DETECTION AND DIAGNOSIS OF RED LEAF DISEASES OF GRAPES *(VITIS SPP)* IN OKLAHOMA

By

SARA ELIZABETH WALLACE

Bachelor of Science in Horticulture

Oklahoma State University

Stillwater, Oklahoma

2016

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2018

DETECTION AND DIAGNOSIS OF RED LEAF

DISEASES OF GRAPES *(VITIS SPP)*

IN OKLAHOMA

Thesis Approved:

Dr. Francisco Ochoa-Corona

Thesis Adviser

Dr. Eric Rebek

Dr. Hassan Melouk

ACKNOWLEDGEMENTS

Thank you to Francisco Ochoa-Corona, for adopting me into his VirusChasers family, I have learned a lot, but more importantly, gained friends for life. Thank you for embracing my horticulture knowledge and allowing me to share plant and field experience.

Thank you to Jen Olson for listening and offering me this project. It was great to work with you and Jana Slaughter in the PDIDL. Without your help and direction, I would not have achieved this degree. Thank you for your time and assistance with the multiple drafts.

Thank you to Dr. Rebek and Dr. Melouk for being on my committee, for your advice, and thinking outside the box for this project. I would like to thank Dr. Astri Wayadande and Dr. Carla Garzon for the initial opportunity as a National Needs Fellow and for becoming part of the NIMFFAB family. I have gained a vast knowledge about biosecurity and an international awareness with guests, international scientists, and thanks to Dr. Kitty Cardwell, an internship with USDA APHIS.

Thank you to Gaby Oquera-Tornkein who listened to a struggling student and pointed me in the right direction. Thank you to fellow grad students who gave me molecular advice and training along the way, especially Fernanda Proana, Liz Pena, Salome Suarez, Patrick Rydzak, Marie Sharp, Omar Arias, and Nathalia Graf. Thank you

to the EPP staff Pam, Sharon, Barbara and Jill who advised and reminded me of forms, dates, and funding.

Thanks to EPPGSA officers, Claudia Diaz, Mansi Gulati, Salome Suarez, Felipe Cevallos, Liz Pena and Patrick Rydzak, for making us dress up for Halloween; I will always remember dressing as a grape cluster, for my project.

And finally, thanks to my husband, George, and sons, J.W. and Jesse who kept us going these past two years: with extra jobs, doing dishes, and sharing a vehicle. I dedicate this thesis to my husband George, whom I could not have done this without. He is my steady rock in this journey of life and I am proud to be his partner and look forward to the future and what adventure awaits us.

Name: SARA ELIZABETH WALLACE

Date of Degree: JULY, 2018

Title of Study: DETECTION AND DIAGNOSIS OF RED LEAF DISEASES OF GRAPES *(VITIS SPP)* IN OKLAHOMA

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Abstract:

The grape industry in Oklahoma was valued at $98 million in 2010. In 2015, symptoms resembling Grapevine Leafroll disease were observed, but Grapevine Leafroll-associated Viruses were not detected using enzyme-linked immunosorbent assay (ELISA). A 2-year Cooperative Agricultural Pest Survey was initiated to determine the etiology of the red leaf symptoms in Oklahoma vineyards. In 2016, a total of 121 symptomatic grapevines from 13 counties were sampled and 96 symptomatic grapevines from 14 counties were sampled in 2017. Each sample was tested for Grapevine Red Blotch Virus (GRBV), *Xylella fastidiosa* (Pierce's Disease), and '*Candidatus* Phytoplasma spp,' by polymerase chain reaction (PCR). ELISA was used to test for Grapevine Leafroll associated Virus (GLRaV) strains 1,3 and 4 strains. Rotbrenner, caused by *Pseudopezicula traceiphila*, (2017 only), can be found in xylem from petioles and the xylem was examined morphologically for signs of fungal structures. In 2016, GRBV was detected in 38% of 121 symptomatic samples, GLRaV-1 and -3 were detected in 16%, GLRaV 4 strains were detected in 2%, and *X. fastidiosa* was detected in 2%. There were no detections of '*Ca* Phytoplasma spp' in 2016 or 2017. In 2017, GRBV was detected in 34% of the 96 samples, GLRaV-1 and -3 were detected in 17%, GLRaV 4 strains were detected in 3%, and *X. fastidiosa* was detected in 3%. Rotbrenner was not detected in any of the samples in 2017. The findings of this survey provide information to Oklahoma grape growers and extension personnel about the cause of red leaf diseases affecting grapevines so that appropriate management strategies can be implemented in the near future.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Grape Production

