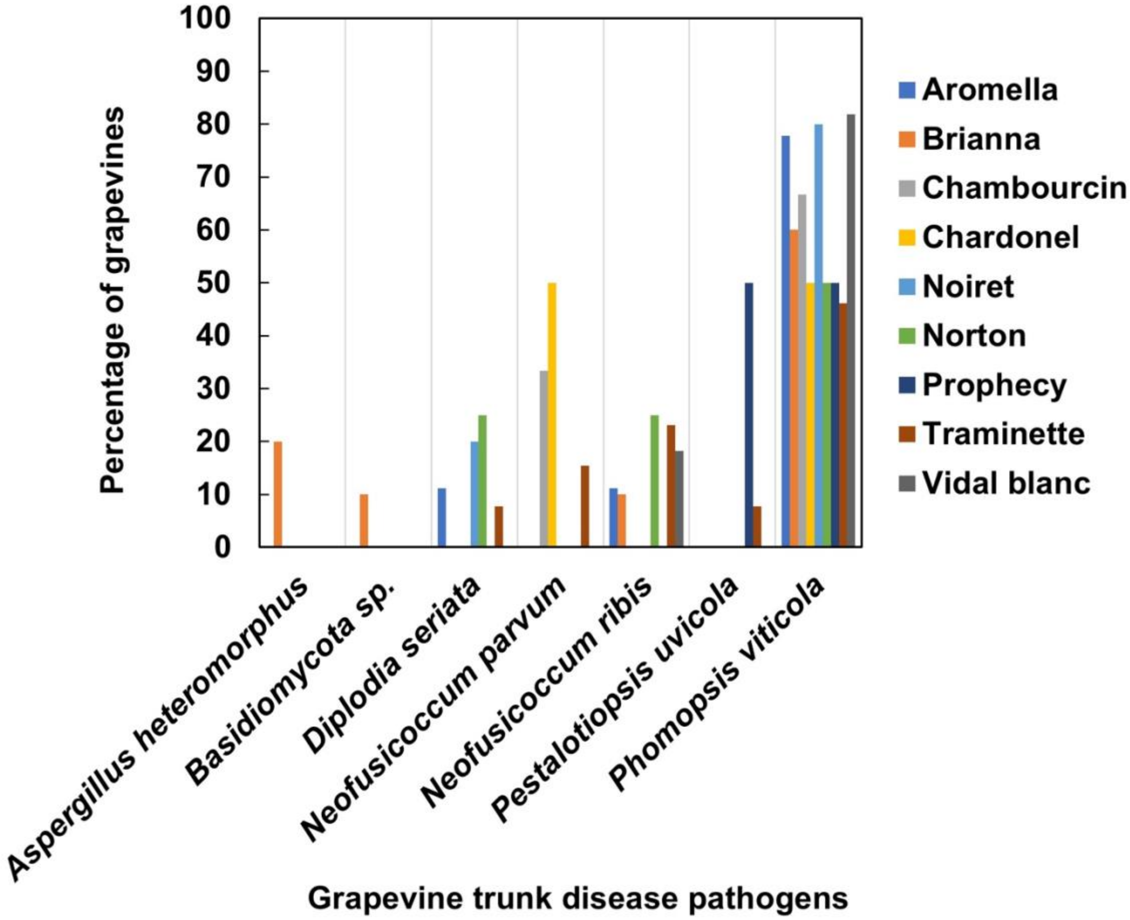

Fig. 3.2. Grapevine trunk disease fungal pathogens identified from *Vitis* interspecific hybrid cultivar pruning cuttings at the Horticulture and Agroforestry Research Center in New Franklin, MO. *Phomopsis viticola* was found in every cultivar surveyed. This figure only takes into account the fungal pathogens associated with grapevine trunk disease in each sample. The sample sizes were: Aromella (n=8), Brianna (n=15), Chambourcin (n=12), Chardonel (n=9), Noiret (n=12), Norton (n=15), Prophecy (n=17), Traminette (n=18), and Vidal blanc (n=18).

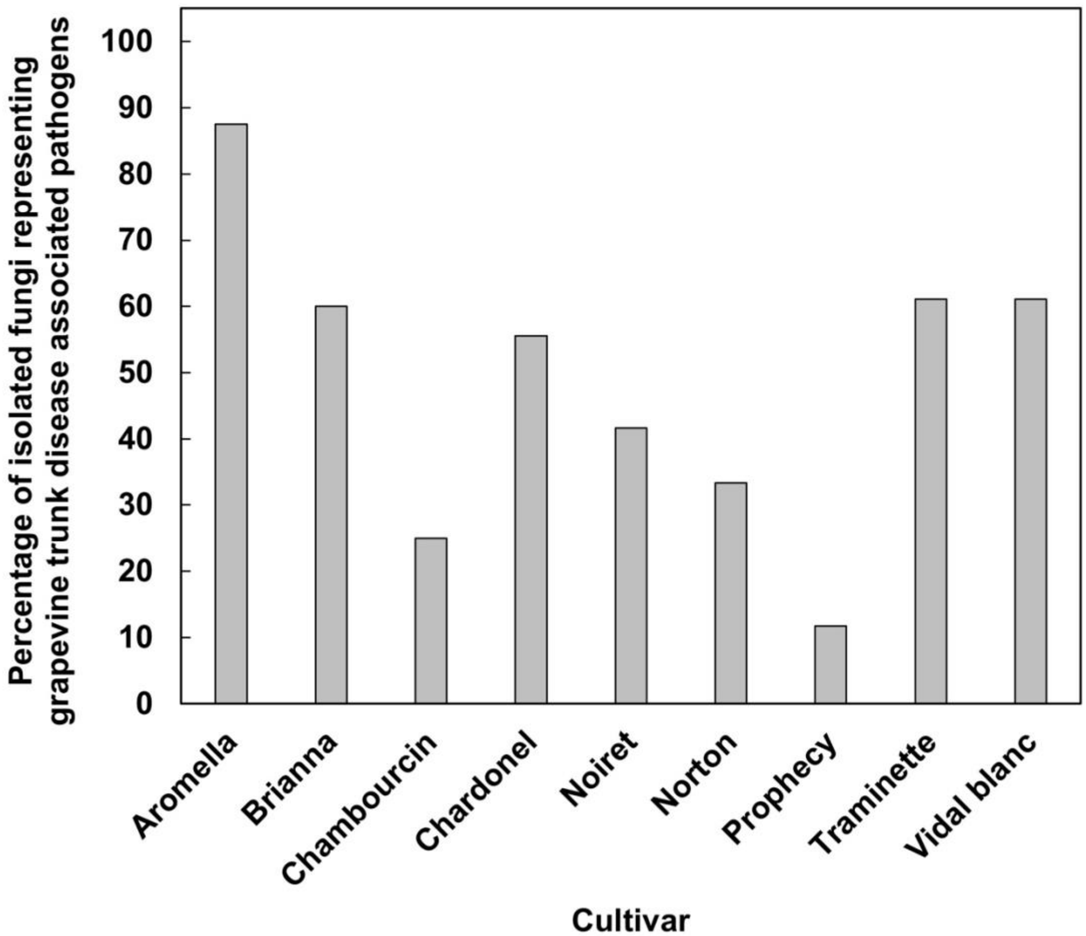

Fig. 3.3. Percentage of isolated fungi representing grapevine trunk disease associated pathogens from samples of *Vitis* interspecific hybrid cultivars selected from the Horticulture and Agroforestry Research Center in New Franklin, MO. The differences between grapevine trunk disease in each cultivar was not statistically significant ($p=0.1701$). The sample sizes for each cultivar were: Aromella (n=8), Brianna (n=15), Chambourcin (n=12), Chardonel (n=9), Noiret (n=12), Norton (n=15), Prophecy (n=17), Traminette (n=18), and Vidal blanc (n=18).

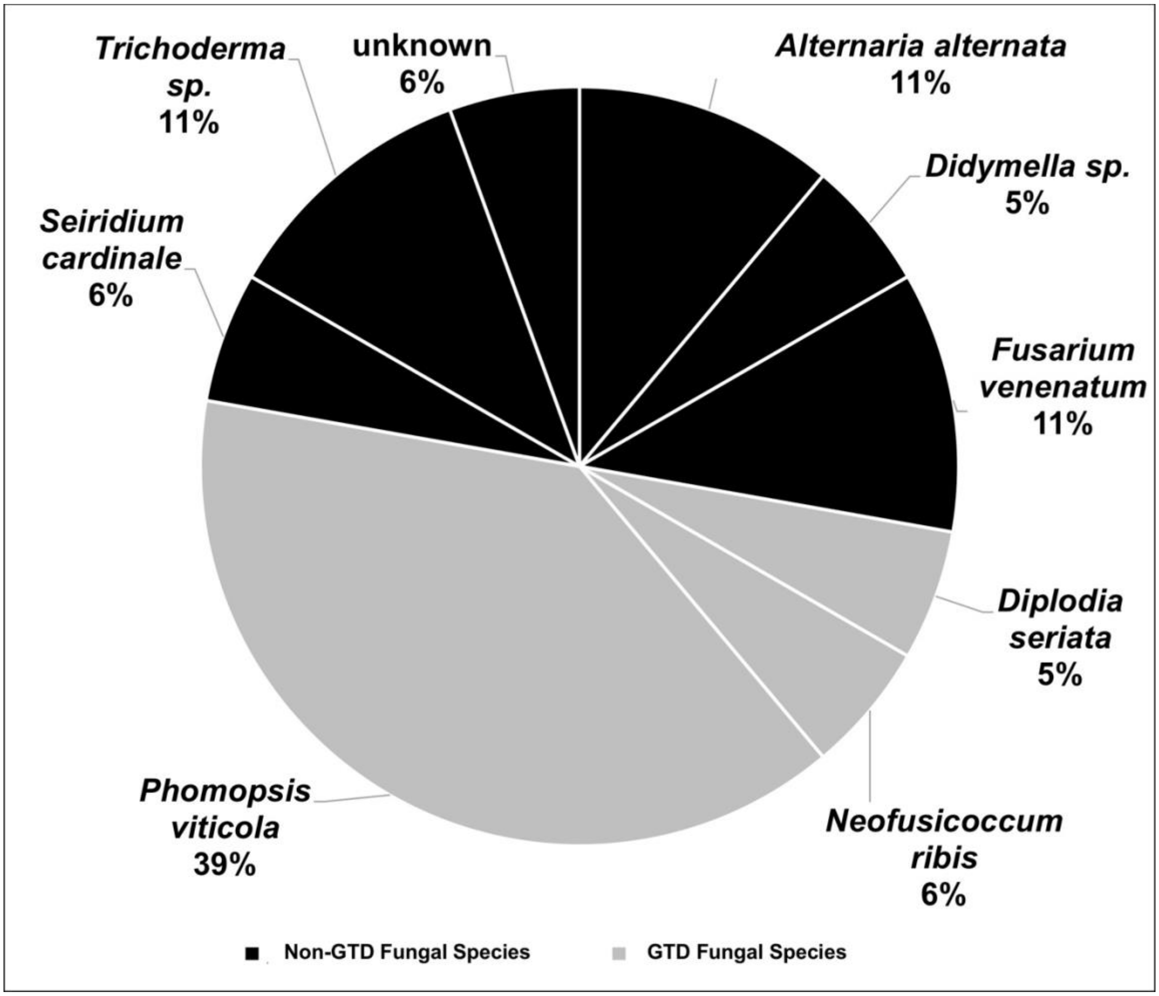

Fig. 3.4. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Aromella at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=8). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

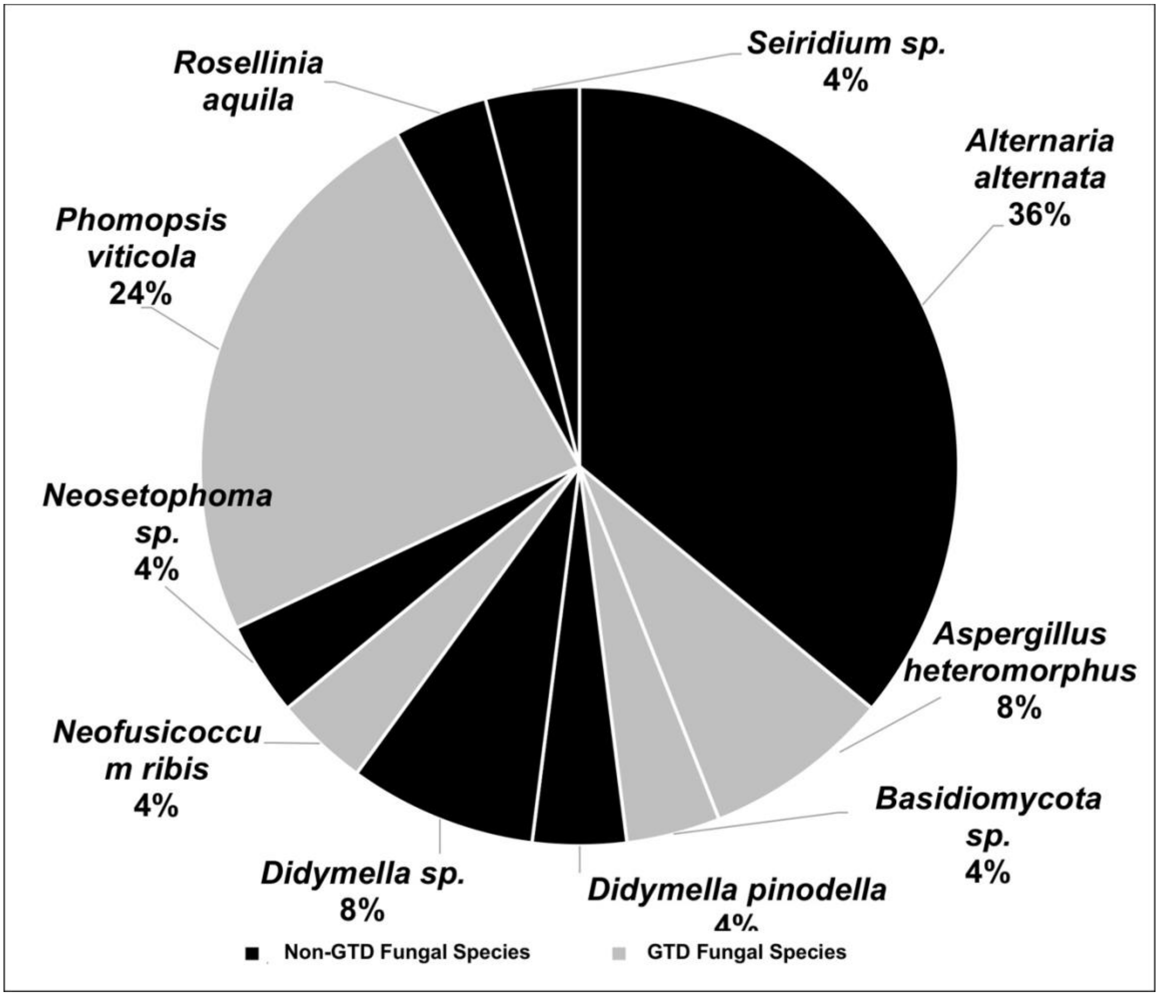

Fig. 3.5. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Brianna at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=15). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

