TRUNK DISEASE OF GRAPEVINES IN TEXAS

A Thesis

by

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ABSTRACT

On a global scale, grapevine trunk diseases (GTDs) remain the greatest limitation to maintaining optimal yield as a vineyard ages. The best method of control is early prevention. However, due to the slow development of symptoms, preventative measures are infrequently employed. Many of the vineyards in Texas are less than ten years old, but with maturation of the winegrape industry will come GTDs. The overall goals of this project were to identify and characterize the fungi responsible for trunk disease in Texas and to raise awareness of these pathogens and promote early prevention as a parameter of disease management. First, three vineyards were surveyed for GTD incidence, severity, and the presence of causative agents. A positive correlation between vine age and disease severity was demonstrated. Second, the project identified the prevalent GTD-causing fungi in Texas wine grape vineyards. Fungi isolated from infected vines showed the presence of all major grapevine trunk diseases previously identified in the US, commonly known as *esca proper*, Botryosphaeria dieback, Eutypa dieback, and Phomopsis cane and leaf spot. Pathogenicity assays demonstrated disease causality and determined that *Lasiodiplodia* sp. were the most aggressive of the tested pathogens. Results from the third objective coincide with previous studies which indicate a correlation between spore dispersal and increased precipitation. The information obtained from these studies will be used to formulate GTD management recommendations for Texas wine grape growers.

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NOMENCLATURE

DNA	Deoxyribonucleic Acid
GSLD	Grapevine leaf stripe disease
GTD	Grapevine Trunk Disease
ul	Microliter
ME	Malt Extract
MEB	Malt Extract Broth
ml	Milliliters
mm	Millimeters
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
spp.	Species
TEB	Tris-Borate-EDTA
TX	Texas

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1. INTRODUCTION

Texas has recently emerged as a leading wine producing state, ranking fifth nationwide. As of 2013, the 286 wineries in Texas produced an economic impact of 1.8 billion dollars (Riverman, 2015). As the Texas wine and grape industries continue to expand, the understanding and control of vine diseases will become increasingly important to insure a reliable supply of high quality Texas-grown grapes. Many vineyards in Texas are less than ten years old and are just now experiencing disease losses commonly found in older vineyards. Grapevine trunk diseases (GTDs) are examples of those diseases, and are considered by some (Gramaje *et al.*, 2015) to be the most destructive of all vine diseases worldwide. As a vineyard ages, the incidence and severity of GTDs increases. Thus GTDs will progressively play a more critical role in the young, but expanding Texas winegrape industry.

Eutypa dieback, Botryosphaeria dieback, the Esca complex, and Phomopsis dieback are the predominant trunk diseases affecting grapevines (*Vitis vinifera*) worldwide (Bertsch *et al.*, 2013; Lawrence *et al.*, 2015). Although all four of these diseases are caused by different pathogens, their shared symptomology leads to a sole common classification as a grapevine trunk disease. The principal symptom is the development of a chronic infection or canker of the vine. A grapevine canker is a localized area of dead woody tissue caused by fungal infection. These infections accumulate and progress within a vine over time. Once infected, the vine begins to experience a slow decline in its overall health, resulting in an increased susceptibility to

a wide variety of other pathogens. Associated symptoms include, but are not limited to, poor early growth and reduced vigor.

Little is known about many aspects of the transmission of GTDs (Bertsch *et al.*, 2009). One possibility is that native plants near vineyards serve as an inoculum sources of the various trunk pathogens (Damm *et al.*, 2009; Rolshausen *et al.*, 2010; Agustí-Brisach *et al.*, 2011). Susceptibility of cultivated vines is influenced by cultural practices such as irrigation, water stress, and seasonal pruning. Vines propagated from cuttings of an infected parent plant can harbor a pathogen that may spread through the vascular tissue of the developing vine throughout its lifetime.

The only research conducted on GTDs in the state of Texas emphasized Botryosphaeria species (Úrbez-Torres *et al.*, 2009a). These species are the causative agents of the disease known as Botrysphaeria dieback. There are at least 21 different fungi associated with Botrysphaeria dieback worldwide (U´rbez-Torres, 2011; Bertsch *et al.*, 2013). Wounds generated from vine pruning are the primary infection court for members of Botryosphaeriaceae. The pathogen enters its host through an open wound, and then slowly spreads though the vascular system. Development of a perennial canker follows the infection. The other vascular pathogens associated with GTDs elsewhere are a considerable threat that need further evaluation in Texas (Bertsch *et al.*, 2013). To adequately prevent and potentially manage GTDs, the causative agents must be identified and understood (Dodds *et al.*, 2010).

Select environmental conditions are an integral component of the virulence of a pathogen and the susceptibility of the host, yet most of the GTD studies have been

completed in other climates (Kloepper, 1996). The lack of research directed at understanding organisms specifically in regards to Texas grapevines is a significant hindrance to maintaining longevity and productivity of the state's vineyards. Continuing Texas research on Botryosphaeriaceae spp. and identifying the role of other vascular pathogens affecting vines is vital for industry progression. While all of the GTD pathogens induce similar host symptoms, their biology and epidemiology are variable, which may indicate the need for varying management practices.

Eutypa species belonging to the Diatrypaceae family are classified as trunk pathogens that lead to soft-rot of the woody host tissue (Rolshausen *et al.*, 2007; Trouillas *et al.* 2010). Soft -rot fungi (Diatrypaceae) predominantly decompose the carbohydrate component of the wood (Worrall *et al.*, 1997; Rolshausen *et al.*, 2008). While these species are known to primarily colonize dead or decaying wood, isolations can be obtained from the margins of necrotic tissues. On symptomatic vines, emerging perithecial ostioles that contain allantoid ascospores are characteristics that enable morphological identification (Kirk *et al.*, 2001). Metabolites biosynthesized by *Eutypa lata* (acetylenic phenols and their heterocyclic analogues) are translocated into shoot and leaf tissue by regulatory host functions (Andolfi *et al.*, 2011). This results in an array of foliar symptoms even though the pathogen can only be isolated from the trunk, canes, or cordons of the grapevine.

The Esca disease complex is an outlier among the GTDs as the biology and epidemiology of these pathogens are vastly different from other GTDs (Pierron *et al.*, 2016; Retief *et al.*, 2006; Ridgeway *et al.* 2005). While many pathogenic fungi have

been associated with esca, the primary causal agents are accepted as *Phaeomoniella* sp. (Chaetothyriales, Herpotrichiellaceae) and *Phaeoacremonium* sp. (Diaporthales, Togniniaceae), along with numerous wood rotting basidiomycetes (Bertsch *et al.*, 2004; Crous *et al.*, 1996; Fischer, 2006). The most common basidiomycete associates are those within the family Hymenochaetaceae, but the diversity and taxonomy of these accompanying organisms is still being scrutinized (Cloete *et al.*, 2016; Zhou *et al.*, 2016; Cortesi *et al.*, 2000). Metabolites produced by *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* (naphthalenone pentaketides) result in symptoms on the fruit and foliage, but the fungi themselves are regarded as tracheomycotic agents, infecting the vascular tissue (Andolfi *et al.*, 2011; Bertsch *et al.*, 2013). With most GTDs, airborne dispersal is the primary mode of inoculum spread, but fungi comprising the esca disease complex may also spread though the soil entering the host through its roots (Eskalen *et al.*, 2004; Gutter *et al.*, 2004; Travadon *et al.*, 2013). Lignin degradation as a result of white-rot disrupts the structural integrity of the vine and increases the susceptibility to damage from heavy winds. White-rot is a type of wood decay caused primarily by basidiomycetes (Hymenochaetaceae, Stereaceae, *Irpex*) and defined by an extensive reduction in lignin content. White-rot fungi may also attack the cellulose and hemicellulose components of the cell wall, but are not classified as extensive degraders of these constituents (Manion, 1981). Remedial vine surgery is a practice employed to limit the spread of a GTD within a single vine (Smart, 2015; Sosnowski. *et al.*, 2011). This is done by cutting away and removing all infected areas

from the vine. These efforts are ineffective if a vine is a host of Esca pathogens, which have a tendency to colonize the cordons as well as the trunk.

Diaporthe ampelina formerly known as *Phomopsis viticola* belongs to a diverse group of taxonomically related fungi (Gomes *et al.*, 2014). *Diaporthe* spp. have an established role as a limiting factor to Texas vineyards (Úrbez-Torres *et al.*, 2009), but research of this complex group has only recently began to increase and expand (Lawrence *et al.*, 2015; Dissanayake *et al.*, 2015). *Diaporthe* spp. are recognized as the causative agents of two separate diseases on grapevine: Phomopsis cane and leaf spot which affects new growth, such as shoots, and Phomopsis dieback which affects the permanent woody structures such as the trunk, cordons, canes, and rachises, leading to fruit loss (Baumgartner *et al.*, 2013; Gomes *et al.*, 2013; Dissanayake *et al.*, 2015; Pscheidt, 1991).

Environmental conditions such as heavy rainfall and high humidity stimulate spore production and dispersal of GTD pathogens (Úrbez-Torres *et al.*, 2010). Native grape species are also suspected to serve as supplemental hosts (Trouillas *et al.*, 2010; Cloete *et al.*, 2011). Once established in a single vine, the pathogen can potentially spread throughout an entire vineyard. Pronounced frequency of disease incidence and severity can result in substantial yield reduction. Susceptibility can hinge on the cultivar and growing conditions (Travadon *et al.*, 2013; Úrbez-Torres *et al.*, 2010). Older vineyards are prone to sustaining higher levels of GTDs. Since many of the varieties of grapes grown in Texas are not native, limitations from abiotic stresses are expected. Plants that are forced to overcome an abiotic stress, such as drought, are more vulnerable

to diseases caused by a biotic pathogen (Bostock *et al.*, 2014). Once a GTD is established in a vineyard, management options are limited. Spores are naturally dispersed by wind and rain, but the spread of pathogen may be compounded as a result of poor vineyard management practices. Sterilization of pruning tools and removal of infected vines upon positive identification are practices that have proven to be critical to evading disease development (Niekerk *et al.*, 2006; Block 2013 *et al.*, Sosnowski *et al.*, 2007; Agustí-Brisach, 2015). The efficacy of fungicides in controlling disease is disputed. Topsin M (United Phosphorous, Philadelphia, PA) is thought to be the best product for controlling trunk diseases but it has several limitations (Rolshausen *et al.*, 2010). To restore yield, a grower has to incur the costs of removing and replacing infected vines. Alternative methods of vine recovery such as retraining of vines have produced varying results.