Grapes are the number one fruit produced in the world. Grapes grow on a perennial, deciduous vine that is trained to a trellis in a vineyard. Grapes are grown in every state of the U.S. for table grapes and wine. There was a resurgence to plant grapes for wine production in Oklahoma in the 1990's. Winery numbers increased from 4 to greater than 60 from 2001 to 2011 (Stafne 2007; Stafne 2013). The Agricultural Census of 2012 recorded 456 acres of grapes in production in Oklahoma (NASS 2015). In 2010, the Oklahoma Grape Industry Council (OGIC) hired a consultant from California to assess the economic value of the grape industry in the state and the industry was estimated to be $98.5 million (Frank, Rimerman + Co. LLP 2010).

Growers acquire grape plant material from other growers, local nurseries, and ordering elsewhere within the United States. Many plants carry unintentional pests or pathogens which can negatively affect the vineyard in the new planting location. Agricultural biosecurity is key to preserving the integrity of the regional and national agriculture sector to sustain the economy of families, trade markets and to export produce around the world. The primary step in protecting American agriculture is exclusion through border security at ports and airports, where imported goods and people could contain or carry in pests and pathogens.

Cooperative Agricultural Pest Survey

Since only 1-2% of the goods imported into the U.S. are inspected for pests (P. Berger, personal communication), Cooperative Agricultural Pest Surveys (CAPS) are conducted with state agricultural department staff and land-grant university laboratories to assess which pests are present in the state. In 2015, grapevine leaves showing red blotch symptoms were collected from several commercial growers and sent to the Plant Disease and Insect Diagnostic Lab (PDIDL) at Oklahoma State University (OSU), where Grapevine Leafroll-associated Viruses (GLRaVs) were not detected. A CAPS project was initiated to test for exotic plant pests in grapes (not known to occur in the USA) and to test for common diseases reported infecting grapes in the US (but not detected yet in Oklahoma) to determine the cause of red leaf diseases and increased mortality of grapes in Oklahoma.

Objectives

With the increasing number of wineries and growth in acreage of grapes, this research project hypothesizes that there are undiagnosed pathogens present in Oklahoma vineyards. Therefore, the objectives of this research are:

Objective 1: Collect samples and survey vineyards representative of the Oklahoma grape industry for red leaf diseases

Objective 2: Test grapevine leaf samples for five different pathogens:

Grapevine Red Blotch Virus

Grapevine Leafroll-associated Viruses

Xylella fastidiosa

'Candidatus Phytoplasma spp.*'*

Pseudopezicula tracheiphila

Objective 3: Determine the primary cause of red leaf disease in Oklahoma vineyards and to show disease distribution in the state

Objective 4: Develop a new set of PCR primers for detection of *Fig Mosaic Virus* needed by plant pathologists

CHAPTER II

REVIEW OF LITERATURE

Grape Production in the USA

Grapes have been the number one fruit produced around the world for centuries with multiple uses: table grapes, wine, raisins, juice, and byproducts (Kurtural 2003). Grapes are the highest value fruit crop and the sixth highest value of all US crops (MFK Research 2010). According to the 2012 Census of Agriculture, over 50% of all land planted to non-citrus fruit trees is planted to grapes; 1.14 million acres (NASS 2015). More than 23,000 farms grow grapes and 90% are farms smaller than 100 acres (MFK Research 2010). Vineyards and wineries offer new opportunities for rural communities, especially where traditional crops were previously grown, and benefit the local economy through increased tourism (MFK Research 2010) and real estate values (Frank, Rimerman + Co. LLP 2010). The grape product value was $5.5 Billion in 2015 (NASS 2016). The full economic impact of US wine, grapes and grape products is valued at $162 Billion which includes jobs, wineries, sales, and revenue from restaurants selling wine, sales of wine, grape sales (wine, fresh, raisin, and juice), wine-related tourism visits, expenditures, federal, state, and local taxes (MFK Research 2010).