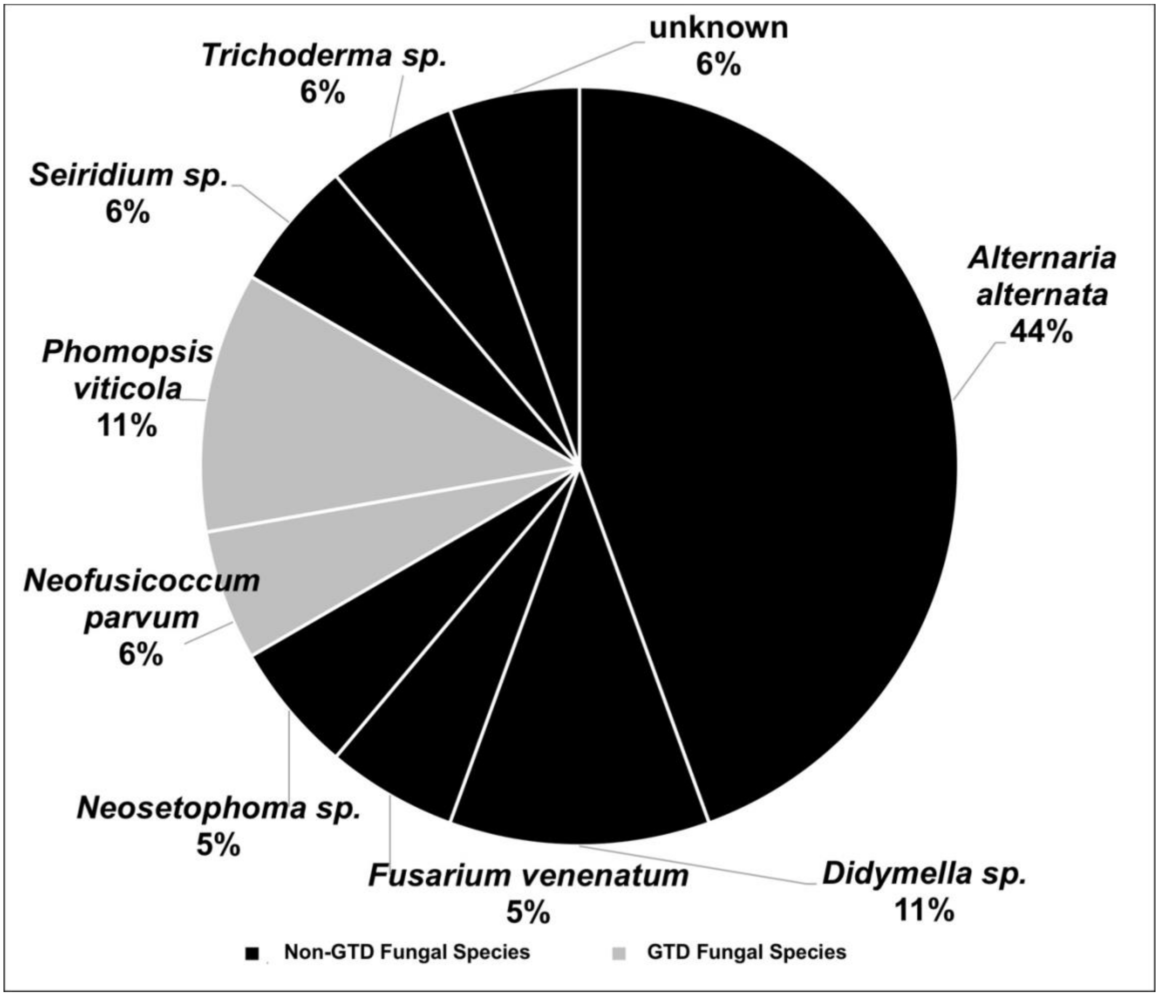

Fig. 3.6. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Chambourcin at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=12). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

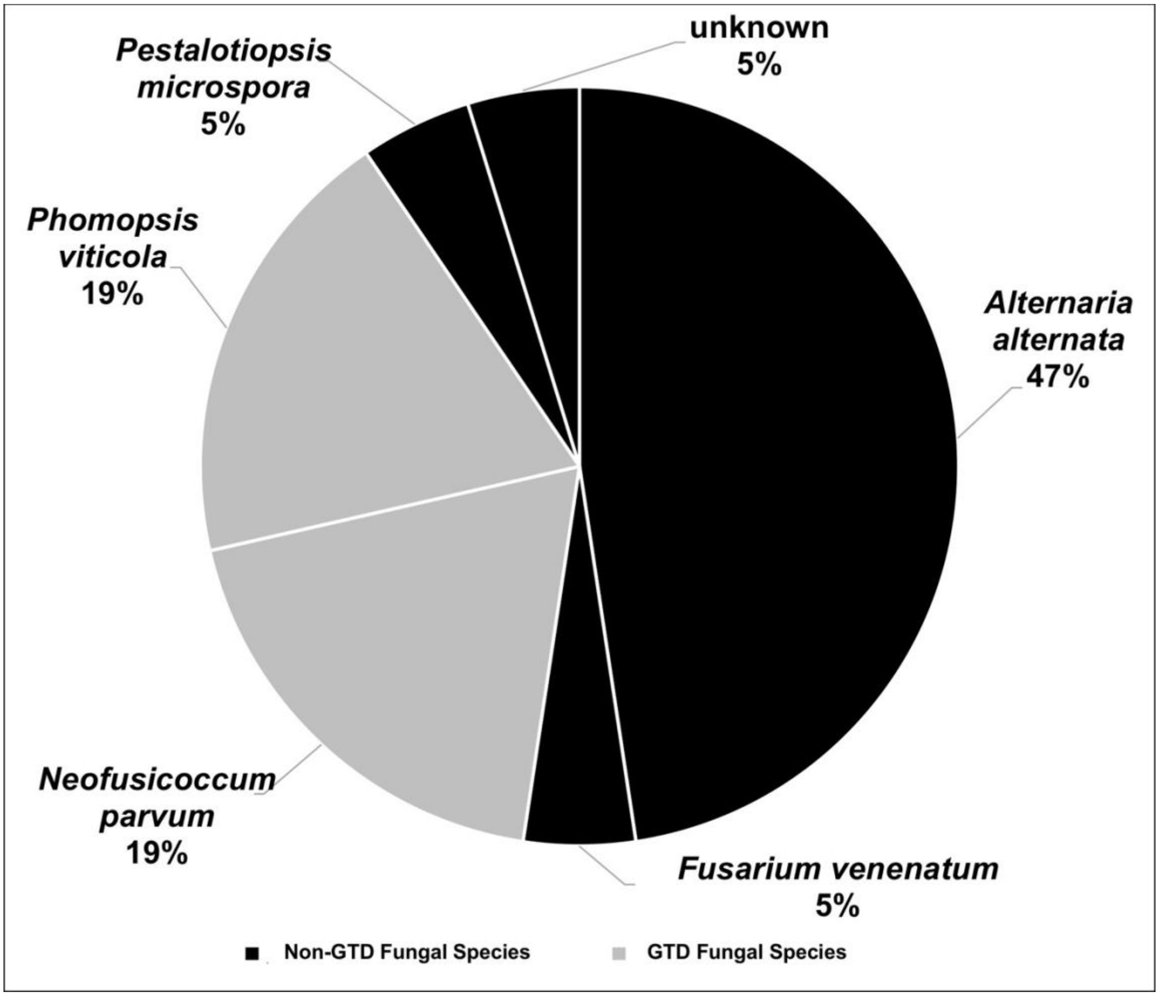

Fig. 3.7. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Chardonel at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=9). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

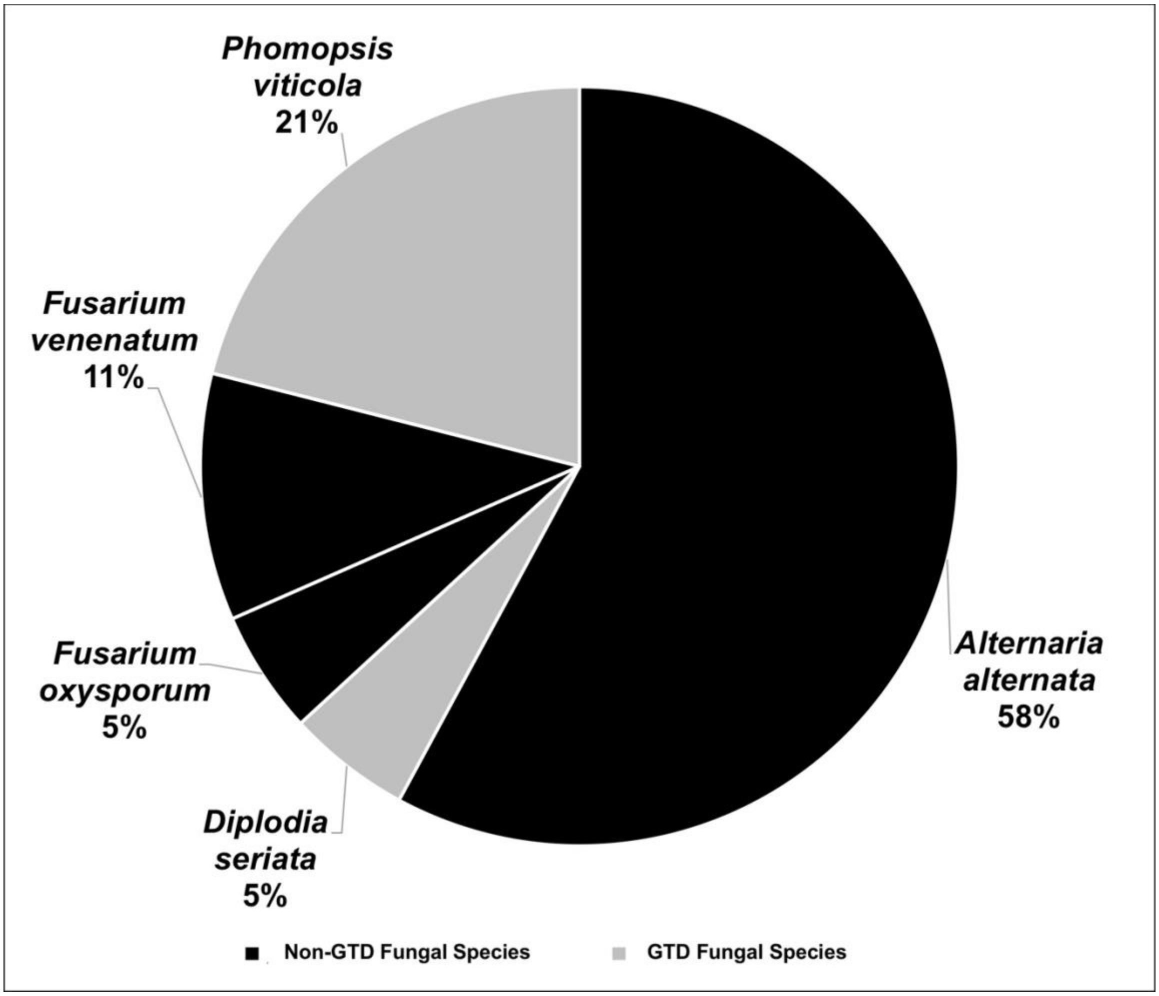

Fig. 3.8. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Noiret at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=12). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