The research conducted for this thesis laid the groundwork for advancing the scientific knowledge pertaining to GTDs within the state of Texas. To significantly benefit researchers and growers alike, there must be greater emphasis placed on continuing to understand each individual GTD. The following objectives were addressed to provide a better understanding of GTDs in Texas:

Objective 1: Survey vineyards for canker incidence, severity and isolate putative pathogens.

Objective 2: Determine the prevalent canker-causing fungi among selected vineyards in Texas.

Objective 3: Evaluate the influence of climatic conditions on spore dispersal of potential canker causing pathogens.

2. MATERIALS AND METHODS

Objective 1

Vineyard Survey

To better understand the progression of GTDs over time, a survey for incidence and severity of disease within established vineyards was conducted. Austin County Vineyards, Flat Creek Estates and the Industry experimental vineyard were selected for the survey based on their variability in age and cultivar. Vines surveyed included 1,586 Blanc du bois and 411 Black Spanish at Austin County Vineyard, 1,027 Sangiovese at Flat Creek Estates, and 247 hybrids at Industry experimental vineyard (Table 1).

A grapevine rating system measuring the severity of GTD symptoms was used to conduct the survey (Table 2). Each vine was individually assessed and given a rating that most accurately represented its condition. At each survey site detailed notes were taken to document observations such as deceased vines, retrained vines, and other unanticipated variables.

Objective 2

Pathogen Identification

A total of 160 samples from diseased vines were retrieved from field sites where GTDs have been confirmed (Table 3). To properly identify the causative agents, samples of symptomatic vines were collected at each of the locations surveyed and assayed for putative pathogens (Table 3).

Table 1 Vineyards selected for survey of grapevine trunk disease in Texas and the number of vines surveyed at each vineyard.

Vineyard/Cultivar	No. of Vines	Age in Years
Austin County Vineyards	2,032	
Blanc Du Bois	1,249	20
Blanc Du Bois	334	4
Black Spanish	411	20
Flat Creek Estates	1,027	
Sangiovese	1,027	16
Industry Experimental Vineyard	247	
Various Hybrid Cultivars	247	6

Table 2 Vine rating system for vineyard survey of grapevine trunk disease.

Rank	Description
0	No signs (fruiting bodies) or symptoms (dead spurs with loss of spur position on cordon or trunk) observed.
1	Signs of pathogen evident. No significant symptoms observed.
2	Dead spurs are identified; less than 5% of overall spur positions are affected. No significant impact on vine health is observed.
3	Shoots or canes appear to be stunted as a result of disease; less than 10% of overall spur positions are affected (dead) by disease.
4	Disease is readily observed; less than half of the spur positions are affected.
5	At least, half of the vine is significantly impacted by disease. All associated symptoms can be identified.
6	More than half of the spur positions are affected. All primary cordons are affected by disease.
7	All major spur positions and cordons are affected. Active shoots are growing in close proximity to dead tissue.
8	Vine health and plant structure is highly limited by disease; less than 20% of shoots are active.
9	Vine has irreversibly succumbed to disease; less than 10% of the vine remains as living tissue.
10	Death of the vine.

Table 3 Vineyards where samples of diseased vines were collected and the number of samples collected from each location is listed.

	County	Vineyard	No. of Samples Collected
1	Austin	Variety Trial at Industry	12
		Austin County Vineyards	18
2	Blanco	William Chris	2
3	Brazos	College Station Research Vineyard	4
4	Burnet	Flat Creek Vineyard and Estates	59
5	Gillespie	Becker Vineyards	6
		Granite Hills Vineyards	4
6	Grayson	Munson Memorial Vineyard	7
7	Hayes	Driftwood Estate Vineyards	24
8	Mason	Robert Clay Vineyards	6
9	Rains	Della Terra Farms	4
10	Real	Frio Canyon Vineyards	3
11	Terry	Hunter Family Vineyard	5
12	Yoakum	Newsom Vineyards	6

Collected samples were bagged, labeled, placed on ice, and returned to the lab for dissection. Tissue pieces less than 5 mm^2 were cut from the margins of necrotic wood and disinfected in 75% ethanol for 1 min, rinsed in sterile distilled water, dried carefully above an open flame and plated in groups of three onto plates of Potato Dextrose Agar (PDA) (Difco) (Cortesi *et al.*, 2000; Larignon *et al.*,1997). The plates were incubated at room temperature until fungal mycelium was observed growing from wood sections. Isolates possessing colony morphology (based on what keys or pictures or what—no way you knew what they looked like) representative of relative pathogenic species were sub-cultured onto water agar and incubated for another five days. Pure cultures were obtained by transferring a single fungal hyphal tip to individual plates of PDA. Processing was conducted as soon as possible with samples stored at 0^oC for no more than month. Figure 1 and Table 3 describe locations where samples were collected along with the number of collected samples (Google Maps, 2016). Preliminary diagnoses were made on the basis of morphological characteristics in order to determine the genus of each isolation. Molecular phylogenetic analysis was conducted to confirm questionable morphological identifications (Essakhi *et al.*, 2008, Úrbez-Torres *et al.*, 2006). DNA of isolates selected for molecular identification was extracted from mycelia of fungi cultivated on PDA. Fungi belonging to Basidiomycota were grown in bottles of Malt Extract Broth (MEB). The medium was amended with benomyl at 5 mg per liter to reduce other fungal contamination. Isolation of Basidiomycota was conducted as described in previous literature (Cortesi *et al.*, 2000). Cultures were then placed in a dark box for incubation.

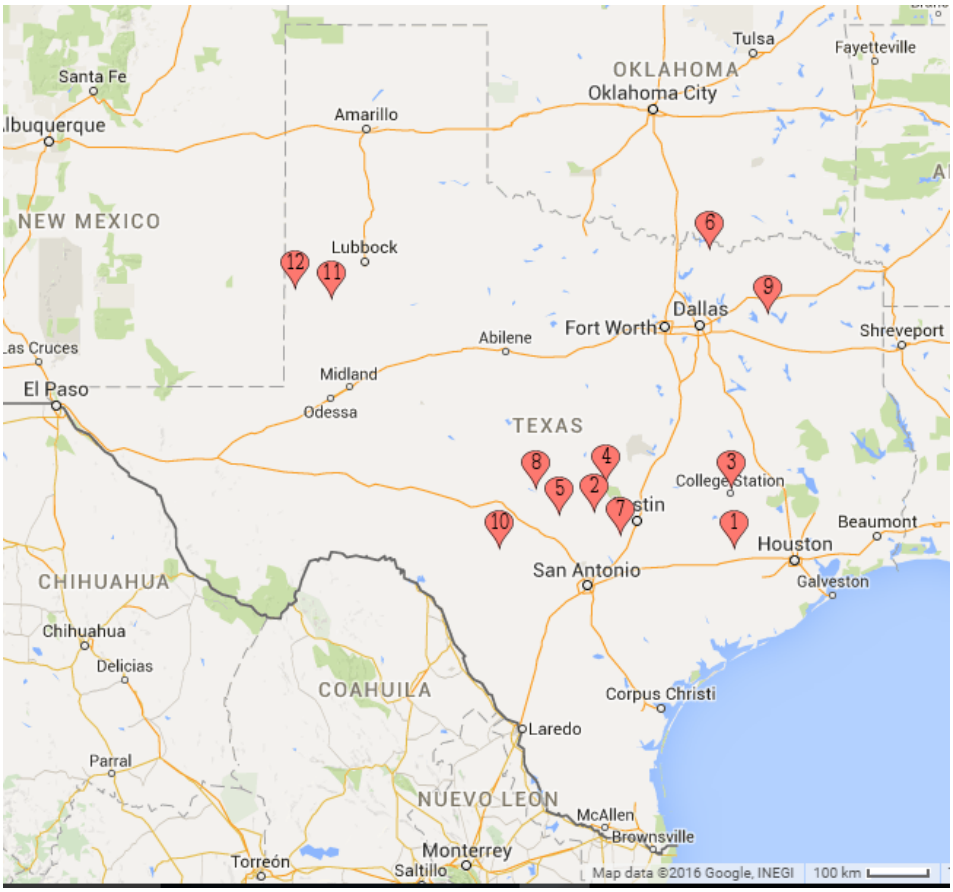

Figure 1 Map of sites selected for sample collection of diseased vines. Locations are numbered according to county (see Table 1 for county designations). Samples were collected between August 2014 and March 2016 (Google Maps, 2016)

Isolated and identified fungi were documented. Three agar plugs of each isolate were stored in individual vials of sterile water at 25^{o}C. Selected isolates were later used in greenhouse experiments.

Molecular Confirmation

DNA extraction of mycelia from pure cultures was performed using ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research Corporation 17062 Murphy Ave. Irvine, CA 92614, U.S.A.), according to manufacturer's instructions. Conventional PCR was performed according to Go Taq Colorless ® directions using Internal Transcribed Spacers (ITS1 and ITS4) primers. PCR parameters were set at 95^{o}C for 3 minutes, (95^{o}Cfor 30 seconds, 55^{o}C for 1 minute, 72^{o}C for 1 minute) for 40 cycles, 72^{o}C for 10 minutes, 4^{o}C. PCR reactions were stored at -20^{o}C before being electrophoresed on a 5% agarose gel with Tris-Borate-EDTA (TBE) buffer. The identification of the isolate was confirmed by sequencing the approximate 500-750 bp (varies among genus) PCR amplicon at the Texas A&M University Gene Technology Lab (http://www.idmb.tamu.edu/gtl/). Molecular identification was then based on the comparison of sequences with reference to ITS regions from known nucleotide collections within GenBanks (http://www.ncbi.nlm.nih.gov) using the Basic Logical Alignment Search Tool (Kaliterna *et al.* 2012).