Table grapes are sweet, eaten fresh, have a short shelf life, and are difficult to transport (Kurtural 2003). California produces about 90% of all fresh grapes consumed in the USA (NASS 2016). Each state grows grapes for fresh consumption and wine production (NASS 2016).

Wine production is the primary use for grapes and these grape cultivars are often chosen for their name and reputation. For example, European varieties, *Vitis vinifera*, are often preferred over the best native cultivars, or for the climate and soil conditions of the growing region (Stafne 2006). California produces 90% of the bottled wine consumed in the United States (NASS 2015). The USA is the fourth largest producer of wine in the world and the number one consumer (Wine Report 2017). Wineries in the US have increased to 11,496 in 2016 from 2230 in 1998 (AgMRC 2015; Wine Statistics 2017) an increase of 415% in 18 years.

Grapes are grown around the world and grape plant material is exported and shared among countries and regions within a country. Most wine is consumed in the country where it is produced, and about 14% is exported (Kurtural 2003). There is tradition, art, and science involved with the wine's production and its chemical properties based on the levels of acid, sugar, sulfur, and sulfites within the wine (AgMRC 2015). Climate and management practices such as irrigation and fertility affect the sugar content (Stafne 2007).

Agritourism

In the USA, 21 million people visit vineyards in California and spend $2 billion annually. (AgMRC 2016). Tourists can attend educational tours, visit wine tasting rooms, restaurants, gift shops, and a few offer overnight stays. Vineyards are an increasingly common wedding venue (J. Olson, personal communication). Nationally, agritourism is defined as an agriculturally based activity that brings people to the farm or ranch. Agritourism is the fastest growing segment of tourism in Oklahoma, and an increasing trend as farmers find ways to diversify farm income and maximize profit. There are also non-economic reasons for farmers to invest in agritourism. Tew and Barbieri (2012) found that farmers desire to retain their rural lifestyle and to raise their families on the family farm, which enhances the quality of life. Agritourism has increased as consumers increasingly buy and enjoy local food and desire to know the origin of their food.

With the expansion of the wine industry in Oklahoma, 'The Wine Trails' was developed offering tours and events at wineries and vineyards along Route 66 and throughout the state (Oklahoma Agritourism 2017). Within agritourism, Pick-Your-Own farms, where the consumer comes to pick produce, is a marketing strategy that decreases the costs of farming in labor and transport, while the consumer enjoys fresh, hand-picked produce that they harvest themselves.

Prohibition

Grapes have been grown in Oklahoma long before statehood. Over 4,000 acres of grapes were planted in the 1890's (Widener 2010). When Oklahoma became a state in 1907, the state constitution forbade wineries to sell their products (Widener 2010). Then in 1918, the United States enacted Prohibition, which made illegal the sale, production, transportation, and consumption of alcoholic beverages (Pinney 2007). Prohibition ended in 1933; however, alcohol was not legalized in Oklahoma until 1959 (Stafne 2007, Widener 2010).

Prohibition was written into the Oklahoma state constitution, so Oklahoma remained "dry" (alcohol-free) even though the rest of the country repealed national prohibition in 1933. However, in reality, Oklahoma was never really "dry" with bootleggers selling alcohol and the upper class having access to alcoholic drinks at country clubs in Tulsa and Oklahoma City (Goldsmith 2015). Oklahoma continued to be a "dry state" until 1959, when Governor Edmonson removed alcohol from the country clubs, increased patrols to catch the bootleggers, to allow the people of Oklahoma to vote in favor of alcohol (Goldsmith 2015).