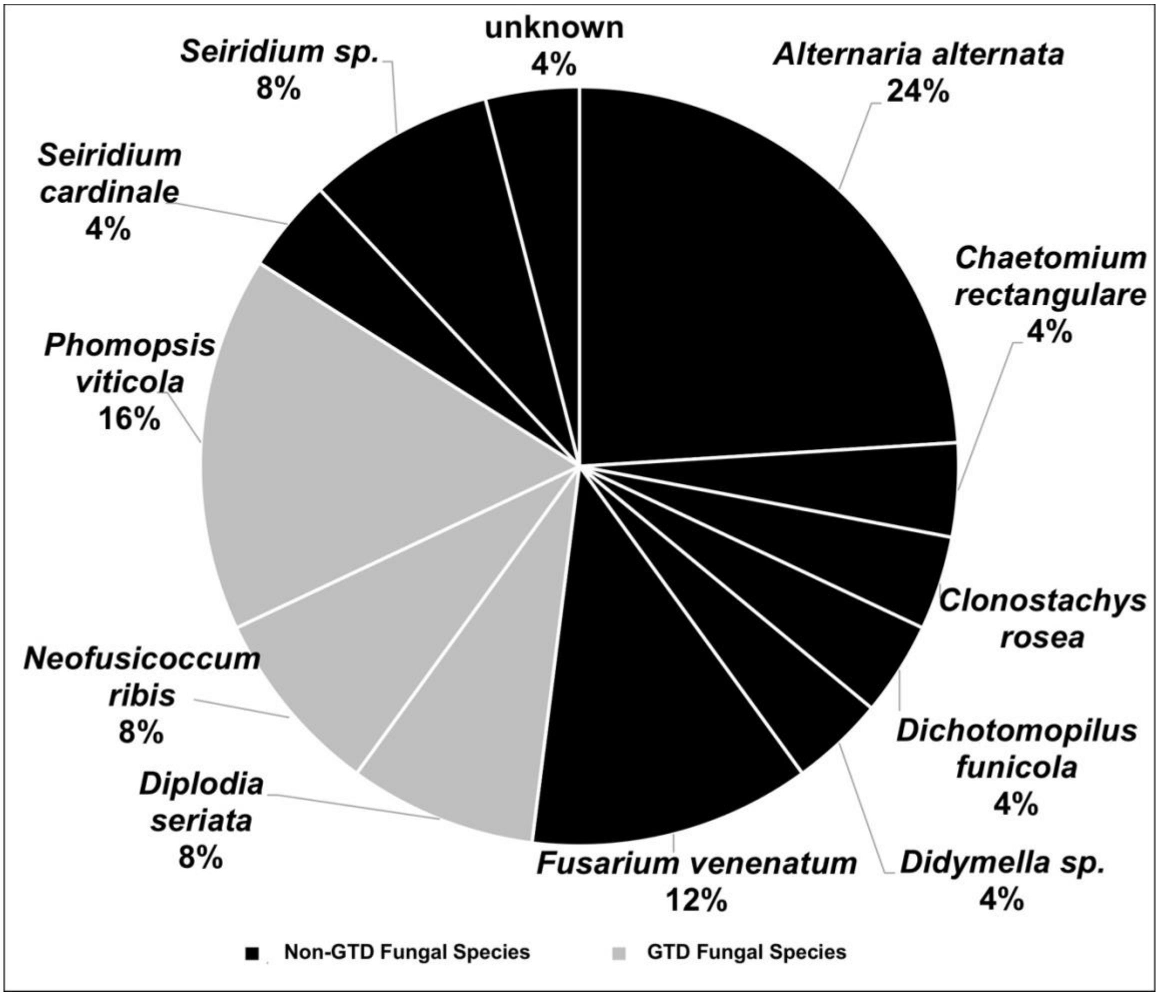

Fig. 3.9. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Norton at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=15). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

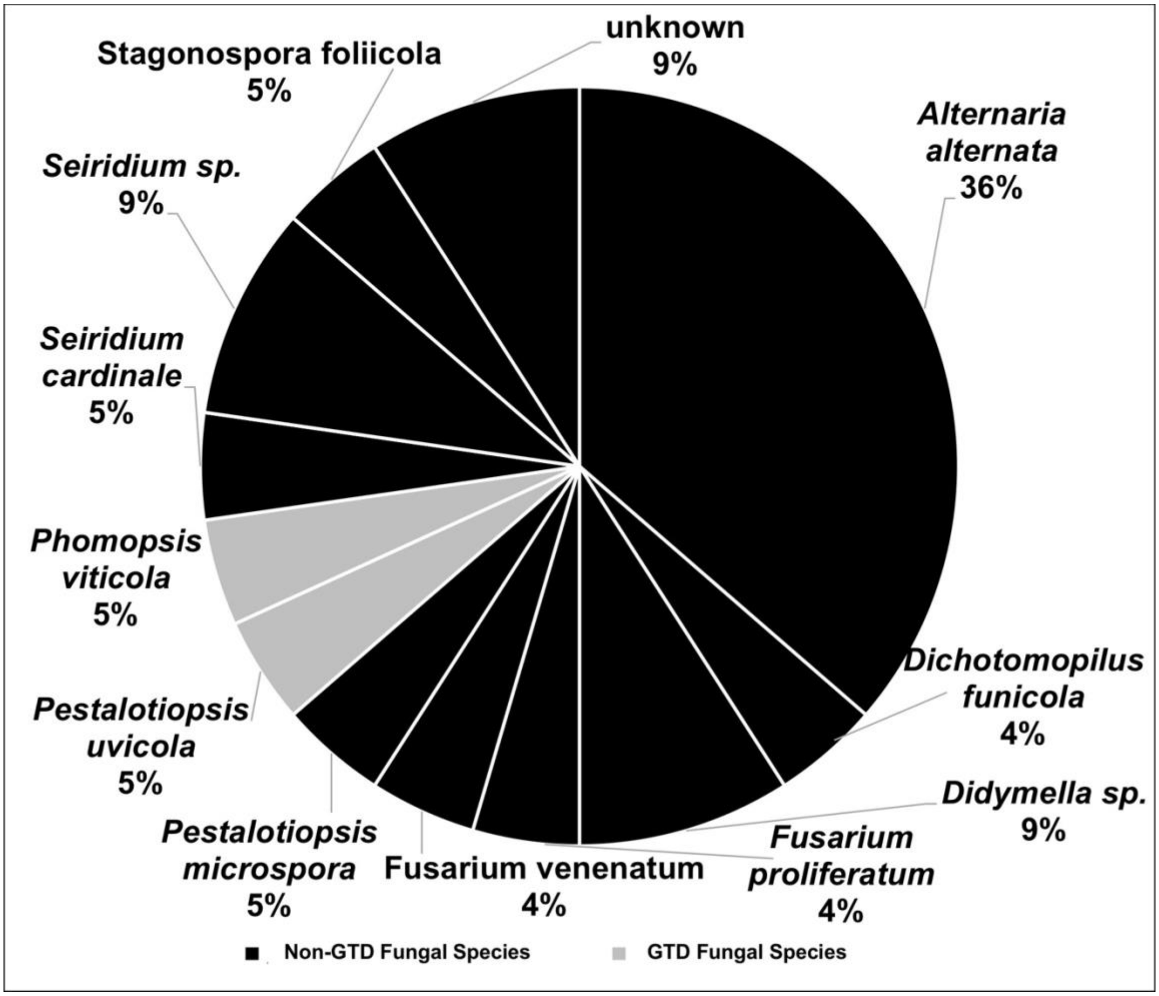

Fig. 3.10. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Prophecy at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=17). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

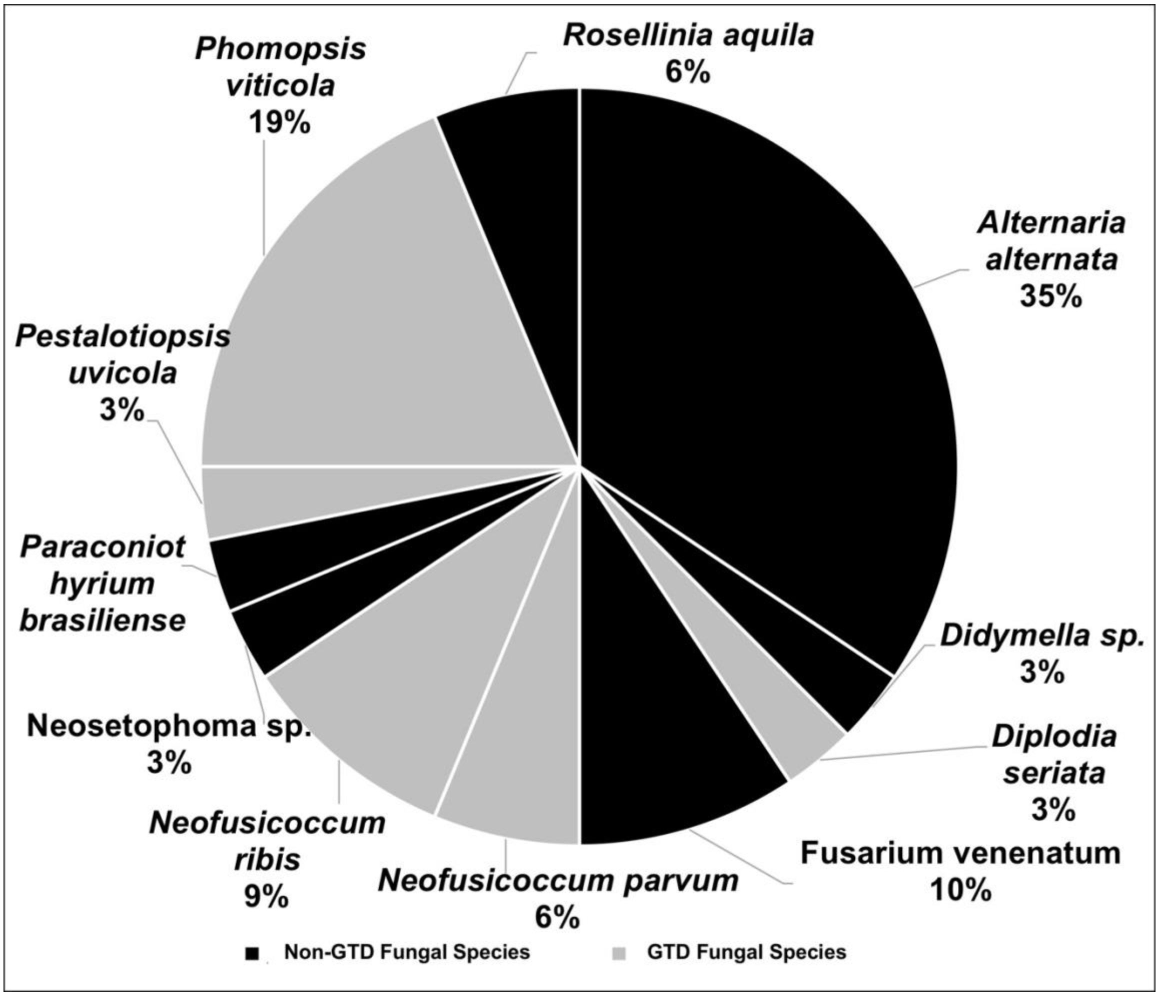

Fig. 3.11. Percentage of fungal species isolated from *Vitis* interspecific hybrid cultivar Traminette at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=18). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

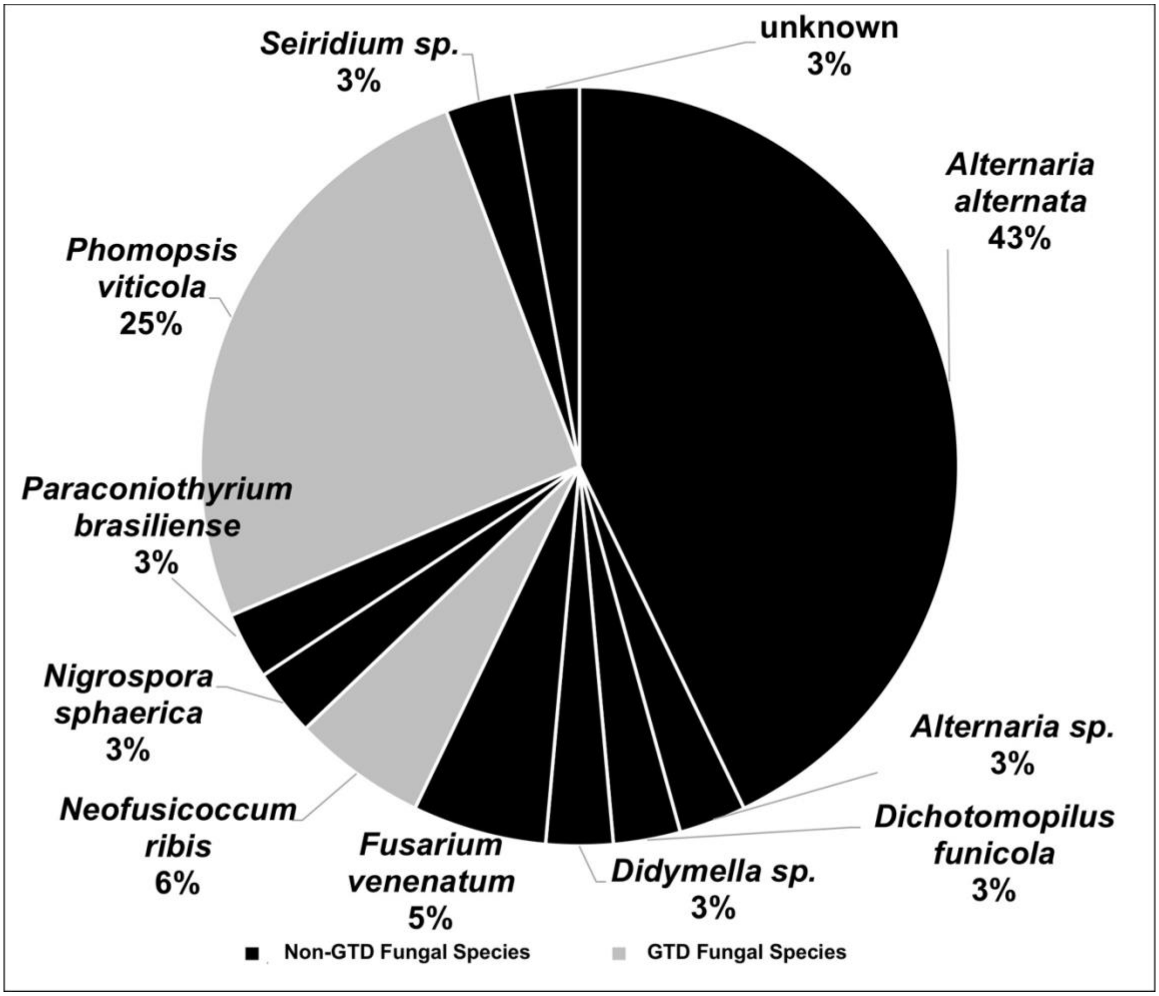

Fig. 3.12. Fungal species isolated from *Vitis* interspecific hybrid cultivar Vidal blanc at the Horticulture and Agroforestry Research Center in New Franklin, MO (n=18). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