Greenhouse Experiments

Merlot, Cabernet Sauvignon, Sauvignon Blanc, Blanc du Bois, and Black Spanish were the grape cultivars selected for vine propagation followed by inoculation. Each of the cultivars were inoculated with fungal isolates of *Eutypella* sp., *Diplodia*

seriata and *Lasiodiplodia* sp., all of which were obtained from the isolations conducted in Objective 2. Inoculations were preformed via two different methods as described below. Isolates were removed from storage and allowed to grow on media for a period of one month before inoculation.

Vine Propagation

Dormant hardwood cuttings were obtained from two Texas vineyards (county locations 6 and 10, Figure 1) after seasonal pruning (Jan. - Feb.) and cut into 12 inch sections. Bundles of cuttings were soaked for twenty minutes in buckets filled with sterilized water heated to 120^{o} F and amended with 5% bleach. After sanitation, the bundles were placed in plastic boxes filled with moist cedar wood chips. The temperature of the boxes was maintained at 80^{o}F and the wood chips were hydrated in order to keep the environment warm and humid. After one month the vines generated callus tissue. They were then potted in Metro-Mix® 380. Vines were adequately watered and fertilized while being allowed to establish for one year in the green house.

Vine Inoculation

On the day of inoculation, vines were pruned leaving only 2 to 3 shoots per vine. Pruning shears were sterilized with 95% ethanol and flamed. The top of the vine was cut off 12-14 inches above the soil line before inoculation. Top inoculations were done by placing a 5mm agar plug of the select pathogen on the stub left from the pruning wound and then sealed by wrapping the site with Parafilm®. An incubation period of 6 weeks was allowed before analysis.

The spatial pattern of vine placement on the greenhouse benches was randomized to remove variances in environmental conditions. Three different pathogens were selected for the inoculation of each of the five cultivars, with five replicates for each cultivar. A control group of each cultivar was inoculated with sterile PDA.

Analysis

Aggressiveness among the three pathogens and tolerance of the cultivars was assessed by measuring the length of vascular discoloration starting from the wounding site after a period of 6 weeks. The bark below the wound generated from inoculation was removed with a sterile blade. Measurements were taken with a standard ruler and recorded. Tukeys's Studentized Range (HSD) Test for length of canker progression was used to assess comparisons between test groups. Koch's postulates for proof of pathogenicity were fulfilled by re-isolating each of the pathogens from inoculated vines and confirming their identity as the fungus that was originally used for inoculation.

Objective 3

Spore traps, in the form of glass microscope slides coated in petroleum jelly, were used to collect and analyze spore dispersal data. The traps were placed on symptomatic vines at vineyards listed in county locations 1, 4, and 7 (Figure 1). Traps were mounted to vines using tie wire and binder clips (Figure 2). They were changed and replaced once every 12-18 days. Within each vineyard, 8 traps were positioned in blocks of symptomatic vines. At the Industry Trial Vineyard traps were placed in a block of hybrid vines. At Flat Creek Estates traps were set in two separate blocks, one in Sangiovese and one in Muscat Blanc. At Driftwood Estates traps were set in three

Figure 2 Glass microscope slide coated in petroleum jelly and attached to vine for the purpose of monitoring spore dispersal. Traps were collected and replaced once every 12 to 18 days over a period of six months.

separate blocks: Cabernet Sauvignon, Syrah, and Chardonnay. Sampling spanned from February of 2015 to June 2015. An additional five traps were set within each block and collected in sterile tubes. These traps were analyzed via an alternative wash method (Úrbez-Torres *et al.,* 2010). The Sangiovese block at Flat Creek estates was selected for further testing where spores were collected for the additional months of December 2015 to April 2016.

Lab Analysis

Spore counts were then be plotted over time to determine trends in inoculum release and associations with rainfall and relative humidity. Spore counts were quantified by using a compound light microscope to individually analyze each slide. Botryosphaeriaceae spores are easily distinguished and became the primary spore type for this investigation. Computations were made with the aid of a hand-held tally counter. A second method of analysis was used to assess spores on the slides collected in the sterile tubes. The following procedures were modeled after previous works (Úrbez-Torres *et al.,* 2010). Approximately 10 ml of distilled water was added into each of the screw cap tubs and sealed before being shaken by hand. Two aliquots of 200 µl each were collected per spore trap, filtered through a .45µm filter and spread on two replicate 85-mm-diameter petri plates containing PDA. Plates were allowed to incubate for 3 days before a single hyphal tip of each fungal colony was transferred to a fresh medium. Isolations were allowed to incubate for a period of 6 weeks before identifications were made via the morphology of produced spores. If incubation did not result in spore production after 6 weeks, agar plugs were transferred to a wood chip media.

Botryosphaeriaceae more readily sporulate when a substrate is provided for pycnidium to develop upon. Wood tissue pieces (1 inch in length and .5 cm thick) were removed from healthy cuttings of grapevines with a sterile blade. Wood pieces were then autoclaved in a sealed glass jar for 25 min at 121°C. Wood pieces were then embed in freshly poured PDA plates before medium solidification.

Rainfall data was obtain from weather stations local to the select field site. The rainfall data relative to the Industry experimental vineyard was sourced from a meteorological station located in Brenham, TX. The rainfall data relative to Flat creek estates was sourced from a meteorological station located near Marble Falls, TX. The rainfall data relative to the Driftwood vineyard was sourced from a meteorological station located in Maxwell, TX. All of the listed weather stations and the resulting data outputs are products of The Weather Company, LLC (https://www.wunderground.com/). Correlations between spore counts and rainfall were made by comparing the average spore counts per block to the rainfall data relative to the month sampled.

3. RESULTS

Objective 1

The results of the survey at Austin County Vineyards are depicted in Figures 3 and 4. Of the two grape varieties grown at this vineyard, Blanc Du Bois comprised the majority of vines (n = 1,583), followed by the Black Spanish (n = 411). There were two plantings of Blanc du Bois at Austin County Vineyards; the first occurred 20 years ago and the second 4 years ago. For all vines growing within this vineyard, regardless of age, a 100% disease incidence of cankers was recorded. However, the severity of cankers in the Blanc du Bois, as assessed with the disease rating system, clearly varied between the two age groups (Figure 3). The 20 year-old-vines produced a normally distributed disease rating that fell between 4 and 10 with the majority of the vines receiving a rating of 7. The four year old vines also produced normally distributed disease ratings that fell between 1 and 5 with the majority of the vines receiving a rating of 3. Similar to that of the Blanc du Bois of the same age, the 20 year old Black Spanish rating ranged from 4 to 10 (Figure 4). There was a special subset of vines in the Blanc du Bois at Austin County Vineyards, consisting of 20 year old vines that have been retrained in an effort to rehabilitate them and restore productivity. The ratings for these vines ranged from 4 to 6, falling in between those scores for the younger and older age groups (Figure 3).

Similar patterns in the frequency distributions of disease vines were consistently observed at the other surveyed vineyards. At the Industry vineyards, the varieties

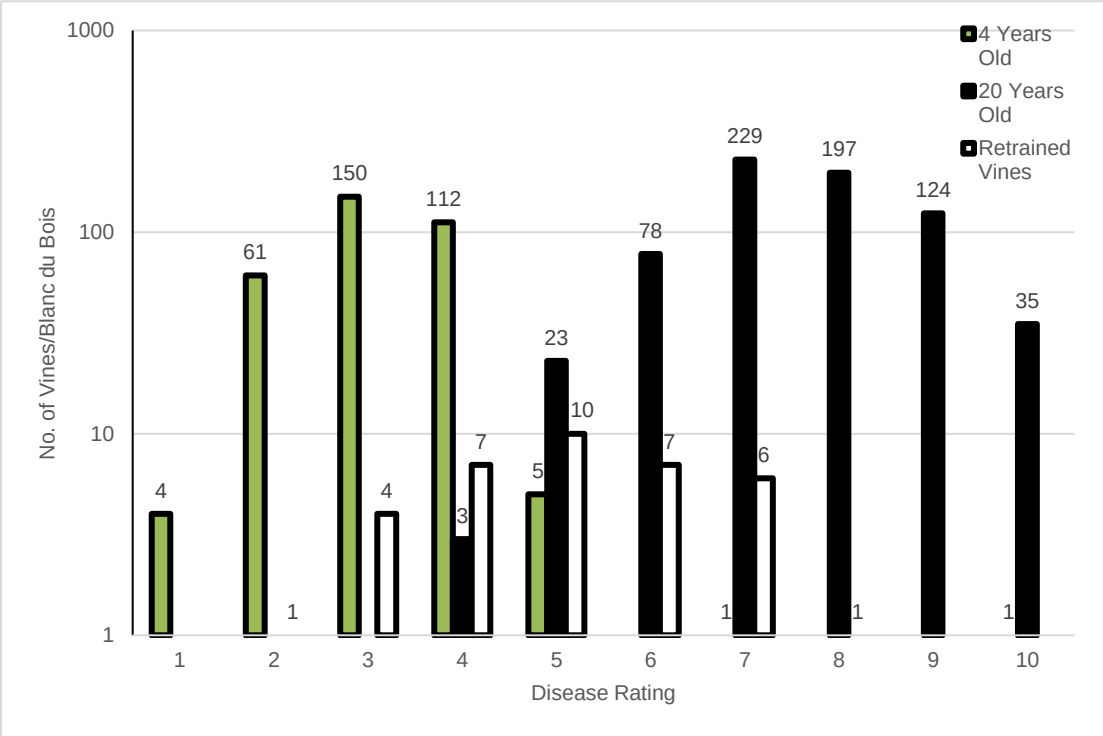

Figure 3 Results from the survey for grapevine trunk disease at Austin County Vineyards. A total of 1,583 Blanc Du Bois vines were surveyed using the vine rating system (Table 3).