In the 90's, Oklahoma experienced a renewed interest in growing grapes and producing wine which increased opportunities for agritourism in the state. The people of Oklahoma voted to allow wineries to sell and ship their products directly to restaurants and retailers in 2000. (Widener 2010). In 2001, there were only four wineries in the state, but by 2015, that number increased to sixty-four wineries. In 2012, there were 139 commercial vineyards with 439 acres of

vines bearing fruit (Oklahoma Farm Report 2012). And in 2018, 69 wineries were registered in Oklahoma according to the Oklahoma Grape Industry Council (OGIC) (C. Duncan, personal communication).

The Economic Impact of Oklahoma Wine Report

In 2010, the OGIC, which represents more than 90% of commercial vineyards and wineries in Oklahoma, hired Frank, Rimerman and Co. LLP of St. Helena, California, to detail the economic impact of Oklahoma's wine and grape industry. Their report estimates the total value of Oklahoma's grape industry at $98.5 million, which includes salaries for 840 jobs, wine sales, and tourism. This $98.5 million also includes $5 million in taxes contributed to the state and local economy and $6 million in federal tax revenue (Frank, Rimerman and Company 2010).

Grape is a perennial crop, grown on woody, deciduous, climbing vines that fruit as early as the third year after establishment. Viticulture requires a considerable initial investment including the cost of vines, trellis materials, labor, pruning machinery and pesticides for the first three years before the vines bear fruit. Irrigation is required in Oklahoma to sustain plants through heat and drought conditions (Stafne 2007). However, once established and maintained, grapevines can produce for 30 years.

Current Diseases of Grapes in Oklahoma

Fungal leaf diseases and cankers are common, but fungicides are available for treatment and prevention. Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa*, is not treatable and was reported in Canadian County in 2008. Those plants were removed but surveys in 2008-2009 indicated that PD is found in many counties in Oklahoma. This disease is spread by insect vectors such as sharpshooters (Rebek and Overall 2013) and exchange of plant material between growers. Grapevine Red Blotch virus was detected in Oklahoma in 2015 which precipitated this survey (Plant Disease and Insect Diagnostic Laboratory, Oklahoma State University).

Cooperative Agricultural Pest Survey

Before 2015, viruses had not been detected in Oklahoma vineyards. In 2014, grape leaves with red blotches and rolling were submitted to the PDIDL and tested negative to Pierce's disease and Grapevine Leafroll-associated Virus. In 2015, additional grape samples were sent to the PDIDL with early reddening of leaves, death of young vines and curling of leaves. The Oklahoma Department of Agriculture, Food, and Forestry (ODAFF) and the PDIDL initiated a Cooperative Agricultural Pest Survey (CAPS) to determine the cause and distribution of red leaf diseases of grapes in Oklahoma. The CAPS, funded by the United States Department of Agriculture (USDA), is a monitoring program that surveys agricultural and environmental resources for pests before they cause economic damage (USDA APHIS, 2018). The diseases and insects of concern are chosen by the USDA to be of regulatory significance and are surveyed by the state's department of agriculture in a partnership with local agricultural growers and the state's Plant Diagnostic Laboratory. USDA's Animal Plant Health Inspection Service (APHIS) has approved methods for detection and confirmation of presence or absence of exotic pests and pathogens. The exotic pests and pathogens included in this survey were Phytoplasma diseases of grapes and rotbrenner. The surveying state can add diseases that pose a regional significance. Therefore, Pierce's Disease, Grapevine Leafroll disease, and Red Blotch were also included. The CAPS survey provided the funding for ODAFF inspectors to sample Oklahoma vineyards for symptomatic plant material, which were tested at the PDIDL.

Grapevine Red Blotch Virus

Grapevine red blotch was first discovered in Napa Valley in California in 2008. The grape plants in California presented with red blotches between the veins with delayed fruit ripening in red cultivars and white or yellow patches in white cultivars (Calvi 2011). In 2010, a vineyard in New York suspected to have declined from Grapevine Leafroll viruses, tested

negative to the leafroll viruses. Researchers at Cornell determined the cause was due to a single-stranded (ss) DNA virus in cooperation with scientists at UC Davis (Al Rwahnih et al 2012; Krenz et al 2014; Yepes 2018). The new virus belongs to *Geminiviridae* and was named Grapevine Red Blotch-associated Virus (GRBaV) (Krenz et al 2012). Koch's postulates were completed in 2018, and the virus was renamed Grapevine Red Blotch Virus (GRBV) (Yepes 2018).