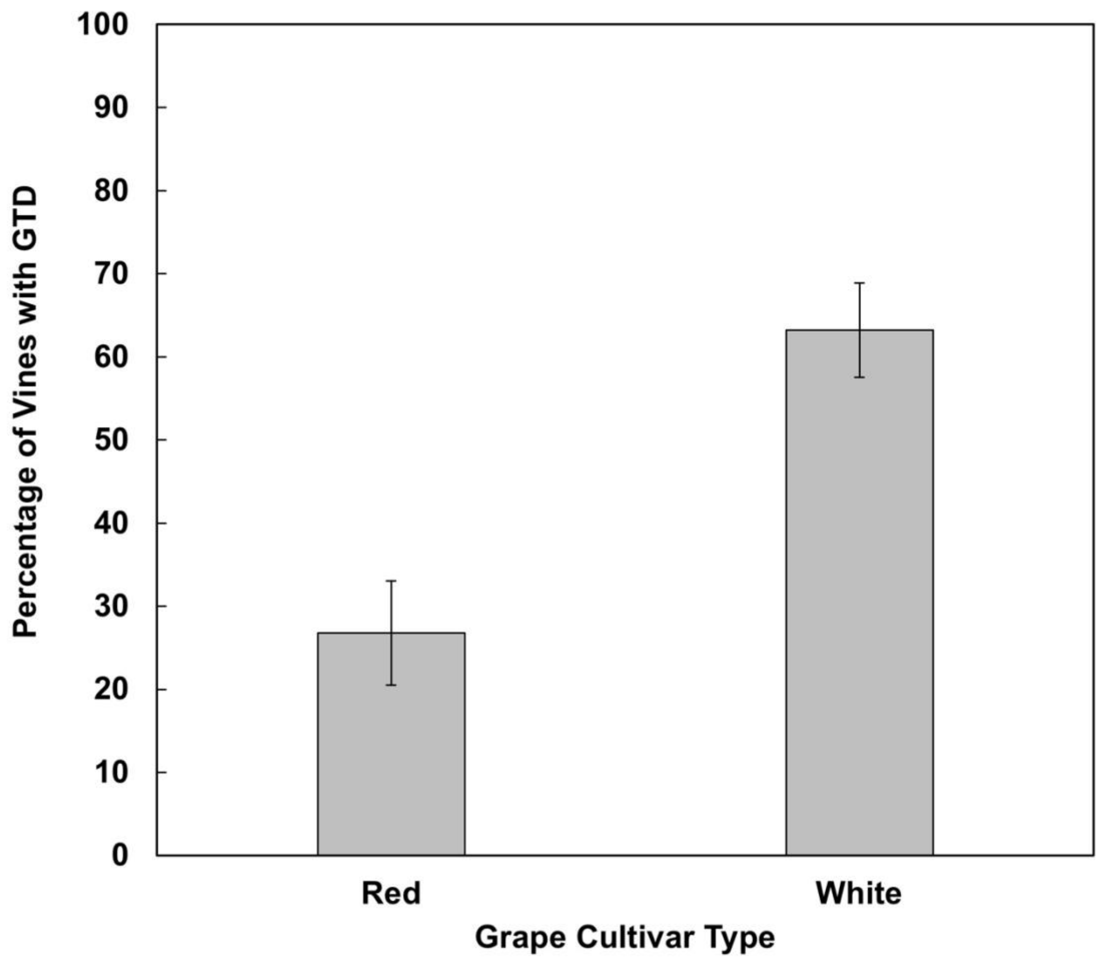

Fig. 3.13. Percentage of *Vitis* interspecific hybrid red grape cultivars (n=56) and white grape cultivars (n=68) with grapevine trunk disease (GTD) at the Horticulture and Agroforestry Research Center in New Franklin, MO. The red grape cultivars include: Chambourcin (n=12), Noiret (n=12), Norton (n=15), and Prophecy (n=17). The white grape cultivars include: Aromella (n=8), Brianna (n=15), Chardonel (n=9), Traminette (n=18), and Vidal blanc (n=18). $p<0.001$.

Chapter 4

Survey of GTD pathogen incidence in symptomatic grapevines

Introduction

Grapevine trunk disease (GTD) is an emerging fungal disease complex consisting of over 100 fungal species across 34 genera. This fungal disease complex has affected grapevines all over the world and can cause vine decline and eventual death (Wicks and Davies 1999). The pathogens are known to infect the xylem of the vines by entering through pruning wounds (Úrbez-Torres et al. 2010). The wide array of causal organisms can spread by weather such as wind and rain and can also spread by arthropods (Moyo et al. 2014). Temperature and rain differences in vineyards can influence pathogen dispersal and the symptoms of each vine (Van Niekerk et al. 2011). Vascular symptoms include brown streaking of wood, brown discoloration of wood, necrotic cankers, white rot, and soft yellowish wood. Foliar symptoms include interveinal chlorosis, shoot tip dieback, shriveling fruit, and gray speckling on berries (Úrbez-Torres et al. 2012).

A few of the commonly known GTD pathogens consist of *Eutypa lata*, *Neofusicoccum parvum*, *Diplodia seriata*, *Phaeomoniella chlamydospora*, and *Phomopsis viticola*. Some are known under diseases such as Eutypa dieback from *Eutypa lata*, Phomopsis cane and leaf spot from *Phomopsis viticola*, and petri disease caused by *Phaeomoniella chlamydospora* (Carter et al. 1983, Larignon and Dubos 1997, Hewitt and Pearson 1988). *Neofusicoccum parvum* causes cankers in the vascular wood of grapevines, which can lead to leaves wilting (Massonnet et al. 2017). Phomopsis cane and leaf spot can affect all green parts of the grapevine and also cause cankers in the wood (Úrbez-Torres et al. 2013). Petri disease impacts young vines, which leads to their decline in plant health (Larignon and Dubos 1997).

Economically, this disease complex can cause losses from individual vines to an entire vineyard. In California, $260 million in losses were estimated in 2001 as a result of GTD while in South Australia, there were $2800 in losses per hectare caused by eutypa dieback (Siebert 2001; Wicks and Davies 1999). Due to Missouri's favorable climate for fungal pathogens, which includes warm, humid weather, the Missouri wine industry has the potential to suffer major losses from GTD.

The purpose of this research is to evaluate the incidence and severity of GTD on *Vitis* interspecific hybrid cultivar Vidal blanc vines showing symptoms of trunk disease in a commercial vineyard in Augusta, MO. By evaluating the fungal species present in the symptomatic vines and comparing them to the visual disease ratings, the severity of damage may be correlated to specific fungal pathogens.

Methods

Site and Sampling

In February 2019, a commercial vineyard in Augusta, MO (Fig. 4.1) was removing two and a half acres of an eight-acre block of 20-year-old *Vitis* interspecific hybrid cultivar Vidal blanc. Each acre contained 550 vines per acre, with a total of 1,375 vines being removed. Ten random vines in each row were evaluated for incidence of grapevine trunk disease and percentage of tissue with visual GTD symptoms (cankers, dark streaking of wood, brown discoloration of wood, white rot, or soft yellowish wood) from the trunk that had been lopped off. A total of 100 vines were surveyed. For each of the randomly selected 100 vines, visual disease incidence and severity ratings were conducted. Incidence ratings were marked 1 or 0 based on if the trunk had visual symptoms of GTD. The symptoms consisted of cankers, dark streaking of wood, brown discoloration of wood, white rot, or soft yellowish wood. To evaluate severity, a visual rating system to estimate how much of the vine's cut surface of the trunk showed symptoms (Fig. 4.2).

Out of the 100 vines selected for incidence and severity ratings, 21 of those were sawed off the top of the trunk at approximately ½ inch in size using a hand saw. The 21 samples were labeled and transported to the lab in a cooler. In the lab, each sample was cut into four one-centimeter-long pieces and the bark was removed using a scalpel.

PCR and identification

The PCR and identification steps used in this experiment were the same as those described in Chapter Three.

Statistics

Data were analyzed using a regression model through the REG procedure in SAS 9.4 (SAS Institute, Cary, NC). The REG procedure is a regression analysis to determine which fungal species predicted damage. The analysis was repeated to determine if the presence or absence of any GTD species affected the average amount of damage.

Results

Of the 100 randomly selected Vidal blanc vines across two and a half acres, 90% showed visual GTD symptoms, which included brown streaking of wood, necrotic cankers, and discoloration of wood. The average disease severity rating was 12.55%.

When fungal species were identified from the twenty-one randomly selected trunk samples, 71% Seventy-one percent of the fungal species were not associated with GTD

(Fig. 4.3). These include *Alternaria alternata* (18%), *Camillea tinctor* (6%), *Fusarium venenatum* (12%), *Stereum complicatum* (12%), *Fusarium oxysporum* (41%), and *Didymella sp*. (12%). The fungal species identified from samples that were associated with GTD were *Phomopsis viticola* (13%), *Botryosphaeria sp.* (8%), *Neofusicoccum ribis* (4%), and *Neofusicoccum parvum* (4%).

Some trunk samples contained more than one fungal species identified from isolation and extraction techniques. The breakdown of fungal species identified was based on the number of fungal species found in each sample (Figs. 4.4-4.7). Only one fungal species was identified in each of 18 out of 21 samples (Fig. 4.4). These fungal species included GTD pathogens and fungal species not associated with GTD. The GTD pathogens included *Phomopsis viticola* in two samples, *Neofusicoccum parvum* in two samples, and *Botryosphaeria sp.* in one sample. The fungal species not associated with GTD included *Fusarium oxysporum* in seven samples, *Stereum complicatum* in two samples, *Didymella sp.* in two samples, *Alternaria alternata* in one sample, and one sample that is unknown. Lack of DNA for extraction and amplification may have caused the unknown sample to remain unidentified through Sanger Sequencing.

One sample contained two fungal species, both of which were GTD fungal pathogens: *Phomopsis viticola* and *Neofusicoccum parvum* (Fig. 4.5). One sample contained three fungal species, one of which was a GTD pathogen: *Neofusicoccum ribis* (Fig. 4.6). The other two fungal species are not associated with GTD but are known to cause other diseases: *Alternaria alternata* and *Fusarium venenatum*. One sample contained four fungal species, one of which was a GTD pathogen *Botryosphaeria sp*. The other fungal species were *Alternaria alternata*, *Fusarium venenatum*, and *Camillea tinctor* (Fig. 4.7).

A regression model was completed to show the severity of damage as a function of species (Table 4.1). The relationship between severity and fungal species recovered from Vidal blanc trunk samples was not statistically significant (p=0.7153).

Discussion

All samples collected for this study had the bark removed prior to plating on PDA petri dishes. This eliminates fungal species that may have colonized the outside of the wood. This allows for proper identification of fungal endophytes colonizing the vascular tissue of the wood, which is consistent with the GTD fungal disease complex. Some of the same fungal species from the study in chapter two that are not associated with GTD were also identified from samples in this study. This could have impacted the types of fungal species identified from each sample. Some of these fungal species not associated with GTD are known to cause diseases in other crops. *Alternaria alternata* is known to cause blights, leaf spots, and rots on potato, tomato, citrus, and pistachio crops (Pryor and Michailides 2002, Weir et al. 1998, Peever et al. 2002), while a species of *Didymella*, *Didymella applanate* can cause spur blight in the canes of red raspberries (Pepin and Williamson 1985). The unknown species found in small quantity was unable to be identified via Sanger Sequencing. This could be due to not enough DNA for extraction and amplification.

There was no statistical significance between the fungal species identified in the trunk samples and the visual severity ratings. The regression model predicted the average damage for the samples to determine no correlation between severity rating and whether the samples contained GTD pathogens. A future study might include a larger sample size of severity ratings for symptomatic vine samples. Another future study can evaluate the GTD infection rate of the newly planted (certified virus-free) Vidal blanc vines grafted onto 3309 rootstocks.

Other studies have shown that *Botryosphaeriacea* fungal species were the most common fungal species found in symptomatic samples throughout Missouri when isolated from wedge-shaped cankers (Úrbez-Torres and Gubler 2011). This study revealed that *Phomopsis viticola* was the most common GTD fungal species identified in symptomatic Vidal blanc vines in the Augusta, MO vineyard. The reasoning for the difference in most commonly found GTD pathogens from Úrbez-Torres's 2011 study could be due to the number of samples, variation in sampling sites, and cultivars surveyed.

This study supports Úrbez-Torres's study that *Phomopsis viticola* is a major GTD fungal pathogen (Úrbez-Torres et al. 2013). *Phomopsis viticola* being the most common GTD pathogen found in this study could be attributed to *Phomopsis*-related diseases emerging in Missouri due to the climate's high humidity levels throughout the growing season. Phomopsis cane and leaf spot is known to affect grapevines and is common in climates with high humidity. It can infect all green parts of the grapevine, including spotting on leaves, brown lesions on canes, brown necrosis on rachis, and shriveled berries (Hewitt and Pearson 1988). Certain factors can impact *Phomopsis viticola*. These include high rainfall during the growing stages of the season and dry conditions before bloom the following year. Additionally, vines that are hedged for multiple growing seasons have a higher potential for *Phomopsis viticola* inoculum to infect (Pscheidt and Pearson 1989).

The other GTD pathogens isolated from symptomatic samples confirms Úrbez-Torres's research that *Neofusicoccum parvum*, *Neofusicoccum ribis*, and *Boyrosphaeria sp.* are commonly found in symptomatic vines in Missouri (Úrbez-Torres and Gubler 2011).

Since *Phomopsis viticola* was the most common GTD pathogen identified from the symptomatic *Vitis* interspecific hybrid cultivar Vidal blanc, more research on *Phomopsis viticola* spore dispersal timing within Missouri vineyards may assist viticulturists to prevent infection in Vidal blanc. Additionally, future research on incidence and severity of GTD on other symptomatic cultivars may reveal a better understanding of the relationship between severity and GTD pathogens present. Research on alternative management practices to reduce spread of *Phomopsis viticola* would be useful to further protect Missouri vineyards from infection.

Fig. 4.1. Satellite image of the commercial vineyard in Augusta, MO. The two and a half acres being removed housed 1,375 vines. The block consisted of eight acres of *Vitis* interspecific hybrid cultivar Vidal blanc. The two and a half acres were being removed due to disease damage. Image provided by Google Maps 2020 satellite data.