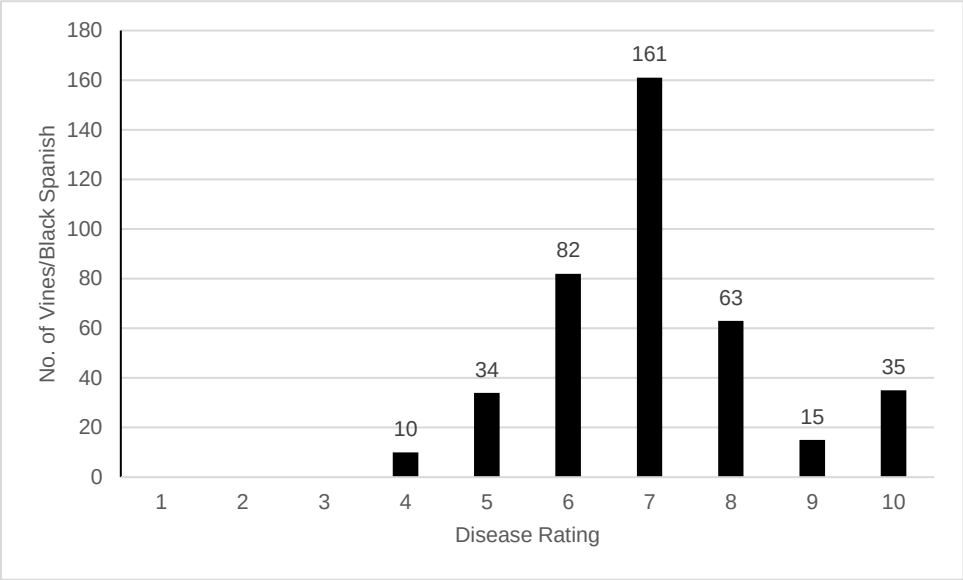

Figure 4 Results from the survey for grapevine trunk disease at Austin County Vineyards. A total of 411 Black Spanish vines were surveyed using the vine rating system (Table 3).

consisted of an array of mixed hybrid vines being produced for resistance to various diseases. There were 247 vines in the survey, all less than 10 years old. Although the disease was identified in the block of hybrid vines, the incidence and severity were in the range of 0 to 4 with very few numbers in the higher disease rating categories (Figure 5). At Flat Creek Estates, the incidence survey entailed 1,027 vines of the variety Sangiovese. These vines were planted 16 years ago, and the frequency distribution of disease incidence ranged from 4 to 9, but with a relatively high number of vines rated 10 (n = 94, figure 6). As at Austin County Vineyards, there was a population of vines also retrained at Flat Creek for the same purpose. They sustained a normally distributed pattern of disease incidence, but with numbers of vines in lower disease categories than that of the untrained vines (Figure 6).

Objective 2

A total of 34 genera of fungi were isolated from the 160 field samples analyzed (Table 1). The primary identifications were made by comparing observed morphological characteristics of cultures to published descriptions in literature. Molecular techniques were used when further confirmation was necessary. Homology to reported isolates was accepted if the percent identity was above 95%. The primary grapevine trunk diseases Esca, Botryosphaeria dieback, Eutypa dieback, and Phomopsis dieback were all identified during the assessment of diseased vines (Table 4). The causal pathogens (*Phaeomoniella* spp. and *Phaeoacremonium* spp., Botryosphaeriaceae, Diatrypaceae, and *Diaporthe* spp.,) represented five separate orders, spanning three classes of ascomycetes.

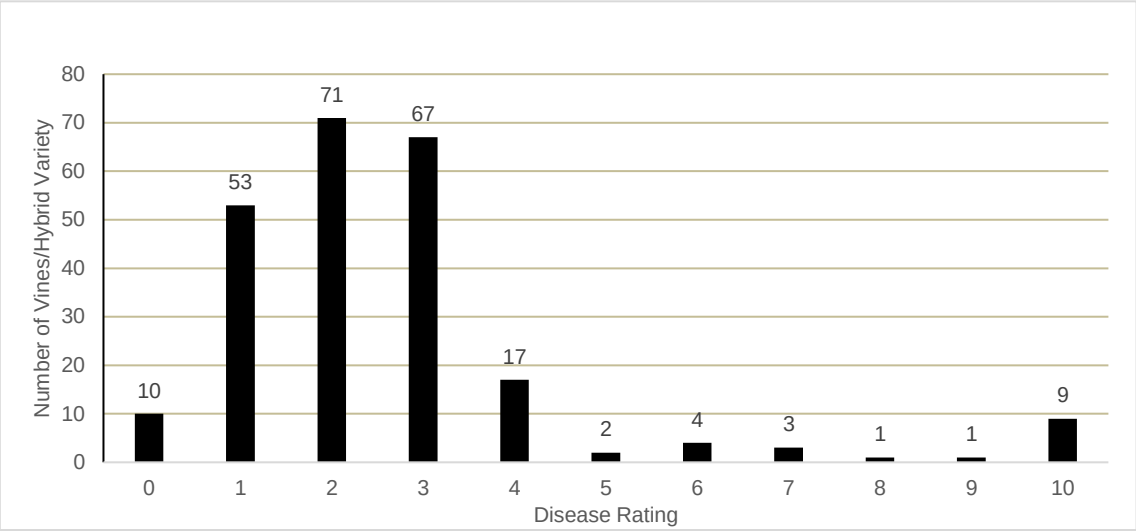

Figure 5 Results from the survey for grapevine trunk disease at the Industry Trial Vineyard. A total of 247 hybrid vines were surveyed using the vine rating system (Table 3).

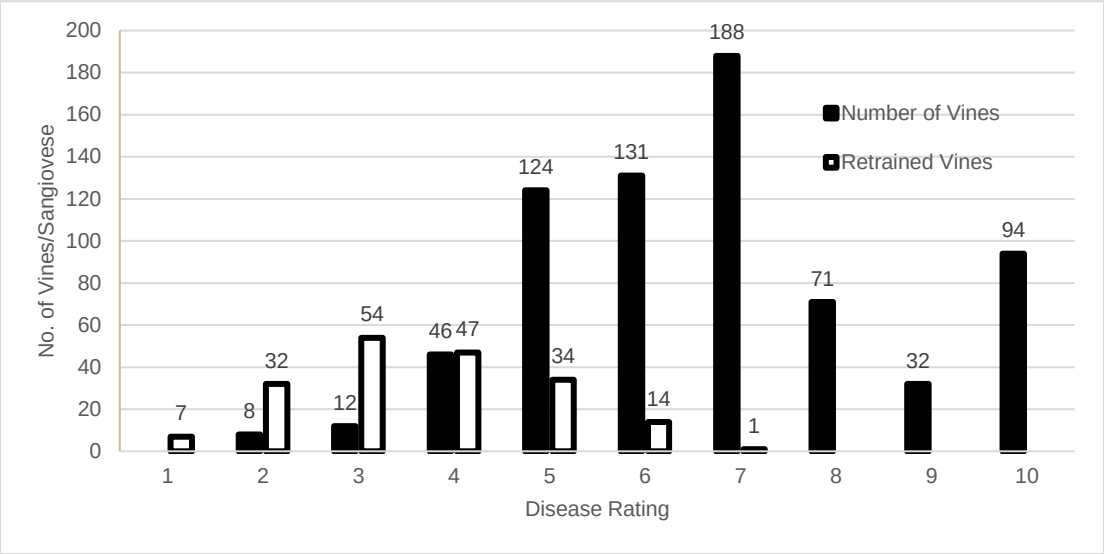

Figure 6 Results from the survey for grapevine trunk disease at Flat Creek Estates Vineyard. A total of 1,027 Sangiovese vines were surveyed using the vine rating system (Table 3).

Table 4 Results of grapevine survey for fungal species colonizing diseased grapevines in Texas. Table includes counties where specific cultivars were sampled and counties from where fungal species were isolated.

			Grapevine Cultivar and Respective County Numbers								
			BdB[b]	BS[c]	Cab[d]	Char[e]	Mus.B[f]	Pino.G[g]	Sang[h]	Syr[i]	Tinta[j]
PHYLUM-CLASS, Order-Family	**Genus**	**Ecological Niche[a]**	**1,3,9**	**1,3,9**	**2,5,7,10,11,12**	**7,8,10**	**4**	**4,7**	**4**	**4,7,8**	**4**
Ascomycota-Dothideomycetes											
Botryosphaeriales-Botryosphaeriaceae	*Diplodia seriata*	Trunk pathogen	1,3,9	1,3,9	2,5,10,11,12	7,8,10	4	4,7	4	4,7,8	4
	Diplodia sp.	-				8		7		8	
	Dothiorella viticola	Trunk pathogen				8			4	8	
	Lasiodiplodia sp.	Trunk pathogen	1		5,11,12	7,8		7	4	4,7,8	
	Neofusicoccum sp.	Trunk pathogen	1,9	1	11,12	8	4	7	4	8	4
Capnodiales-Cladosporiaceae	*Cladosporium* sp.	Endophyte/Saprobe			5				4	4,7	
Pleosporales-Didymellaceae	*Didymella glomerata*	Saprobe			2,5,7	8	4	7	4	8	4
Pleosporales-Montagnulaceae	*Paraconiothyrium* sp.	-	1	1,9	5			7	4		
Pleosporales-Pleosporaceae	*Alternaria* sp.	-	1,3,9	1,3,9	2,5,7,10,11,12	7,10	4	4,7	4	4,7,8	4
	Alternaria alternariae	-							4		
	Alternaria tenuissima	-							4		
	Epicoccum nigrum	Saprobe	1	1	5,7	7,8		7	4	8	
Ascomycota-Eurotiomycetes											
Eurotiales-Aspergillaceae	*Aspergillus niger*	-	1,9	1,3	5			7	5		
	Penicillium sp.	Saprobe	1,3,9	1,9	10,12	8		4,7	4	4	
Phaeomoniellales-*Family not assigned*	*Phaeomoniella chlamydospora*	Trunk pathogen	1		5,7	7,8	4	4,7	4	4,7	
	Phaeomoniella sp.	-	1	1	2,5,7	7,8	4	4,7	4	4,7,8	4

Table 4 (continued)