The virus is transmitted by grafting and spread through propagation material. In 2016, Bahder determined that the three-cornered alfalfa treehopper *Spissistilus festinus* (Hemiptera: Membracidae) is a vector of GRBV (Bahder 2016) and earlier, Poojari et al (2013) in greenhouse studies, reported the virus to be transmitted by the Virginia Creeper leafhopper *Erythroneura ziczac* Walsh (Hemiptera: Cicadellidae).

The symptoms of red blotch vary between cultivars; the most common symptom consists of red blotches between the veins that mimics nutritional deficiencies. Therefore, visual inspections of symptoms should not be relied upon for diagnosis (Cieniewicz 2017). Symptoms include red blotched leaves with red/pink veins (on red varieties), starting with basal (lower) leaves, and chlorotic and transparent leaves (on white varieties). During initial infection stages, acceptable yields are harvested, yet the grapes do not ripen evenly and have decreased sugar content (˚BRIX) which affects tannins, flavor, and fermentation for wine (Sudarshana et al 2015, Al Rwahnih et al 2015). Unfortunately, the continual lack of even ripening in grapes can result in loss of quality and total loss of yield (Sudarshana et al 2015; Herrick 2017).

Grapevine Red Blotch Virus has been found in California, New York, Pennsylvania, Virginia, Maryland, New Jersey, and Washington in the United States as well as Switzerland (the plant was bought in 1985 from California) and Canada (Krentz 2014). Much of the biology and epidemiology of this virus is still unknown. GRBV has been detected in nearby wild grapes,

(*Vitis californica x V. vinifera*), wild blackberry plants (*Rubus armeniacus*) (Bahder 2016) and found in riparian habitats close to infected vines (Perry et al 2016). Grapevine Red Blotch Virus was also found infecting the national germplasm by Al Rwahnih et al in 2015.

Polymerase chain reaction (PCR) is currently the most accurate detection and diagnostic test for this virus (Sudarshana 2015). This method uses specific PCR primer sets by Krentz et al (2014). Al Rwahnih et al (2015) found that the virus is distributed throughout the plant, and the most reliable diagnostic plant material is ground bark scrapings for virus detection any time of the year. The virus can also be detected in other parts of symptomatic plants including leaf petioles (J. Olson, personal communication).

Pierce's Disease

Pierce's disease is caused by *Xylella fastidiosa,* a gram-negative, rod-shaped bacterium in the Xanthomonadaceae family and an economically important plant pathogen. *Xylella fastidiosa* is limited to the xylem or water and nutrient-conducting tissue of the plant where it multiplies and clogs these tissues. The first symptoms in the plant are similar to drought and heat stress (leaf scorch), yet plants have adequate soil moisture, and later symptoms include petioles left on canes with leaf drop (matchsticks), distinct patches of green on typically brown canes, and shriveled, unusable fruit (Janse and Obradavic 2010).

Hot and dry weather can enhance the symptoms and severity varies from whether it is the first or second year of the disease (Appel et al 2010). Grape cultivars express the disease differently; in susceptible cultivars, there is no treatment and the duration of time until death varies (Stafne 2007). In resistant varieties, these grapevines may be asymptomatic. However, the asymptomatic infected plants can contain inoculum for insect vectors that spread the infection.