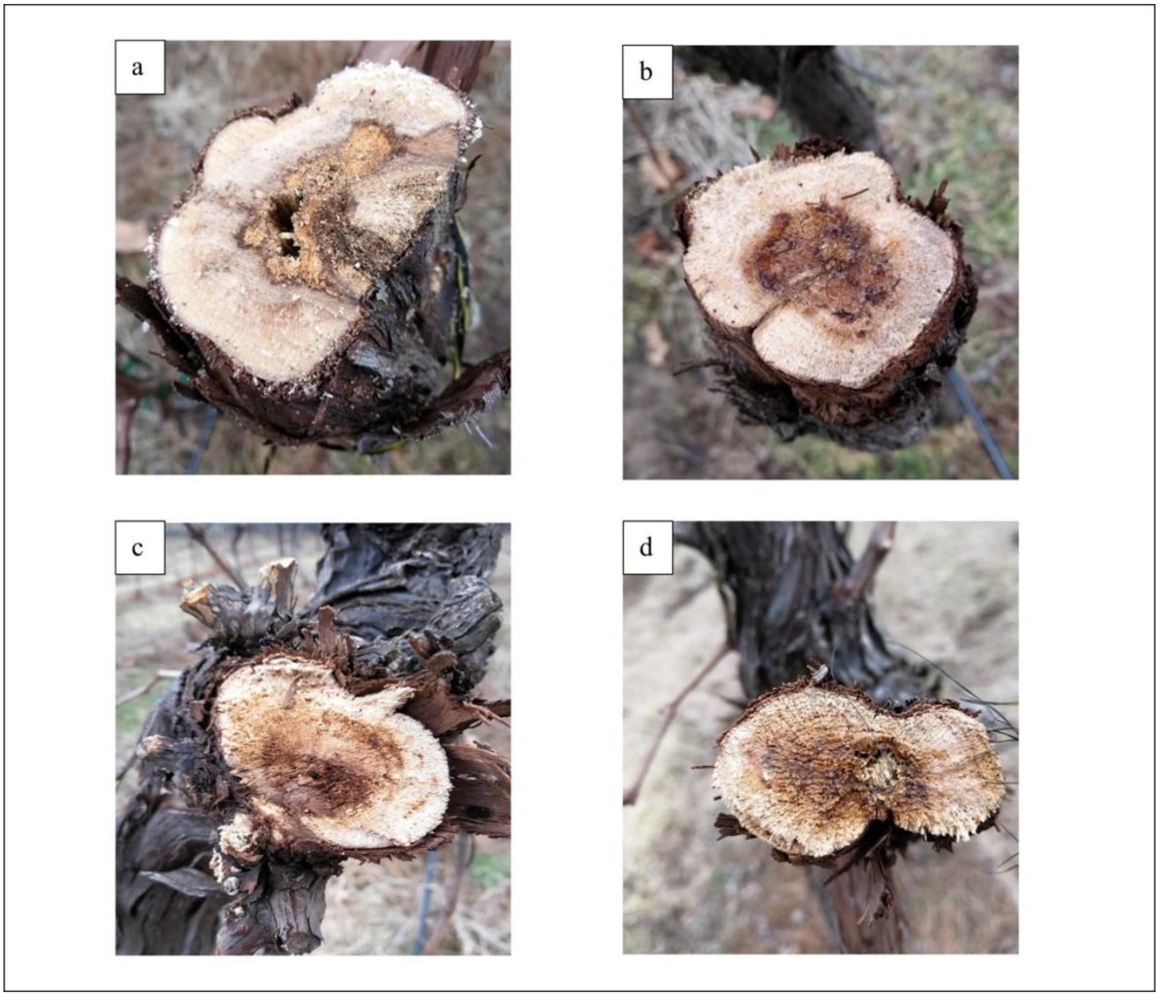

Fig. 4.2. Representation of visual severity ratings for *Vitis* interspecific hybrid cultivar Vidal blanc vines surveyed for grapevine trunk disease symptoms at a commercial vineyard in Augusta, MO (n=100). Grapevine trunks with a small portion of disease symptomology were rated 10% severity (a), 20% severity (b), and 30% severity (c). Grapevine trunks with almost half of the wood showing grapevine trunk disease symptoms were rated 40% severity (d).

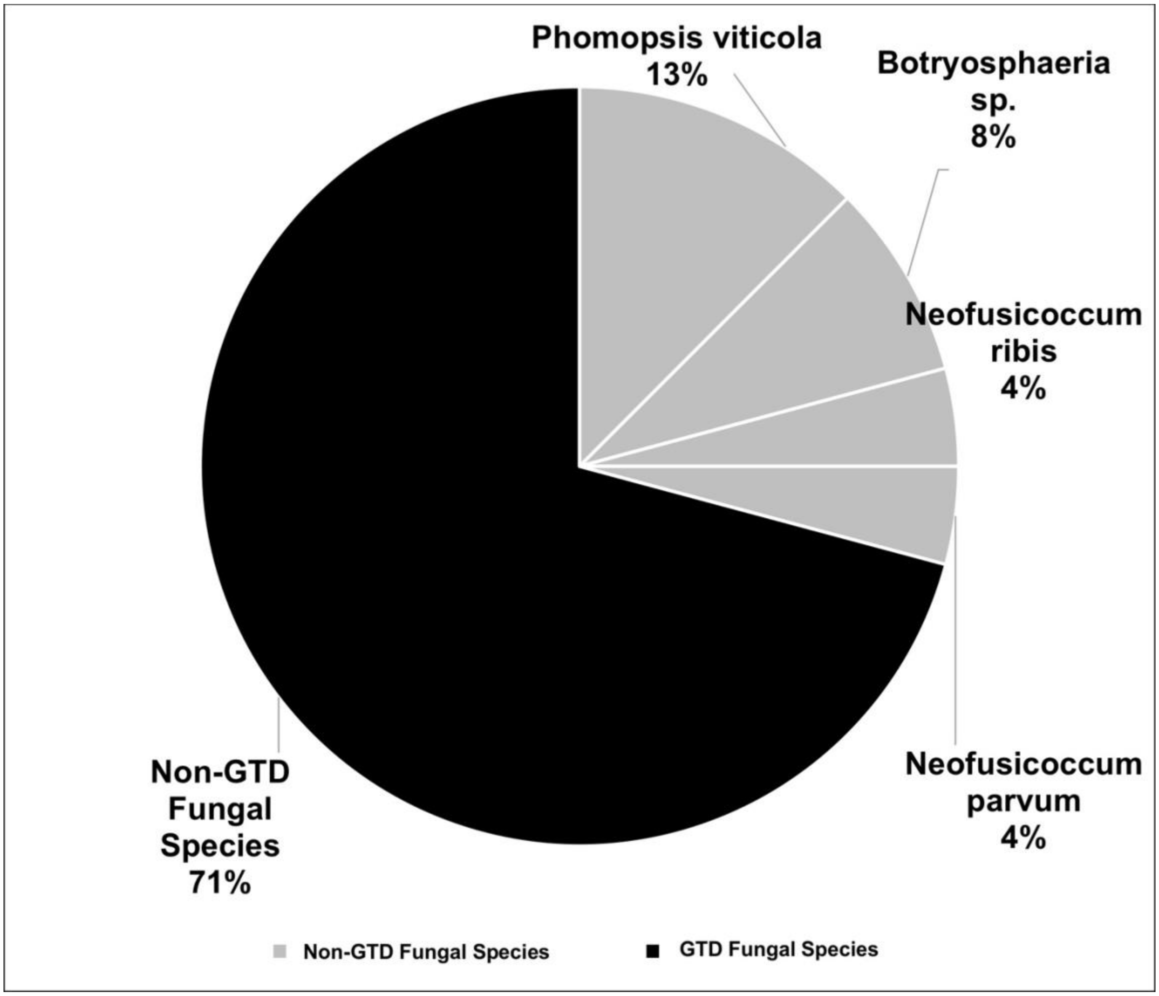

Fig. 4.3. Percentage of fungal species identified in *Vitis* interspecific hybrid grapevine cultivar Vidal blanc samples showing symptoms of grapevine trunk disease in a commercial vineyard block in Augusta, MO (n=21). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex. Some samples contained multiple fungal species. Non-grapevine trunk disease fungal species include: *Alternaria alternata*, *Camillea tinctor*, *Fusarium venenatum*, *Stereum complicatum*, *Fusarium oxysporum*, and *Didymella sp*.

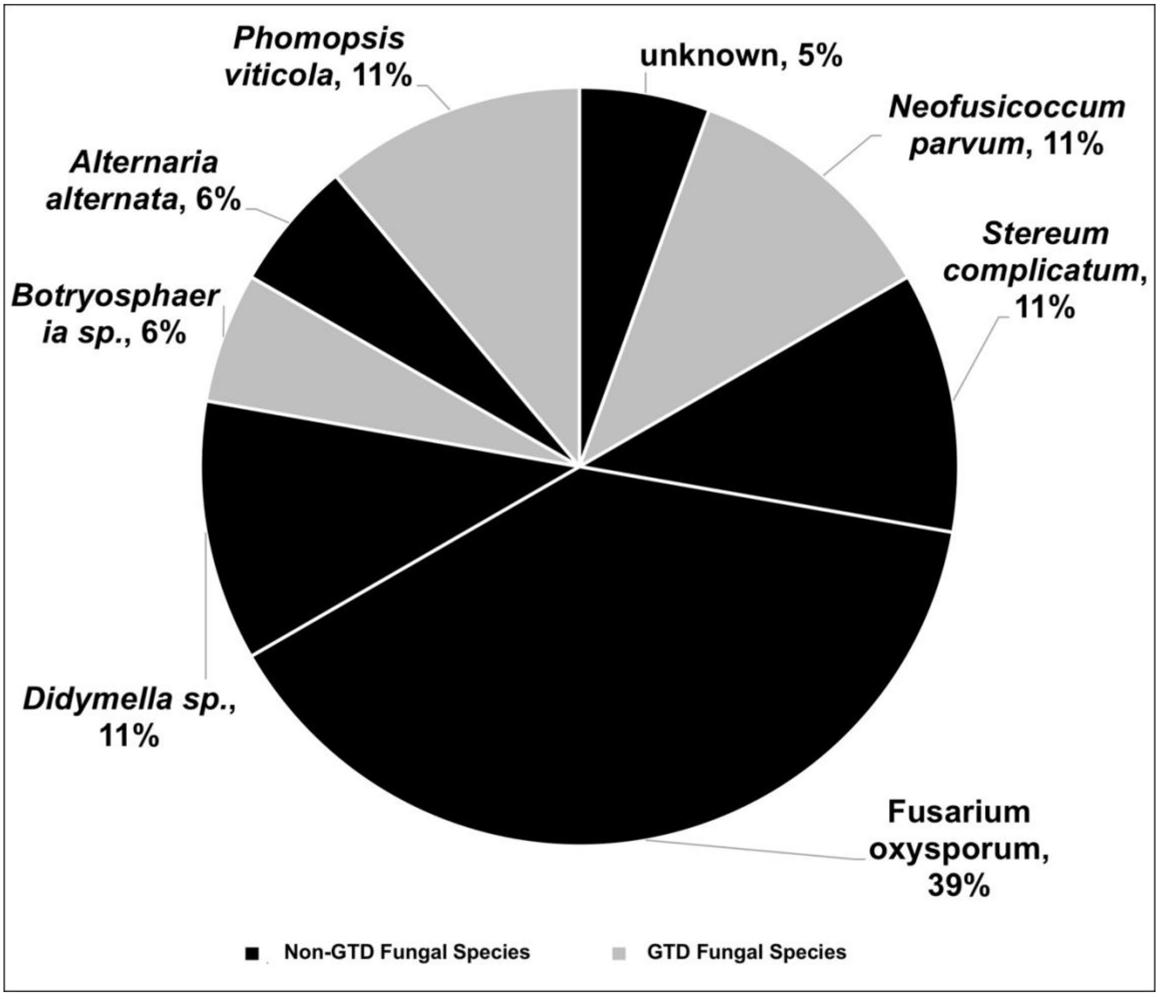

Fig. 4.4. Percentage of fungal species identified in *Vitis* interspecific hybrid grapevine cultivar Vidal blanc samples showing symptoms of grapevine trunk disease in a commercial vineyard block in Augusta, MO. These samples contained only one fungal species (n=18). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