			Grapevine Cultivar and Respective County Numbers								
			BdB[b]	BS[c]	Cab[d]	Char[e]	Mus.B[f]	Pino.G[g]	Sang[h]	Syr[i]	Tinta[j]
PHYLUM-CLASS, Order-Family	**Genus**	**Ecological Niche[a]**	**1,3,9**	**1,3,9**	**2,5,7,10,11,12**	**7,8,10**	**4**	**4,7**	**4**	**4,7,8**	**4**
Ascomycota-Sordariomycetes											
Diaporthales-Diaporthaceae	*Diaporthe* sp.	Trunk pathogen	9	9	11,12				4		
Diaporthales-*Family not assigned*	*Greeneria uvicola*	Trunk pathogen	1,9	9	2,5,7,10	7,8,10		4,7	4		4
Hypocreales-Hypocreaceae	*Trichoderma* sp.	-	1	1	2,5,7	7,8	4	4,7	4	4,7,8	4
Hypocreales-Nectriaceae	*Fusarium* sp.	-	1	1	5			7	4	4	
Sordariales-Chaetomiaceae	*Chaetomium globosum*	Soft-rot fungus	1	1				7	4		
Sordariales-Sordariaceae	*Sordaria* sp.	-	1					7	4		
Togniniales-Togniniaceae	*Phaeoacremonium minimum*	Trunk pathogen	1	1	5				4	4	
	Phaeoacremonium sp.	-	1	1	5,7	7,8			4	4,7,8	4
Trichosphaeriales-Trichosphaeriaceae	*Nigrospora* sp.	-	1	1	5	8				4,8	4
Xylariales-Amphisphaeriaceae	*Pestalotia* sp.	-	1,3,9	1,3,9	2,5,7,10,11,12	7,8,10	4	4,7	4	4,7,8	4
Xylariales-Diatrypaceae	*Eutypella vitis*	Trunk pathogen	1	1	2,5,7,11,12	7,8		7	4	4,7,8	
	Eutypa sp.	-			5,11,12	7,8,10		7	4	4,7,8	4
Xylariales-Sporocadaceae	*Pestalotiopsis sp.*	Trunk pathogen	1	1	5	7,8		7	4	4	
Basidiomycota-Agaricomycetes											
Hymenochaetales-Hymenochaetaceae	*Fomitiporia sp.*	White-rot fungus	1	1	2,5,7	7,8	4	7	4	4,7,8	4
	Tropicoporus tropicalis	White-rot fungus		1					4		
Polyporales-*Family not assigned*	*Irpex lacteus*	Saprobe/ White-rot fungus	1	1	5,11,12	7,8		4,7	4	4,7,8	4
Polyporales-Phanerochaetaceae	*Phlebiopsis flavidoalba*	White-rot fungus	1	1	5,11,12	7,8		4,7	4	4,7	4
Russulales-Stereaceae	*Stereum hirsutum*	White-rot fungus	1	1,9	2,5,7,11,12	7	4	4,7	4	4,7	4

Ecological niche[a], species relationship with grapevine based on previous literature; BdB[b] , Blanc du Bois; BS[c], Black Spanish; Cab[d], , Cabernet Sauvignon; Char[e], Chardonnay; Mus.B[f], Muscat Blanc; Pino.G[g], Pinot Gris; Sang[h], Sangiovese; Syr[i], Syrah; Tinta[j], Tinta Madeira.

Furthermore, the most abundant canker-causing fungi among selected vineyards in Texas proved to be *Diplodia seriata*, having been identified from every vineyard sampled and within 100% of the select cultivars (Table 4). A variety of basidiocarps, from resupinate to effused, were observed on diseased vines in the assessed vineyards (Figure 7). The presence of two genera of Esca-associated white-rot fungi *Inonotus*, and *Stereum* are new reports in Texas. The physiological effects of the esca disease complex were shown to encompass the entire vine producing symptoms on the foliage, vascular tissue and fruit (Figure 8; Figure 9). Other notable Basidiomycota identified were *Tropicoporus tropicalis* (Hymenochaetales) and *Irpex lacteus* (Polyporales). Isolating basidiomycetes from diseased vines proved to be difficult and supplementary measures were taken to promote their growth in pure culture. Their initial growth was significantly slower than that of the ascomycetes and as a result were frequently outcompeted. If the cultures were exposed to light their growth was significantly reduced. MEB was a suitable medium for their growth. However, the culture bottles required wrapping in foil to prevent light from hindering growth when placed on a shaker. Adjustments to the media's pH were attempted but not deemed necessary.

Unexpected, yet notable identifications of canker causing organisms include *Didymella glomerata* and *Greeneria uvicola* (Table 4). *Didymella glomerata* is known to cause the decline of other fruit crops, but has yet to be associated with diseases of grapevine in North America (Aveskamp *et al.*, 2010; Chohan *et al.*, 1980).

Figure 7 Basidiocarps associated with trunk diseases of grapevine in the state of Texas. (a) effused-reflexed fruiting body typical of *Phellinus* sp.; (b-e) resupinate fruiting bodies produced by *Fomitiporia* sp.; (e) resupinate fruiting body lining a cavity created by a boring insect; (f) fruitbodies of *Stereum hirsutum* on grapevine bark; (g) Auriculariaceae fruit body on a decaying portion of a still living vine; (h) pileate to effused-reflexed basidocarp representative of the Hymenochaetales.

Figure 8 Symptoms of grapevines affected by the Esca complex in Texas. (a) Heart rot of a living vine; (b-e) trunk cross-sections showing internal wood symptoms, such as white-rot, derived from basidiomycete infections; (f) colonization of the stele by fungal hyphae; (g) cross section of wood featuring zones lines produced by incompatible basidiomycetes; (h) vascular discoloration surpassing the graft union.

Figure 9 Symptoms associated with the Esca disease complex. (a) Heart rot of the vine's trunk surrounded by vascular discoloration; (b) vascular streaking; (c, d) foliar symptoms of esca (tiger stripes), scope of necrosis and chlorosis varies according to cultivar which exacerbates as the leaf tissue dries; (e) speckled discoloration of the fruit (black measles); (f) sudden wilting and decline of the vine (vine apoplexy).

Greeneria uvicola is an Ascomycete (Diaporthales) that is well documented for causing bitter-rot of grape (Longland *et al.*, 2008), but the frequency of isolation from the vascular tissue of diseased vines, evaluated in this experiment, raise questions concerning its role as a vascular pathogen.

The three species of fungi selected for the greenhouse inoculation studies were chosen according to their incidence in Texas and prior recognition as an aggressive pathogen of grapevine. The group of vines inoculated with *Lasiodiplodia* produced vascular streaking with a mean length (68.2mm) that was significantly greater from other pathogens (Table 5). Figure 10 shows the visual difference between a Blanc du Bois vine inoculated with *Lasiodiplodia sp.* and a control vine. The mean value of vascular streaking developed on vines inoculated with *Eutypella* sp. was significantly different than that of the control (Table 5). Vines inoculated with *Diplodia sp.* developed lengths of vascular streaking that were not significantly different from that of the control (Table 5).

Objective 3

Previous literature discusses the diversity of the grapevine microbiome (Pancher *et al*., 2012; Pinto *et al.*, 2014; Zarraonaindia *et al*., 2016). Not surprisingly, there were a variety of fungal endophytes, saprophytes, and pathogens observed when analyzing the collected spore traps. Recognized as important pathogens of grapevines, species within Botryosphaeriaceae have been well documented and their morphology defined. For the sake of clarity and efficacy, the only data represented here is that pertaining to Fungi within Botryosphaeriaceae.

Table 5 Results from Tukeys's Studentized Range (HSD) Test for length of canker progression. Means with the same letter are not significantly different. Results were obtained using 0.1 for the value of α.

Inoculum	**N**	**Mean (mm)**	**Tukey Grouping**	
Lasiodiplodia	25	68.3	A	
Eutypella	25	29.1	B	
Diplodia	25	25.7	B	C
Control	25	8.6		C

Figure 10 Symptoms of vascular streaking (right) on Blanc du Bois 6 weeks after being inoculated with *Lasiodiplodia*. Control (left) is displayed to highlight the difference between the experimental groups.

All presented data is derived from the traps where spores counted microscopically. The wash method proved to be inviable, due to the abundance of spores produced by saprophytic fungal species. Alternaria sp., which rapidly outgrow other fungal species were found in an unexpected abundance. At the Industry trial vineyard, Botryosphaeriaceae spp. spore release coinciding with rain events was demonstrated by comparing the varying levels of spore dispersal (Figure 11) to that of monthly totals of precipitation (Figure 12).

At the Industry trial vineyard spores were collected between the dates of February 3rd and June 13th 2015. During the month of May, rainfall was the highest (12.2 in), which corresponded to the greatest number of spores (117.2) observed over the spore collection period. During the month of February, the lowest levels of rainfall were detected (.41). Coincidentally, the average number of spores collected was much less in the month of February. During the month of June rainfall and spore dispersal decreased.

At the Flat Creek Estates, Botryosphaeriaceae spp. spore release coinciding with rain events is demonstrated by comparing the varying levels of spore dispersal (Figure 13; Figure 14) to that of precipitation on the appropriate collection dates (Figure 15). During the duration of trapping at Flat Creek Estates, the most spores were collected from the block of Sangiovese. The highest average number of spores (821) was observed on May 13th for the Sangiovese. For the Muscat the highest average number of spores (651) was observed on April 22nd. For both cultivars, the lowest numbers were recorded in the months of February and June. There was an observable drop in the appearance spores between the dates of March 22nd and April 8th.