This disease can be transmitted by multiple xylem-feeding insect vectors including sharpshooters (Hemiptera: Cicadellidae), spittlebugs (Hemiptera: Cercopoidea), and Cicadas

(Hemiptera: Cicadidae) (Mahfoudhi et al 2009). These vectors acquire the bacteria when feeding on infected plants and have circulative transmission with immediate ability to infect (Purcell 1997). Once infected with *X. fastidiosa*, the juvenile vectors lose pathogenicity through molts, and the pathogen does not spread through eggs (no transstadial and no transovarial transmission) (Janse, Hill and Purcell 1995). Adult insects remain infective during their adult lives. Dormant vines can also be infected (Almeida 2005). It is imperative to continually monitor and control vectors in the vineyard and through riparian vegetation management (Rebek and Overall 2017).

The introduction and movement of new vectors and known vectors, including the glassy-winged sharpshooter (GWSS) *Homalodisca vitripennis* (Hemiptera: Cicadellidae) remains a threat to the wine industry, especially in California. The GWSS flies further distances, feeds on a variety of hosts, and is found in more diverse habitats than the native sharpshooters who do not fly as far, feed on fewer hosts, and are easier to manage. Overall and Rebek (2015) found that the sharpshooter *Graphocephala versuta* is the most common native sharpshooter in Oklahoma vineyards that can transmit *X. fastidiosa*.

In Oklahoma, *X. fastidiosa* infections were detected in American elm (*Ulmus americanus* L), oak (*Quercus spp*), mulberry (*Morus spp*), and sycamore (*Platanus occidentalis*) (Smith 2010, Olson 2018). The first report of Pierce's disease in Oklahoma came from Canadian County in August of 2008 (Smith 2010). The samples were tested in Oklahoma State's PDIDL and confirmed positive for *Xylella fastidiosa* through endpoint PCR, ELISA, and automated sequencing which showed "99%-100% homology with the gyrB gene from a Pierce's Disease strain of *X. fastidiosa* 'Temecula'" (Smith 2010). The first infected plants were rogued and destroyed, but the disease has spread within Oklahoma. At this time, there is no treatment other than removal of the infected plant.

Prior to this *X. fastidiosa* detection in 2008, von Broembsen and Olson (2005) surveyed Oklahoma vineyards distributed throughout the state in 2003-2004 and had no detections. However, noted that X. fastidiosa had been detected in counties in Texas bordering Oklahoma (J. Olson, personal communication, Broembsen and Olson 2005). Hail et al (2010) detected X. fastidiosa in northern Texas counties that are close to the Oklahoma border: Camp county, Tarrant County, and Witchita County. These detections were found in the insect vector, *Homalodisca vetripennis* Germar (Hemiptera: Cicadellidae) in Texas vineyards that have tested positive to the *X. fastidiosa* pathogen (Hail et al 2010).

Research at Texas A&M is currently investigating PD-resistant grapes and recommends cultivars like 'Black Spanish' and 'Blanc du Bois' (Appel et al 2010). It is essential to keep plants healthy because stressed or weak plants are more susceptible to insects and disease (Appel et al 2010; Stafne 2007).

Xylella fastidiosa is found as an endophyte on many plant hosts including 75 different families, 204 genera, 359 plant species and 9 hybrids (Gardi 2016). Indigenous plant species (including native grapes) may harbor the bacterium without symptoms (Appel et al 2010). *Xylella fastidiosa* also affects other agricultural crops, causing diseases that include: plum leaf scald, phony disease in peach, almond leaf scorch, bacterial leaf scorch, coffee leaf scorch, mulberry leaf scorch, oleander leaf scorch, periwinkle wilt, ragweed stunt, alfalfa dwarf disease, citrus variegated chlorosis, and crespera disease (Janse and Obradovic 2010). Interestingly, the *X. fastidiosa* grape strain does not affect peach, and the peach strain does not affect grape (Appel et al 2010). Schaad et al (2004) found that there are different strains of *X. fastidiosa* for different hosts. The strain that affects grapes has been called *X. fastidiosa* subsp. *fastidiosa*. Alternative hosts of the *X. fastidiosa* subsp. *fastidiosa* strain includes wild grapes, ragweed, alfalfa, and almond trees (Appel et al 2010).