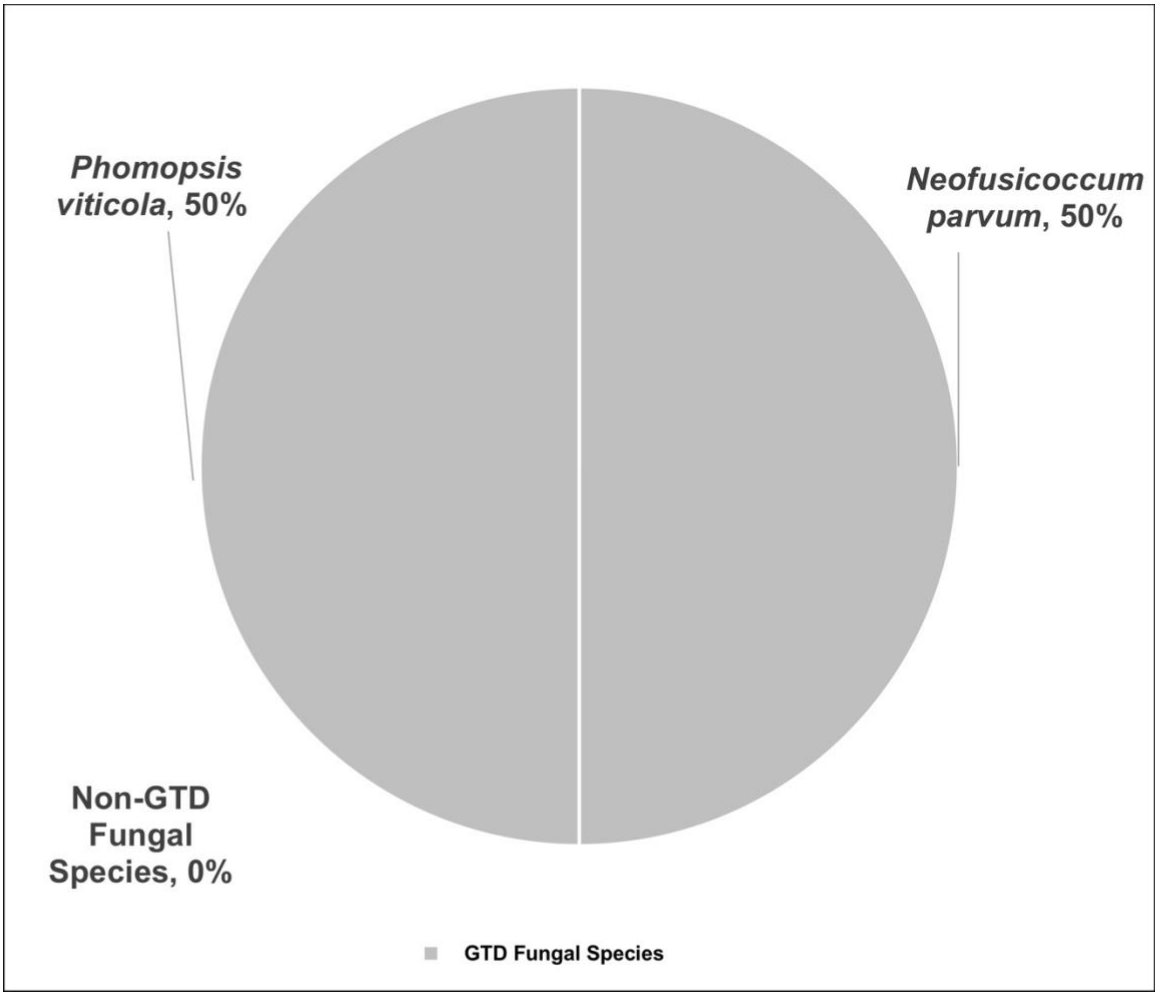

Fig. 4.5. Percentage of fungal species identified in *Vitis* interspecific hybrid grapevine cultivar Vidal blanc samples showing symptoms of grapevine trunk disease in a commercial vineyard block in Augusta, MO. The sample contained two fungal species (n=1). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

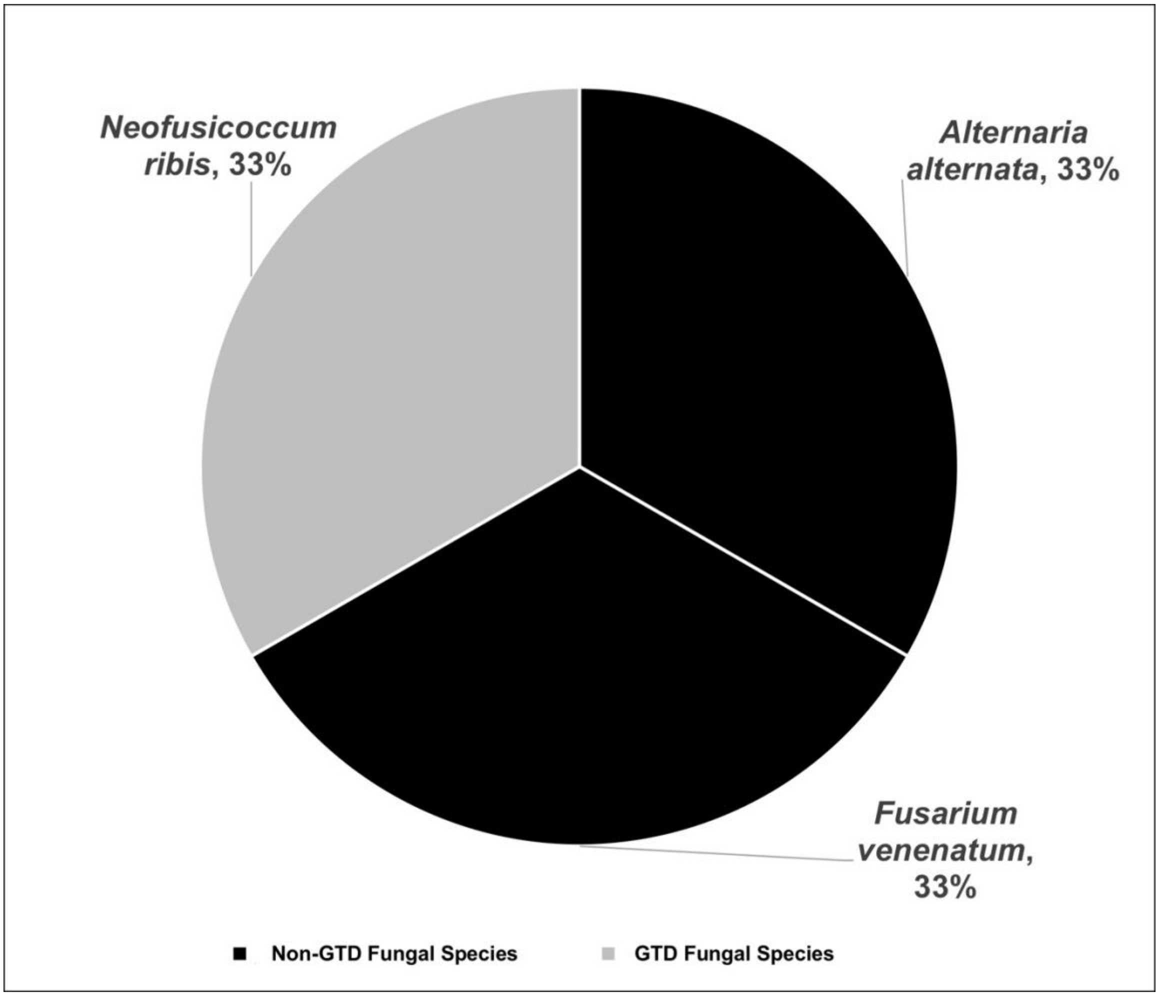

Fig. 4.6. Percentage of fungal species identified in *Vitis* interspecific hybrid grapevine cultivar Vidal blanc samples showing symptoms of grapevine trunk disease in a commercial vineyard block in Augusta, MO. The sample contained three fungal species (n=1). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non-grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

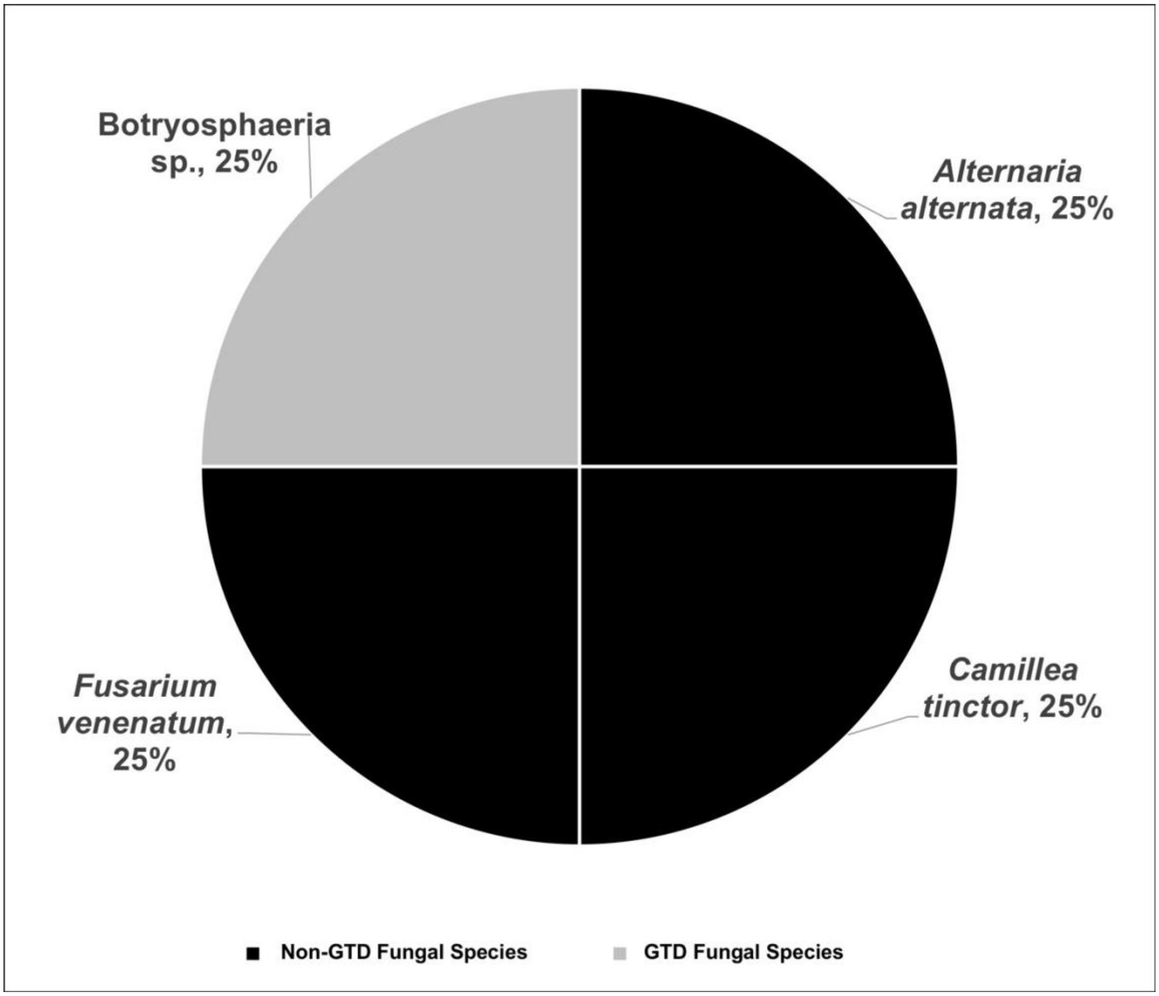

Fig. 4.7. Percentage of fungal species identified in *Vitis* interspecific hybrid grapevine cultivar Vidal blanc samples showing symptoms of grapevine trunk disease in a commercial vineyard block in Augusta, MO. The samples contained four fungal species (n=1). Grapevine trunk disease fungal species (GTD Fungal Species) include the pathogens associated with the disease complex. Non- grapevine trunk disease fungal species (Non-GTD Fungal Species) include fungal species not associated with the disease complex.

Fungal Species	p-value
Botryosphaeria sp.	0.1954
Neofusicoccum parvum	0.3448
Neofusicoccum ribis	0.1954
Phomopsis viticola	0.2486
Alternaria alternata	0.786
Camillea tinctor	0.7352
Didymella sp.	0.1651
Fusarium oxysporum	0.2457
Fusarium venenatum	0.6151
Stereum complicatum	0.1210
Unknown	0.1474

Table 4.1. Regression model p-values of severity of damage as a function of species for *Vitis* interspecific hybrid cultivar Vidal blanc samples taken from a commercial vineyard block in Augusta, MO. The regression model p=0.7153. Grapevine trunk disease pathogens were: *Botryosphaeria sp*, *Neofusicoccum parvum*, *Neofusicoccum ribis*, and *Phomopsis viticola*. Fungal species not associated with grapevine trunk disease were: *Alternaria alternata*, *Camillea tinctor*, *Didymella sp.*, *Fusarium oxysporum*, *Fusarium venenatum*, and *Stereum complicatum*.

Chapter 5

Koch's Postulates on common GTD pathogens from Missouri vineyards

Introduction

The disease complex impacting grapevine trunks is known as grapevine trunk disease (GTD) and consists of over 133 fungal species in 34 genera (Gramaje et al. 2018). This fungal disease complex impacts grapevines worldwide and includes species of *Cylindrocarpon*, *Botryosphaeria*, *Eutypa*, and more (Bonfiglioli 2008). The disease is caused by fungal pathogen species entering the vines through pruning wounds and can be dispersed by arthropods, rain, or wind (Mugnai et al. 1999, Úrbez-Torres et al. 2010, Moyo et al. 2014).

With the large number of fungal species associated with GTD, not all of them have been tested as causal agents under Koch's Postulates. Koch's Postulates consists of four steps to determine causality of a pathogen on a plant host. This includes: (1) identifying the pathogen present on the plant host, (2) isolating the pathogen onto pure culture such as PDA, (3) inoculating the pathogen onto a healthy host plant and replicating the disease symptoms, and (4) recovering the pathogen from the inoculated host and re-isolating the pathogen onto a pure culture.

Koch's Postulates has been completed for some *Vitis vinifera* and *Vitis* interspecific hybrid cultivars but not on all fungal species associated with GTD. In Arkansas and Missouri, Úrbez-Torres and Gubler conducted a study on four mature *Vitis* interspecific hybrid cultivars with 14 fungal species. Koch's Postulates were completed after inoculated mature cuttings were left for 24 weeks and included the species: *Botryosphaeria dothidea*, *Diatrypella sp*., *Diplodia seriata*, *Dothiorella americana*, *Eutypa lata*, *Eutypella vitis*, *Lasiodiplodia missouriana*, *Lasiodiplodia viticola*, *Neofusicoccum ribis*, *Neofusicoccum vitifusiforme*, *Pestalotiopsis sp.*, *Pestalotiopsis uvicola*, *Phaeomoniella chlamydospora*, *Phomopsis viticola*, and *Togninia minima* (Úrbez-Torres and Gubler 2011). Úrbez-Torres also conducted a study on a mature *Vitis vinifera* cultivar Syrah grown in California (Úrbez-Torres et al. 2013). Koch's Postulates were also completed on other *Vitis vinifera* cultivars in China, Croatia, Spain, Iran, and more, but these studies were conducted on mature vines that ranged from four to 35 years of age. The GTD fungal species identified as causal agents in these studies were strains of *Diaporthe*, *Phaeoacremonium spp*., *Phaemoniella chlamydospora*, *Cylindrocarpon liriodendra*, *Diplodia seriata*, *Neofusicoccum parvum*, and *Pleurostoma richardsiae*.