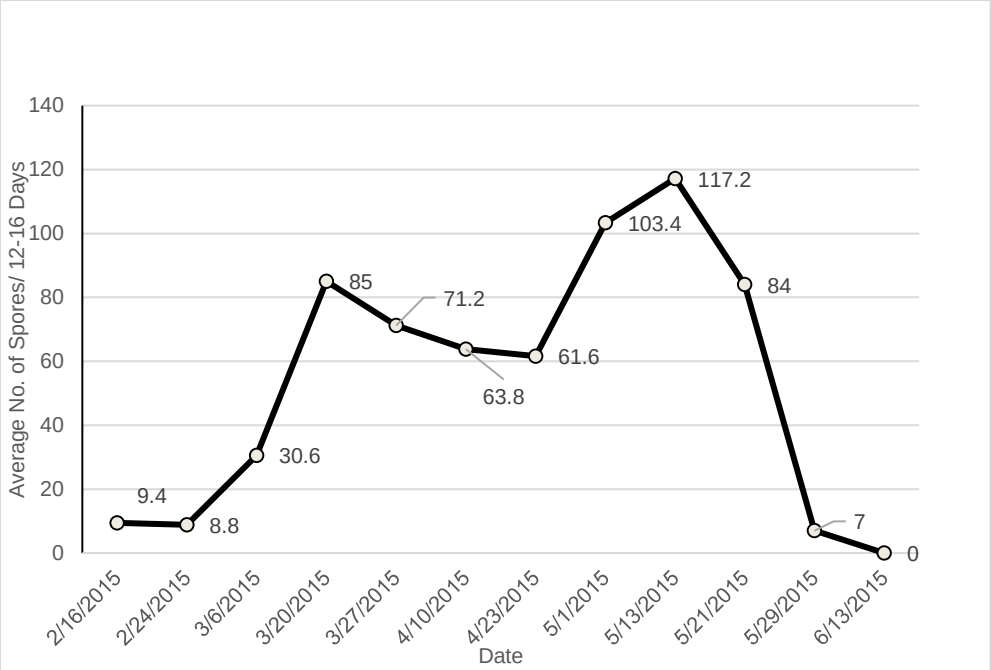

Figure 11 Number of Botryosphaeriaceae spores observed on sampling dates between February 2015 and June 2015 at the Industry trial vineyard. Number represents the average of eight spore traps, set and collected in a block of hybrid vines.

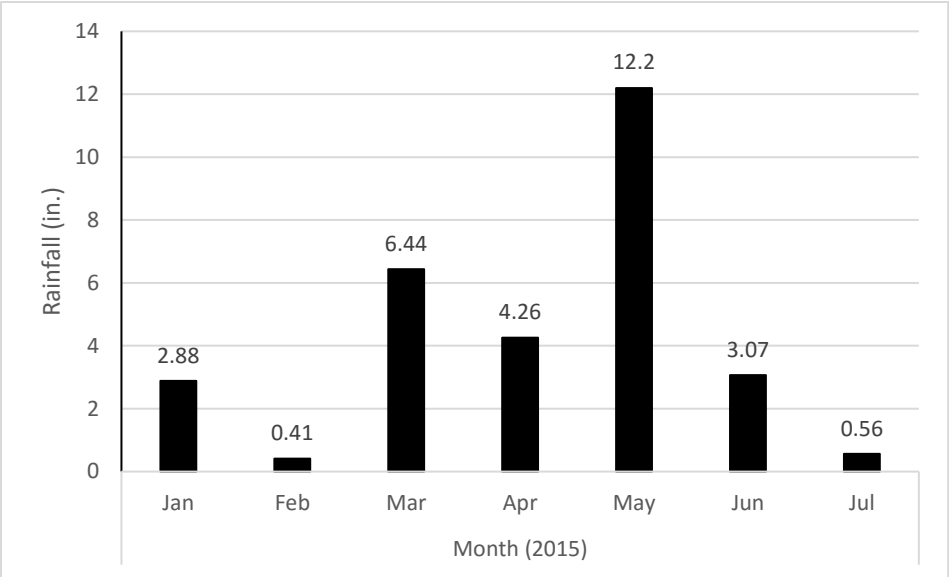

Figure 12 Rainfall detected at meteorological station local to Industry Trial Vineyard, on a monthly basis between January 2015 and July 2015.

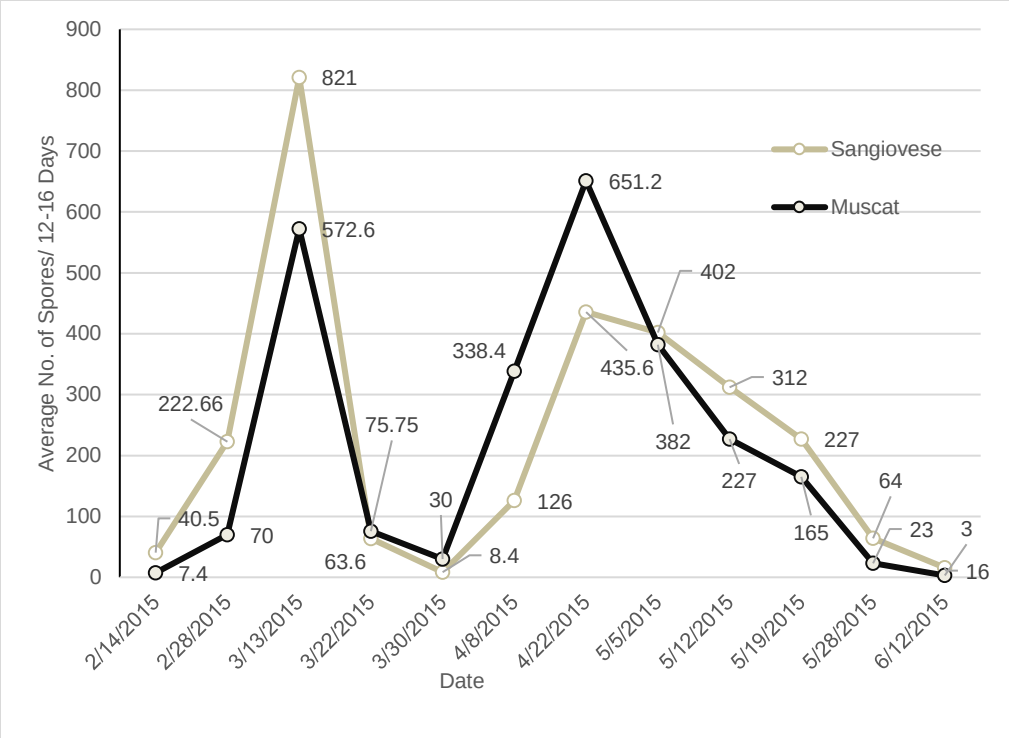

Figure 13 Number of Botryosphaeriaceae spores observed on sampling dates between February 2015 and June 2015 from Flat Creek Estates. Number represents the average of eight spore traps, set and collected in each of the blocks of Sangiovese and Muscat grapevines.

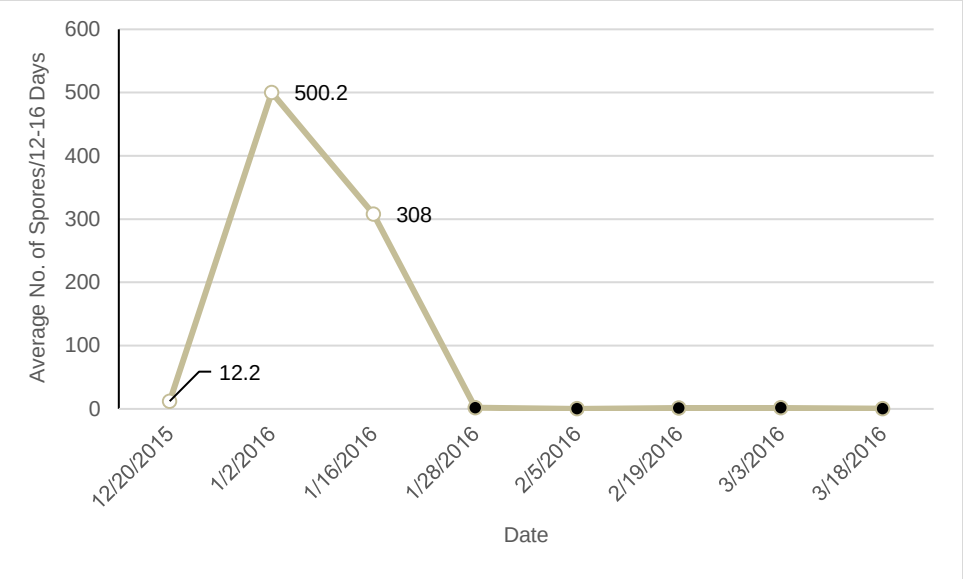

Figure 14 Number of Botryosphaeriaceae spores observed on sampling dates between December 2015 and March 2016 at Flat Creek Estates. Number represents the average of eight spore traps, set and collected in the Sangiovese block. All vines in this block were retrained after the collection date on January 16th 2016.

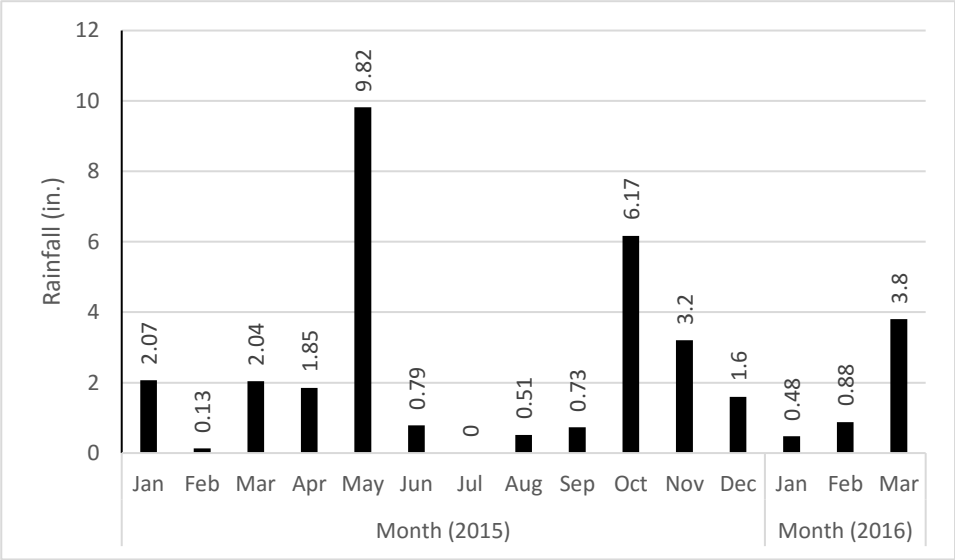

Figure 15 Rainfall detected at meteorological station local to Flat Creek Estates. Rainfall was assessed on a monthly basis between January 2015 and March 2016.