The purpose of this research is to identify the causality of four GTD pathogens on young *Vitis* interspecific hybrid cultivar Vignoles by completing Koch's Postulates. The difference between this Koch's Postulates study and that of Úrbez-Torres et al. (2013), was that the latter was completed on a mature 14-year old cordon rather than own-rooted cuttings and was left for two years before identifying the discoloration in the vascular tissue. In this study, Koch's Postulates were completed on young (less than two-year old) *Vitis* interspecific hybrid cultivar Vignoles and left for 10 days prior to identifying visual GTD symptomology and identifying the pathogens as causal agents of GTD. Understanding the causality of GTD in young vines is important to better manage the spread of disease early on in a vineyard and to understand that GTD affects both young and old vines and can rapidly develop visual symptoms.

Methods

Site and Sampling

Koch's Postulates were conducted in a greenhouse at the University of Missouri in Columbia, Missouri during January-February 2020. The greenhouse conditions during the experiment were 24°C and 32% RH. Methods used were modified from Yan et al. (2011). Six vines of *Vitis* interspecific hybrid cultivar Vignoles were obtained from Double A Vineyards, Inc. (Fredonia, NY) and grown in a 50/50 mixture of perlite and Pro-Mix BX/W Mycorrhizae in 8.694 liter blow-molded containers. Four isolates associated with GTD (*Diplodia seriata*, *Neofusicoccum parvum*, *Neofusicoccum ribis*, and *Pestalotiopsis uvicola*) were used to inoculate shoots of *Vitis* interspecific hybrid cultivar Vignoles. These fungal species were found on diseased grapevine hosts at the Horticulture and Agroforestry Research Center in New Franklin, MO. The species were present on trunk tissue which were identified in the lab.

The four steps of Koch's Postulates were to: (1) find GTD pathogens present on and in diseased hosts, (2) isolate pathogens on PDA petri dishes, (3) inoculate healthy young shoots with the isolated GTD pathogens and observe GTD symptoms, and (4) isolate the GTD pathogens from the inoculated host and compare with the pathogen first isolated.

PCR and Identification

The identification of fungal species and PCR steps were the same as those presented in Chapter Three. The confirmed cultures of the four common GTD-associated pathogens identified were re-isolated onto fresh PDA petri dishes to maintain a healthy fungal colony until inoculation.

Inoculations

Young dormant shoots of *Vitis* interspecific hybrid cultivar Vignoles were inoculated with colonized agar plugs of the four fungal species: *Diplodia seriata, Neofusicoccum parvum, Neofusicoccum ribis*, and *Pestalotiopsis uvicola*. Sterile PDA plugs were used as the control. One of each fungal isolate and one control were inoculated onto each of six vines with five inoculations on each shoot, totaling 30 inoculations. Inoculations were completed using a 4-mm cork borer (2 mm deep) into the dormant shoot, placing a colonized agar plug on the wound, then wrapping the inoculation wound in Parafilm (Bemis Company, Inc, Oshkosh, WI). After 10 days, inoculated shoots were removed from the greenhouse vine via pruning shears and brought back to the lab for analysis of visual disease symptoms and to isolate the fungal species from the inoculated host onto PDA. The isolates were then compared with the isolates originally identified in diseased hosts.

Results

All six replicates of each of the GTD fungal species *Diplodia seriata*, *Neofusicoccum parvum*, and *Pestalotiopsis uvicola* showed brown discoloration of wood outside the wound site, consistent with GTD symptomology. Five out of the six replicates of the GTD fungal species *Neofusicoccum ribis* showed brown discoloration of wood outside the wound site. All six replicates of the control inoculation showed no symptoms of GTD. All fungal species were recovered from samples onto PDA plates 10 days after inoculation. The results reveal that *Neofusicoccum parvum*, *Neofusicoccum ribis, Pestalotiopsis uvicola*, and *Diplodia seriata* are causal agents of trunk disease in young *Vitis* interspecific hybrid cultivar Vignoles.

Visual symptoms can be seen from the inoculation site of the inoculated vines (Fig. 5.1). *Diplodia seriata* (b) revealed dark brown at the wound site and brown discoloration spreading outwards from the wound site, indicating necrosis. *Neofusicoccum parvum* (c) inoculation showed light brown at the wound site and brown discoloration spreading outside the wound site. *Neofusicoccum ribis* (d) inoculation showed gray-brown coloration at the wound site and brown discoloration and streaking emerging outside the wound site. *Pestalotiopsis uvicola* (e) inoculation showed gray-brown coloration at the wound site and brown streaking spreading outwards outside the wound site.

Cross-sections of the inoculated vines revealed visual symptoms of the inoculated vines 10 days after inoculation (Fig. 5.2). The control (a) showed no discoloration. The cross section of *Diplodia seriata* (b) revealed brown necrotic canker covering almost half of the vine cutting. *Neofusicoccum parvum* (c) and *Neofusicoccum ribis* (d) showed brown discoloration of the wood, indicated by the red arrows. *Pestalotiopsis uvicola* (e) had brown discoloration in the cross section surrounded by white rotting sections which is also consistent with GTD symptoms.

Discussion

Fungal species were recovered from surrounding wound sites 10 days after inoculation. This rapid development of visual symptomology and fungal isolates recovered from samples reveals that GTD can spread rapidly in vines exposed to the fungal species associated with GTD. Five out of the six *Neofusicoccum ribis* inoculations showed symptoms of GTD in the photo taken 10 days after inoculation, but all fungal species were recovered from samples. This could mean that the causal agent could be asymptomatic.

This study confirms that the four fungal species, *Diplodia seriata*, *Neofusicoccum parvum*, *Neofusicoccum ribis*, and *Pestalotiopsis uvicola* are causal agents of GTD in young (less than two-year-old) *Vitis* interspecific hybrid cultivar Vignoles vines. These causal agents cause symptoms in young vines in less than ten days. This means that these fungal species can infect and begin harming the vines in a very short time period. This should be taken into consideration when establishing management strategies and preventative measures to control GTD in the vineyard. To our knowledge, this is the first time these four GTD fungal pathogens were tested using Koch's Postulates on young (less than two-year-old) *Vitis* interspecific hybrid cultivar Vignoles within a 10 day period. Testing young vines for GTD pathogens is important in order for viticulturists to plant vines free of pathogens to prevent infection early. Since GTD pathogens can infect young vines, it can also impact nursery stock and ultimately decimate newly planted vineyards.

Previous studies have shown that *Pestalotiopsis* spp. is associated with wedge-shaped cankers, dark streaking of wood, and light-brown discoloration and central necrosis on mature vines. This study confirms that *Pestalotiopsis uvicola* causes brown streaking, and light brown discoloration of wood in young hybrid vines. *Neofusicoccum ribis* and *Neofusicoccum parvum* are closely related, and previous studies of GTD pathogens in Missouri revealed that *Neofusicoccum ribis* had high virulence as the second most aggressive fungus identified from grapevine isolates (Úrbez-Torres 2012).

There are over 130 fungal species associated with trunk disease, but not all of them have been tested with Koch's Postulates to determine causality of GTD. Five common isolates recovered from samples in Missouri vineyards were tested with Koch Postulates on young hybrid cultivar Vignoles. More studies on common GTD isolates recovered from more vineyards across Missouri are needed to see if all fungal species identified are causal agents of GTD in young vines.

Foliar symptomology was not assessed due to the study being conducted on dormant wood. Because this was conducted in the winter months, there were few leaves present to identify symptoms within 10 days.

This study was completed in a greenhouse setting. Future research on the causal agents of GTD on young vines can be tested on young vines in the field to determine similar infection rates. Future research with spore trapping in vineyards could quantify fungal spores present during varying weather conditions in Missouri vineyards and determine if certain levels of spores present in the air correlate with number of vines infected with GTD in the same vineyards.

Knowing that some vines may be more susceptible, there are some strategies that growers can use to manage GTD. The first step would be to make sure that all plants are disease-free prior to planting in the ground, to the best of the grower's knowledge. This can be completed by using reputable clean grapevine plant programs that test for viruses prior to distributing from a nursery (Rowhani et al. 2005). However, there is currently no clean plant program for fungal pathogens and some pathogens may be present without showing symptoms in the vines until stressed. Managing the site and understanding the site's environmental conditions can help determine proper planting conditions to limit stress on the vines (Gramaje et al. 2018). A recommended strategy for controlling pathogens is to not plant a vineyard near apple and pear orchards because they have been known to carry GTD pathogens (Cloete et al. 2011). Once a vineyard is established with mature vines, sanitation is key to limiting the spread of fungal spores. For example, after pruning, it is advisable to remove the cuttings from the ground and destroy all diseased wood. A more environmentally conscious option to burning the material is to compost it, which has been shown to eliminate the pathogens *Diplodia seriata*, *Phaeomoniella chlamydospora*, and *Eutypa lata* after six months (Lecomte et al. 2006). Additionally, growers can protect their pruning wounds on vines by administering fungicide applications, such as Topsin M (thiophanate-methyl; to prevent spores from spreading) as well as learning the ideal conditions with which the GTD fungal pathogens spread (to manage pruning times and prevent spore dispersal) (Rolshausen et al. 2010, Gramaje et al. 2018). Fungal spores tend to be more prevalent after periods of heavy rainfall (Lehoczky 1974). Pest management, especially that of millipedes, cocktail ants, and spiders should be controlled in the vineyard since they were known to vector GTD pathogens (Moyo et al. 2014).

This study revealed that GTD symptoms can develop rapidly in young *Vitis* interspecific hybrid cultivar Vignoles after being inoculated with four common GTD pathogens isolated from Missouri vineyards: *Diplodia seriata*, *Neofusicoccum parvum*, *Neofusicoccum ribis*, and *Pestalotiopsis uvicola*. Understanding the rapid development of symptoms in young vines is important for prevention of infection within nursery stock as well as newly planted vineyards. Future research should be conducted to understand the causality of other fungal endophytes that may be causal agents of GTD. Additionally, future research completing Koch Postulates with common GTD pathogens on various young *Vitis* interspecific hybrid cultivars can establish a better understanding of GTD symptomology on other economically important cultivars.

Fig. 5.1. Koch's Postulates inoculation wound site 10 days after inoculation. The wound sites reveal symptoms of grapevine trunk disease, including discoloration of wood. The control was inoculated with sterile PDA (a) and the grapevine trunk disease pathogens inoculated were: *Diplodia seriata* (b), *Neofusicoccum parvum* (c), *Neofusicoccum ribis* (d), and *Pestalotiopsis uvicola* (e).

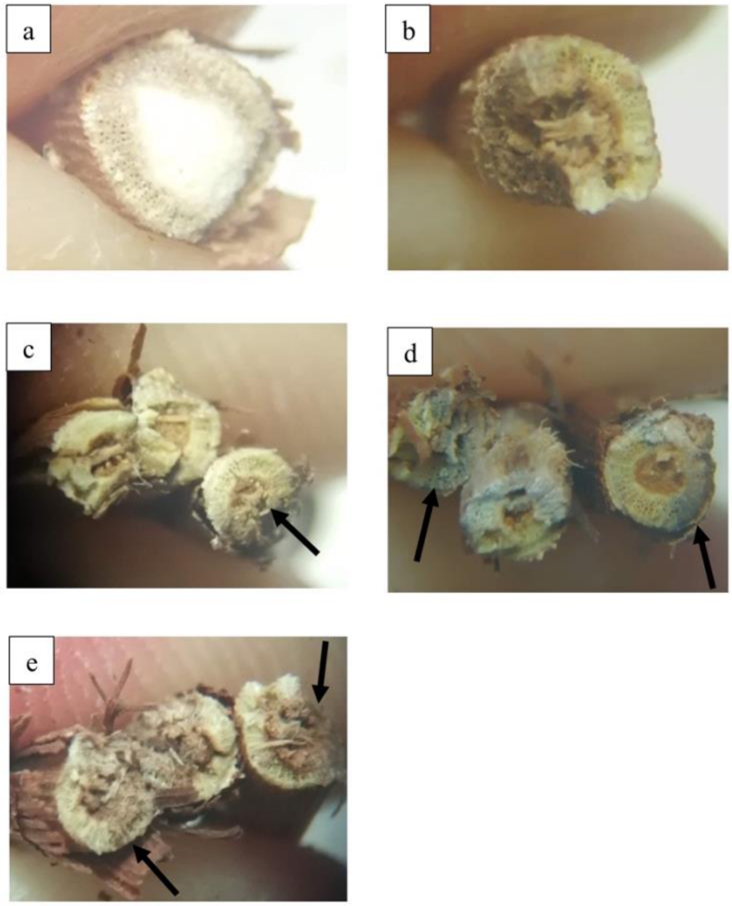

Fig. 5.2. Cross section of Koch's Postulates grapevine trunk disease inoculations 10 days after inoculation. The arrows point to key grapevine trunk disease symptomology on the inoculated shoots, such as discoloration of wood and necrotic canker. The control was inoculated with sterile PDA (a) and the grapevine trunk disease pathogens inoculated were: *Diplodia seriata* (b), *Neofusicoccum parvum* (c), *Neofusicoccum ribis* (d), and *Pestalotiopsis uvicola* (e).

LITERATURE CITED

Agrios, G. N. 2005. Koch's postulates. Pages 26-27 in: Plant Pathology, Fifth Edition. Elsevier Academic Press, Burlington, MA.

Andolfi, A., Mugnai, L., Luque, J., Surico, G., Cimmino, A., Evidente, A. 2011. Phytotoxins produced by fungi associated with grapevine trunk diseases. Toxins (Basel). 3:1569-1605.

Barnes, I., Roux, J., Wingfield, M. J., Coetzee, M. P., Wingfield, B. D. 2001. Characterization of *Seiridium* spp. associated with cypress canker based on ß-tubulin and Histone sequences. Plant Dis. 85:317-321.

Benítez, T., Rincón, A. M., Limón, M. C., Codon, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. J. Microbiol7:249-260.

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C., Fontaine, F. 2013. Grapevine trunk diseases: Complex and still poorly understood. Plant Pathol. 62:243-265.

Bonfiglioli, R. 2008. The spores are out there: Observations from the 6th International Workshop on Grapevine Trunk Diseases. Aust. N.Z. Grapegrow. Winemak. 538:42-45.

Carter, M.V., Bloay, A., Rappaz, F. 1983. An annotated host list and bibliography of *Eutypa armeniacae*. Rev. Plant Pathol. 62:251-258.

Carter, M. V. 1991. The status of *Eutypa lata* as a pathogen. Int. Mycol. Inst. Pytopathol. Pap. 32.

Cloete, M., Fourie, P. H., Damm, U., Crous, P. W., Mostert, L. 2011. Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. Phytopathol Mediterr. 50:S176-S190.

Cobos, R., Mateos, R. M., Álvarez-Pérez, J. M., Olego, M. A., Sevillano, S., González-García, S., Garzón-Jimeno, E., Coque, J. J. R. 2015. Effectiveness of natural antifungal compounds in controlling infection by grapevine trunk disease pathogens through pruning wounds. Appl Environ Microbiol. 81:6474-6483.

Creasap, J. E., Burr, T. J. 2006. Grape Crown Gall. Integrated Pest Management. Retrieved on 20 August from: http://nysipm.cornell.edu/ factsheets/grapes/diseases/crown_gall.pdf.

Dami, I., Ferree, D. C., Kurtural, S. K., Taylor, B. H. 2005. Influence of crop load on 'Chambourcin' yield, fruit quality, and winter hardiness under midwestern United States environmental conditions. Acta Hortic., 689:203-208.

Dissanayake, A. J., Liu, M., Zhang, W., Chen, Z., Udayanga, D., Chukeatirote, E., Hyde, K. D. 2015. Morphological and molecular characterisation of *Diaporthe* species associated with grapevine trunk disease in China. Fungal Biol. 119:283-294.

Fourie, P. H., Halleen, F. 2004. Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. Australas. Plant Pathol. 33:313-315.

Gao, F., Dai, R., Pike, S. M., Qiu, W., Gassmann, W. 2014. Functions of EDS1-like and PAD4 genes in grapevine defenses against powdery mildew. Plant Mol. Biol. 86:381-393.

Giménez-Jaime, A., Aroca, A., Raposo, R., García-Jiménez, J., Armengol, J. 2006. Occurrence of fungal pathogens associated with grapevine nurseries and the decline of young vines in Spain. J Phytopathol. 154:598-602.

Gramaje, D., Baumgartner, K., Halleen, F., Mostert, L., Sosnowski, M.R., Úrbez-Torres, J.R., Armengol, J. 2016. Fungal trunk diseases: a problem beyond grapevines. Plant Pathol. 65:355-356.

Gramaje, D., Úrbez-Torres, J. R., Sosnowski, M. R. 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Dis. 102:12-39.

Gu, S., Wilker, K.L., Dharmadhikari, M. R., Moore, J. F., Howard, S., Edson, C. E., Bonney, T. M., Walsh, M. K. 2000. Evaluation of grapevine cultivars and breeding lines for adaptability to Missouri conditions. Acta Hortic. 528:569-574.

Hewitt, W. B., Pearson, R. C. 1988. Phomopsis cane and leaf spot. Pages 17-18 in: Compendium of Grape Diseases. American Phytopathological Society Press, St. Paul, MN.

Kaliternam, J., Milicevic, T., Bencic, D., Duralija, B. 2013. First report of *Neofusicoccum parvum* associated with grapevine trunk diseases in Croatia. Plant Dis. 97:1656-1656.

Khanizadeh, S., Rekika, D., Porgès, L., Levasseur, A., Groleau, Y., Fisher, H. 2008 Soluble Solids, Acidity, Canopy Fruit Distribution, and Disease Susceptibility of Selected Grape Cultivars in Quebec. Int. J. Fruit Sci. 8:200-215.

King, R., Brown, N. A., Urban, M., Hammond-Kosack, K. E. 2018. Inter-genome comparison of the Quorn fungus *Fusarium venenatum* and the closely related plant infecting pathogen *Fusarium graminearum*. BMC Genomics. 19:269.

Klodd, A. 2019. Early Season Control of Phomopsis on Grapes. University of Minnesota Grape Breeding and Enology. Retrieved 20 August 2020 from: http://enology.umn.edu/news/early-season-control-phomopsis-grapes.

Kovács, C., Balling, P., Bihari, Z., Nagy, A., Sándor, E. 2017. Incidence of grapevine trunk diseases is influenced by soil, topology and vineyard age, but not by *Diplodia seriata* infection rate in the Tokaj Wine Region, Hungary. Phytoparasitica. 45:21-32.

Larignon, P., Dubos, B. 1997. Fungi associated with esca disease in grapevine. Eur. J. Plant Pathol. 103:147-157.

Lecomte, P., Louvet, G., Vacher, B., Guilbaud, P. 2006. Survival of fungi associated with gapevine decline in pruned wood after composting. Phytopathol. Mediterr. 45:127-130.

Lehoczky, J. 1974. Black dead arm disease of grapevine caused by *Botryosphaeria stevensii* infection. Acta Phytopathol. Hung. 9:319-327.

Loschiavo, A., Sosnowski, M., Wicks, T. 2007. Incidence of Eutypa dieback in the Adelaide Hills. Aust. N.Z. Grapegrow. Winemak. 519:26-29.

Massonnet, M., Figueroa-Balderas, R., Galarneau, E. R., Miki, S., Lawrence, D. P., Sun, Q., Cantu, D. 2017. *Neofusicoccum parvum* colonization of the grapevine woody stem triggers asynchronous host responses at the site of infection and in the leaves. Front. Plant Sci. 8:1117.

Mohammadi, H., Banihashemi, Z., Gramaje, D., Armengol, J. 2013. Fungal pathogens associated with grapevine trunk diseases in Iran. J Agr Sci Tech-Iran.15:137-150.

Morales, A., Latorre, B. A., Piontelli, E., Besoain, X. 2012. *Botryosphaeriaceae* species affecting table grape vineyards in Chile and cultivar susceptibility. Ciencia e investigación agrarian. 39:445-458.

Moyo, P., Allsopp, E., Roets, F., Mostert, L., Halleen, F. 2014. Arthropods Vector Grapevine Trunk Disease Pathogens. Phytopathol. 104:1063-1069.

Mugnai, L. Sosnowski, M. Eskalen, A. n.d. Diseases and Pathogens. International Countil on Grapevine Trunk Disease. Retrieved on 20 August 2020 from: https://icgtd.ucr.edu/Diseases&pathogens.html.

Mugnai, L., Graniti, A., Surico, G. 1999. Esca (Black Measles) and Brown Wood-Streaking: Two Old and Elusive Diseases of Grapevines. Plant Dis. 83:404-418.

Murolo, S., Romanazzi, G. 2014. Effects of grapevine cultivar, rootstock and clone on esca disease. Australas Plant Path. 43:215-221.

National Center for Biotechnology Information (NCBI). 1988. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Retrieved from 20 August 2020 from: https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Peever, T. L., Ibanez, A., Akimitsu, K., Timmer, L. W. 2002. Worldwide phylogeography of the citrus brown spot pathogen, *Alternaria alternata*. J. Phytopathol. 92:794-802.

Pepin, H. S., Williamson, B. 1985. Some factors influencing spur blight symptom development in red raspberry. IV International Rubus and Ribes Symposium 183:131-136.

Polesani, M., Bortesi, L., Ferrarini, A., Zamboni, A., Fasoli, M., Zadra, C., Polverari, A. 2010. General and species-specific transcriptional responses to downy mildew infection in a susceptible (*Vitis vinifera*) and a resistant (*V. riparia*) grapevine species. BMC genomics. 11:117.

Pouzoulet, J., Scudiero, E., Schiavon, M., Rolshausen, P. E. 2017. Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeomoniella chlamydospora* in grapevine. Front. Plant Sci. 8:1442.

Pryor, B., Michailides, T.J., 2002. Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with Alternaria late blight of pistachio. J. Phytopathol. 92:406–16.

Pscheidt, J. W., Pearson, R. C. 1989. Effect of grapevine training systems and pruning practices on occurrence of Phomopsis cane and leaf spot. Plant Dis. 73:825-828.

Reddick D. 1914. Dead-arm disease of grapes. New York State Agriculture Experimental Station, Geneva, NY. Bull. 389:463-490.

Reisch, B. I., Pool, R. M., Robinson, W., Henick-Kling, T., Watson, J., Kimball, K., Morris, J. 1990. 'Chardonel'Grape. HortScience. 25:1666-1667.

Reisch, B. I., Pool, R. M., Peterson, D. V., Martens, M. H., Henick-Kling, T. 1993. Wine and juice grape varieties for cool climates. Coop. Ext. Info. Bull. 233, Cornell Univ., Ithaca, NY.

Reisch, B., Pool, R. M., Robinson, W., Henick-Kling, T., Gavitt, B., Watson, J., Barrett, H. 1996. 'Traminette'Grape. New York Food and Life Sciences Bulletin 149. N.Y. State Agricultural Experiment Station, Geneva.

Reisch, B. I., Luce, R. S., Bordelon, B., Henick-Kling, T. 2006. 'Noiret'Grape. New York Food and Life Sciences Bulletin 160. N.Y. State Agricultural Experiment Station, Geneva.

Reisch, B. I., Luce, R. S., Mansfield, A. K. 2014. ‘Aromella’—An Aromatic White Wine Grape. HortScience. 49:676-678.

Rolshausen, P.E., Úrbez-Torres, J.R., Rooney-Latham, S., Eskalen, A., Smith, R.J., Gubler, W.D. 2010. Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. Am. J. Enol. Viticult. 61:113-119.

Rowhani, A., Uyemoto, J. K., Golino, D. A., Martelli, G. P. 2005. Pathogen testing and certification of *Vitis* and *Prunus* species. Ann. Rev. Phytopathol. 43:261-278.

Siebert, J.B. 2001. Eutypa: The economic toll on vineyards. Wines and Vines. April: 50-56.

Smiley, L., Domoto, P., Nonnecke, G., Miller, W. 2016. Cold climate cultivars: A review of cold climate grape cultivars. Iowa State University Extension.

Retrieved 20 August 2020 from: https://www.extension.iastate.edu/viticulture/cold-climate-cultivars.

Schloemann, S., Garofalo, E. 2019. Relative Disease Susceptibility and Chemical Sensitivity for selected grape cultivars. New England Small Fruit Management Guide. University of Massachusetts Amherst Extension Fruit Program. Retrieved on 20 August 2020 from: https://ag.umass.edu/fruit/ne-small-fruit-management-guide/grapes/diseases/table-55-relative-disease-susceptibility.

Sosnowski, M., Ayres, M., Wicks, T., McCarthy, M. 2013. In search of resistance to grapevine trunk diseases. Am. J. Enol. Vitic. 28:55-58.

Travadon, R., Rolshausen, P. E., Gubler, W. D., Cadle-Davidson, L., Baumgartner, K. 2013. Susceptibility of cultivated and wild *Vitis* spp. to wood infection by fungal trunk pathogens. Plant Dis. 97:1529-1536.

Úrbez-Torres, J. R., Leavitt, G. M., Voegel, T. M., Gubler, W. D. 2006. Identification and distribution of Botryosphaeria spp. associated with grapevine cankers in California. Plant Dis. 90:1490-1503.

Úrbez-Torres, J. R., Battany, M., Bettiga, L. J., Gispert, C., McGourty, G., Roncoroni, J., Smith, R. J., Verdegaal, P., Gubler, W. D. 2010. *Botryosphaeriaceae* Species Spore-Trapping Studies in California Vineyards. Plant Dis. 94:717–724.

Úrbez-Torres, J. R., Gubler, W. D. 2011. Susceptibility of grapevine pruning wounds to infection by *Lasiodiplodia theobromae* and *Neofusicoccum parvum*. Plant Pathol. 60:261–270.

Úrbez-Torres, J. R., Peduto, F., Striegler, R. K., Urrea-Romero, K. E., Rupe, J. C., Cartwright, R. D., Gubler, W. D. 2012. Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. Fungal Divers. 52:169-189.

Úrbez-Torres, J. R., Peduto, F., Smith, R. J., Gubler, W. D. 2013. Phomopsis dieback: A grapevine trunk disease caused by *Phomopsis viticola* in California. Plant Dis. 97:1571-1579.

USDA National Agricultural Statistics Service Missouri. 2016. Missouri Grape Facts. Retrieved on 20 August 2020 from: https://missouriwine.org/sites/default/files/2016%20Grape%20Facts_0.pdf.

Van Niekerk, J. M., Calitz, F. J., Halleen, F., Fourie, P. H. 2010. Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa. Eur J Plant Pathol. 127:375-390.

Van Niekerk, J. M., Bester, W., Halleen, F., Crous, P. W., Fourie, P. H. 2011. The distribution and symptomatology of grapevine trunk disease pathogens are influenced by climate. Phytopathol Mediterr. 50:S139-S150.

Weir, T. L., Huff, D. R., Christ, B. J., Romaine, C. P. 1998. RAPD-PCR analysis of genetic variation among isolates of *Alternaria solani* and *Alternaria alternata* from potato and tomato. Mycologia. 90:813-821.

White, T. J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR Protocols, A guide to methods and applications. Academic Press, San Diego. 215-322.

Wicks T., Davies K. 1999. The effect of *Eutypa* on grapevine yield. Aust. Grapegrow. Winemak. 406a:15-16.

Wilcox, W. F., Hoffman, L. E. 2015. Black rot. Compendium of Grape Diseases, Disorders, and Pests, Second Edition. American Phytopathological Society Press, St. Paul, MN. 28-33.

Wunderlich, N., Ash, G. J., Steel, C. C., Raman, H., Savocchia, S. 2011. Association of *Botryosphaeriaceae* grapevine trunk disease fungi with the reproductive structures of Vitis vinifera. Vitis. 50:89-96.

Yan, J. Y., Li, X. H., Kong, F. F., Wang, Z. Y., Gong, L. Z., He, H. P. 2011. Occurrence of grapevine trunk disease caused by *Botryosphaeria rhodina* in China. Plant Dis. 95:219-219.