During 2015 rainfall was highest in May (9.82) and lowest in the months of February (.13) and July (0) (Figure 15). During 2016, observed rainfall was at its highest during the month of March (3.8). There were only two dates in 2016 in which spores were observed, the greater of the two was from January 2nd averaging 500.2 spores per slide. At the end of January 2016 the sangiovese block at Flat creek estates was completely retrained and spore collection dropped to negligible numbers (Figure 14). Rainfall during the month of January was relatively low at this site location (Figure 15).

At Driftwood Vineyard, Botryosphaeriaceae spp. spore release coinciding with rain events was demonstrated by comparing the varying levels of spore dispersal (Figure 16) to that of precipitation on the appropriate collection dates (Figure 17). On every collection date between February and May 2015 the spore traps in the block of Cabernet Sauvignon produced the greatest average number of spores. For all three vineyards, the highest average number of spores (835.2) counted on single collection date was observed in the Cabernet block at Driftwood Vineyard in March. For all three cultivars, there was an observable spike in spore production on March 22nd and May 5th. Spore production was at its lowest in all three blocks during the months of February and June. Rainfall for the Driftwood vineyard was extremely variable between January and July 2015 (Figure 17). The greatest amount of rainfall was observed during the month of April totaling 3.95 inches. There was no rain during the months of June and July and very little during February (.2).

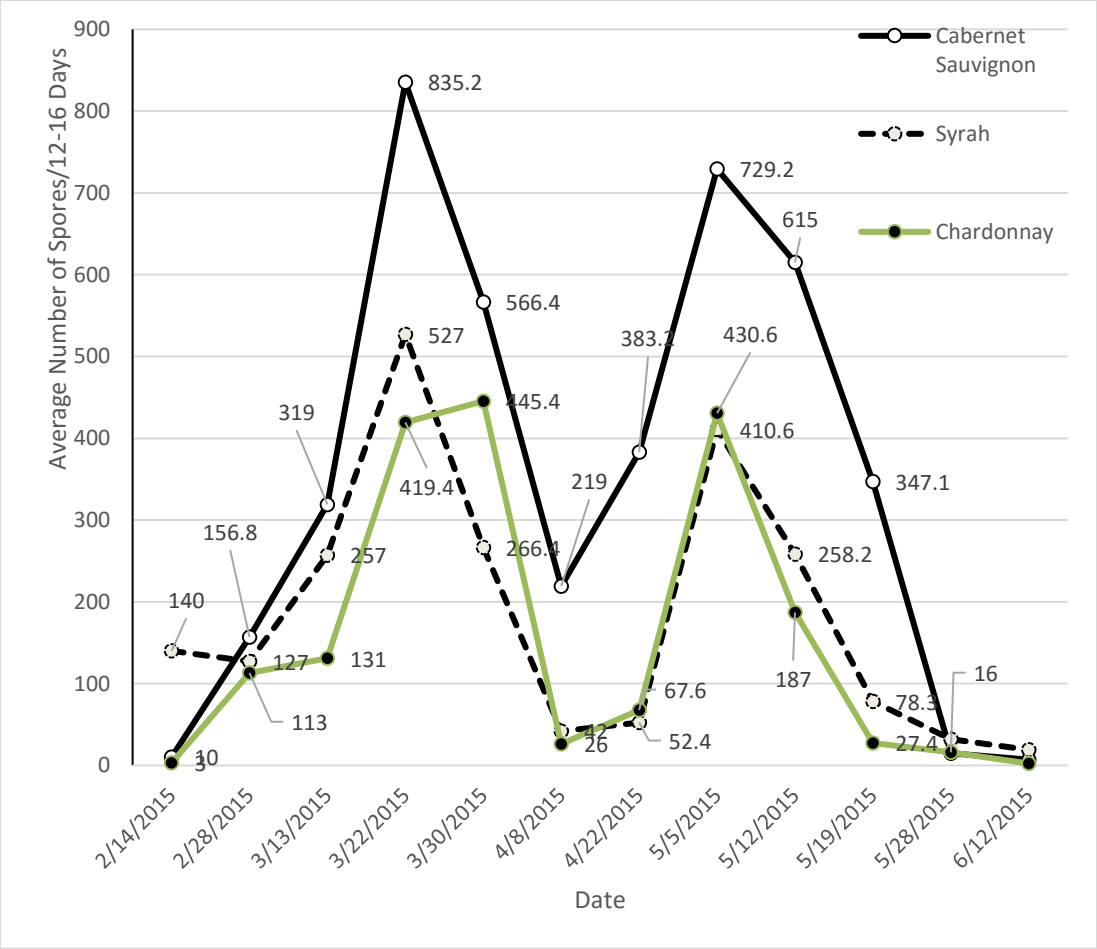

Figure 16 Number of Botryosphaeriaceae spores observed on sampling dates between February 2015 and June 2015 at Driftwood Vineyard. Number represents the average of eight spore traps, set and collected in each of the blocks of Cabernet Sauvignon, Syrah, and Chardonnay grapevine varieties.

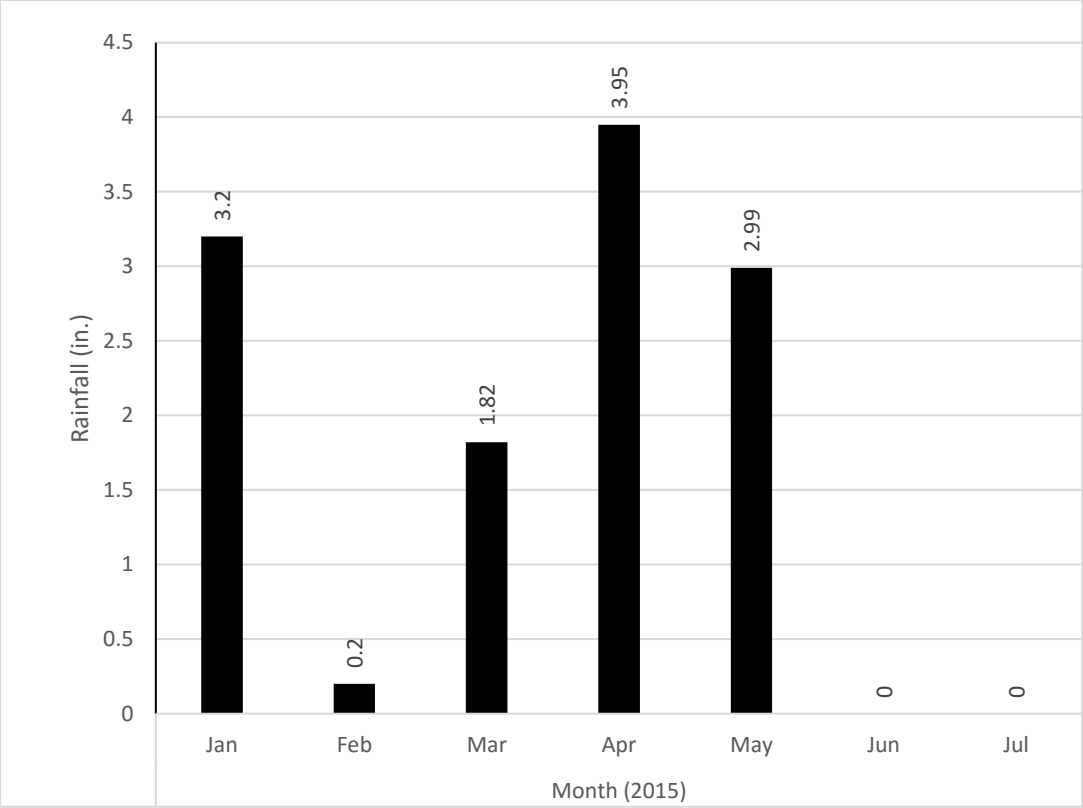

Figure 17 Rainfall detected meteorological station local to Driftwood Vineyard on a monthly basis between January 2015 and July 2015.

Results of our trapping studies indicated that Driftwood vineyard had the greatest amount of inoculum during the course of the study. The levels at Flat Creek Estates fell closely second. The lowest number of spores were collected at the Industry Trial Vineyard.

4. DISCUSSION AND CONCLUSION

The first attempt to identify and characterize the fungal species associated with grapevine trunk disease in Texas was conducted in 2009 (Úrbez-Torres *et al.,* 2009a). In that work, Botryosphaeriaceae spp. were emphasized as the primary constituents of vine decline. To date, that research project comprises the only subsequent investigation in Texas of GTDs, which are considered by some to be one of the most serious worldwide problems on wine grapes. Based on the inoculation studies (Objective 2), *Lasiodiplodia* sp. were the most virulent among all the fungi tested in both the current and previous study (Table 5; Úrbez-Torres *et al.*, 2009a). *Diplodia* sp. were the predominant fungi isolated from cankers within Texas vineyards. However, *Dipoldia* sp. proved to be the least aggressive of the tested fungi, which is in agreement with Úrbez-Torres *et al.*, 2009a. *Eutypella* spp., and *Pestalotiopsis* spp. are two other organisms, identified in Objective 2, that were notably discussed in Úrbez-Torres *et al.*, 2009a.

Observations of vineyards throughout the world, reveal that symptoms of grapevine trunk diseases typically do not appear in vines younger than 7 years of age (Baskarathevan *et al* 2012*;* Rolshausen *et al.,* 2010; Úrbez-Torres *et al.*, 2009b). At Austin County vineyard, the 20 year old Blanc du Bois vines on average received higher disease ratings than that of the 4 year old vines. The majority of the vines surveyed at the Industry Trial vineyard received a disease rating of two and were only 6 years in age. The vines at both Flat Creek Estates and Austin County vineyards which were older than 16 years, received an average rating of 7 or greater. These results demonstrate a

correlation between disease severity and the age of affected vines. Accordingly, the association of age with the incidence and severity of GTDs in Texas is proceeding as would be expected from results throughout winegrape regions elsewhere.

Viticultural Practices

As the Texas wine grape industry has grown and ages of vineyards increase, grapevine trunk diseases have proven to be implemental in limiting the vitality and productivity of vineyards in Texas. There are no known methods of eradication and only limited alleviation of disease provided by fungicide application which makes disease management integral in routine viticultural practices. In the present study, peak periods of spore dispersal by species within the Botryosphaeriaceae at three Texas vineyards coincided with high rainfall during the months of March and May. Seasonal pruning of grapevines is conducted during the spring months, when dozens of pruning wounds may be made on an individual vine. Preventative practices are the best means to controlling trunk disease (Agusti-Brisach *et al.*, 2015; Hillis *et al.*, 2015). Pruning during the spring is necessary to maximize grape quality and yield for the following growing season. However, pruning wounds serve as the primary infection court for many of the trunk disease pathogens. In California, pruning techniques aimed at minimizing risk of infection have been developed (Úrbez-Torres *et al.*, 2010). For example, delayed pruning is simply pruning later in the season when temperatures have increased and rainfall events have subsided. This indirectly correlates to lower inoculum counts and increased vine vitality. Double pruning is another approach to disease prevention that involves conducting a first pass, in the early stages of the dormant season, leaving 8 to

10 inches of wood on each cane and a final pass immediately preceding bud break (Weber *et al.*, 2007). Dieback pathogens are slow growing and the final pass allows for the removal of potentially infected tissue. Double pruning permits growers to avoid infection while still adequately managing many acres of grapes.

In an attempt to increase fruit quality, growers conduct practices that predispose vines to disease. These include drought stressing vines and over-fertilization. Nitrogen is a limiting factor for fungal growth and documented to have a stimulating effect on fungal wood decay (Bolton, 2009; Findlay, 1953). Frequent applications of nitrogen-based fertilizer (organic and inorganic) in excess amounts affects nitrogen mobilization and can lead to residual buildup in storage organs, which wood colonizing fungi can utilize (Findlay, 1953; Fernández-Escobar *et al.*, 2011). Viticulturists should attempt to follow the guidelines of knowledgeable crop advisors. Drought stressing vines causes an increase in susceptibility to disease as the vines vitality is decreased and disease responses are compromised (Bostock *et al.,* 2014). The potential to obtain a desired increase in fruit quality may not always justify the risk of inducing a chronic infection that will limit yield indefinitely.

If dormant wood cuttings are to be used for vine propagation, the source must be that of a healthy vine. Grapevine trunk pathogens reside within the vascular tissue so the use of cane cuttings from infected vines serves as a means of pathogen spread. Sanitation of cuttings is recommended to further reduce the risk of infection.

Notable Findings

Greeneria uvicola, the cause of bitter rot of grapes, was found in 14 of the 23 grapevine blocks and 8 of the 12 considered counties assessed in Texas (Table 4). This pathogen lowers crop yield and the quality of wine produced from affected grapes. There is even a report of it triggering the loss of the California retail market as the result of a quarantine (Chitambar, 2016) in addition to being on the 'Harmful Organism List' for China (PCIT, 2015). Viable spores of this pathogen were found overwintering on fruit that remained on the vine in Texas vineyards. In the spring, mummied fruit, pedicles, and other decaying tissues serve as sources of inoculum. Berries are susceptible to direct infection from conidia. This pathogen is highly reproductive, with the ability to cause primary and secondary infections. Bitter rot management options have been described in previous literature as it is a common disease in the southeastern United States (Milholland 1991; Miranda, 2005). Disease development favors the climatic conditions in Texas but, if enacted, management techniques are shown to provide favorable control of the disease (Wilcox *et al.*, 2015). Confirmation of *G. uvicola* in Texas vineyards should trigger extension efforts to educate growers on the incidence of disease and available means of management.

The spore trapping studies revealed several unexpected findings. There was an inconsistency between the pathogens isolated from diseased tissues and the morphology of Botryosphaeriaceae spores found on local traps. If a pathogen was isolated from a diseased vine, there was no guarantee that spores of that species would be collected in the trap. Spores of *Neofusicoccum* spp. were collected from traps on vines, where the

pathogen could not be isolated from wood tissue. The collection method is not assumed to be inadequate. Airborne dispersal of spores may vary among species or the extent of pathogen colonization may determine spore production (van Niekerk *et al.,* 2010). Interestingly, insect borer excrement was collected on traps and found to contain copious amounts of viable Diatrypaceae spores. The role that arthropods play in promoting the spread of grapevine trunk disease has only recently been investigated (Moyo *et al.*, 2014). In Texas, the frequent association of boring insects with diseased vines suggests that insects are promoting the rate at which a pathogen spreads within a single vine (personal observation, Jim Kamas, Dept. of Horticulture, TAMU). Borer entry wounds were observed to be lined with the fruiting bodies of white-rot basidiomycetes (Figure 7). By chewing through the vascular tissue an arthropod is not only generating wounds, which serve as entry points for the pathogen, but they may also spread inoculum as they travel through the host.

Relationships among the microorganisms in vines colonized by trunk pathogens are poorly understood. Typically, multiple pathogens infect a single vine and sometimes even a single wound. To date, there are very few scientific studies that allude to effects of co-inoculation (Pierron *et al.*, 2016; Whitelaw-Weckert *et al.*, 2013), but the primary model for disease is one of succession (Mugnai *et al.*, 1999). The best example of this is The Esca disease complex. Petri disease, Grapevine leaf stripe disease (GSLD), and Black Goo are all diseases of young vines that are associated with the pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* (Edwards *et al.*, 2004; Fourie *et al.*, 2004; Surico *et al.*, 2009). *Phaeoacremonium aleophilum* is also credited

as a causative agent of Esca-associated symptoms (Wilcox *et al.*, 2015). These species were identified in Texas in addition to several other species of *Phaeomoniella* and *Phaeoacremonium*. *Phaeomoniella* spp., found in 15 of the 23 grapevine blocks assessed, were identified in greater abundance than *Phaeoacremonium spp.,* which was only identified in 11 of the 23 grapevine blocks (Table 4). Vines that play host to these pathogens can be subsequently colonized by wood-rotting Basidiomycetes upon aging. Following infections by the ascomycetes, wood-rotting basidiomycetes cause extensive decay columns, and the resulting disease is redefined as *esca proper*. Unlike the other trunk diseases which cause localized infections, *esca proper* results in a systemic array of symptoms that affect the physiology of the entire vine. There are numerous past reports of basidiomycetes in Esca-symptomatic vines within Australia, Europe, New Zealand, South Africa, and South America (Fisher, 2006), but there are only two reports of basidiomycetes causing Esca-associated white-rot in North America (Cloete *et al.*, 2015). *Phaeomoniella* spp., *Phaeoacremonium* spp., and wood-rotting basidiomycetes were all found in association within diseased vines, in Texas. Conclusively, *esca proper* is the correct descriptor for the Esca-associated disease in Texas.

The microbial and enzymatic degradation of wood has been investigated in many species of hardwood and softwood trees, but corresponding research pertaining to vines is lacking. As a result, many of the explanatory concepts concerning wood-rots of vines in Texas were derived from studies of the same genus that is found to colonize trees. *Phellinus* sp. (Hymenochaetaceae), a known pathogen to native Texas trees, is spread via wind-blown basidiospores and, most importantly, by root-to-root contact (Wilson *et al.*,

2004; Sullivan *et al.*, 2007). The 100% disease incidence observed in vineyards where Hymenochaetaceae was confirmed suggests that transmission via root-to-root contact is probable. The information provided in Table 4 consists of identifications that were made to a 95% level of confidence. There were many fungal species, primarily within the Hymenochaetaceae, that were not identified to the level of genus and therefore not listed in the table. Taxonomy is commonly based on traditional characterizations such as annual or seasonal basidiocarps and the type of exhibited hyphal system (Zhou *et al.*, 2016). Multiple species were found colonizing single vines, which presented complications when trying to identify isolated fungi. Another way to classify these organisms is by their ecological characteristics in the field. The ability of fungi within Hymenochaetaceae to spread via root-to-root contact varies among genera. The genus *Phellinus* is not assumed to be the only causative agent of white-rot in Texas vineyards, but its spread within tree stands simply serves as an explanatory model for transmission of the pathogen within vineyards.

Wood rot, both white and soft, progresses slowly allowing the development of internal decay columns before external symptoms are revealed. These fungi present a serious threat to the longevity of vines, as infection decreases structural integrity leading to an increase in susceptibility to wind and freeze damage. None of the Basidiomycetes identified in this project have been previously reported on grapevines in Texas. *Tropicoporus tropicalis* (Hymenochaetaceae) was isolated from basidocarps found on Esca-symptomatic vines in Texas. Although the presentation of symptoms is uniform with those of *esca proper* in other parts of the world the genus of the causative agent is

novel. This is not surprising as the global taxonomy of fungi within the Hymenochaetaceae has yet to be thoroughly described (Fisher, 1996; Vlasák *et al.*, 2013; Zhou *et al.*, 2016). *Tropicoporus tropicalis* has recently been described as a pathogen of hardwood trees in Brazil and China, but it has never before been reported on grapevine (Zhou *et al.*, 2016).

Remarks

The results of this project serve as a baseline for further research needed to better understand grapevine trunk diseases in Texas. Growers must implement recommended preventative practices if the control of disease is to succeed. Similar procedures have recently been proposed in California, attempting at educating growers on the importance of early onset disease management (Hillis *et al.*, 2015). Community outreach by extension operatives will prove to be implemental in the control of trunk disease in Texas. Many of the identified causative agents have been recognized in previous works from other locations, although the taxonomy of the Esca-associated wood-rotter pathogens remains undescribed and *esca proper* requires further consideration. As the vineyards in Texas age, the effect of trunk diseases worsen demanding improved management options derived from academic involvement in grower education.

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