Current detection methods show that PCR was shown to be 100x more sensitive (Banks et al 1999) than DAS-ELISA (Minsavage et al 1994). However, ELISA is the preferred method for its reliability, quickness and allows larger sample processing volumes. The method has been used since 1976 for plant pathogens (Nome et al, 1980). The serological assay is effective for end-of-season, symptomatic (leaf scorching) plant material (Schaad, 2002), but does not work well on early infections because it is less sensitive or affected by patchy distribution of the targeted pathogen in the plant (Bextine et al 2004). Bextine et al (2004) compared whole tissue vs. xylem fluid and found that for asymptomatic plant material, xylem fluid improved the sensitivity of both PCR and DAS-ELISA. However, for symptomatic tissue, there was no increase or difference in sensitivity found between xylem fluid and whole tissue (Bextine et al, 2004).

Early detection of *X. fastidiosa* is desired to diagnose infection in asymptomatic plants. Schaad et al (2002) found that 2 weeks after bud break (early in the season) is the best time to test xylem fluid during sap flow. Disease symptoms typically appear later in the season; confirming infection earlier allows removal of the plant before vectors can further spread the disease in the vineyards. This technique was developed for mapping PD infections in vineyards and to rogue plants that were infected in previous seasons (Schaad et al, 2002).

In Oklahoma, immuno-capture polymerase chain reaction IC-PCR was used to test insects carrying *X. fastidiosa* (Overall 2013). This method is more sensitive than conventional PCR, and the titer of the bacteria is lower in insects than symptomatic plants. In this study, conventional PCR was used since symptomatic plants were sampled.

'*Candidatus* Phytoplasma spp'

Phytoplasmas are prokaryotic microscopic bacteria that lack cell walls and have small genomes. These pathogens are transmitted by grafting and phloem-feeding insect vectors

including leafhoppers, planthoppers, and psyllids. Phytoplasmas are obligate intracellular parasites that are found in the phloem of infected plants. Phytoplasmas are in the class Mollicutes, within the order Acholeplasmatales, and in the Acholeplasmataceae family. Recently, the genus was renamed *Candidatus* Phytoplasma since they cannot be cultured (Firrao et al 2004).

Phytoplasmas in the USA

In 1977, phytoplasmas were found in a New York vineyard showing signs of Grapevine Yellow disease (Davis 2015). In the 1990's, phytoplasmas were found in Virginia vineyards and then another outbreak in Virginia in 2009-2011 (Beanland, 2006). Davis et al, (2015) re-evaluated the phytoplasmas causing North American Grapevine Yellows (NAGY) that affect this valuable agricultural crop which included samples from Missouri, Maryland, Pennsylvania, Ohio, New York and Virginia. The Davis team molecularly identified these samples as '*Candidatus* Phytoplasma pruni' by 16S with 2 variant strains within the NAGY samples (Davis et al, 2015).

***'Candidatus* Phytoplasma australiense'**

'Candidatus Phytoplasma australiense' is the causal agent for Australian grapevine yellows (AGY). Molecularly, '*Ca*. P. australiense' is closely related to other European and Iranian strains (Mirchenari 2015). However, Davis (1997) compared these phytoplasma strains and found that this Australian strain was "unique and represented a new taxa" as it was more closely related to the phytoplasmas causing disease in Papayas such as Papaya Dieback (PDB) and Phormium yellow leaf (PYL) in Australia (Gibb, 1999).

The symptoms of Australian grapevine yellows include reddening of leaves, leaf rolling, and yellowing in red varieties. Symptoms in white cultivars are leaf rolling and yellowing (Mirchenari 2015). *'Ca*. P. australiense' is a quarantine pest because of its economic importance

to many fiber, forage, and food crop hosts including potatoes *(Solanum tuberosum*), however, is contained to Australia and New Zealand (Liefting et al, 2011).

'Candidatus Phytoplasma vitis'

'Candidatus Phytoplasma vitis' ('*Ca*. P. vitis') is well known in Europe as *flavescence doree* (FD), which causes grapevine yellows disease in vineyards. *Flavescence doree* is a quarantine pest because it causes rapid deterioration in grape and wine production with plants dying in a few years. Wine production represented 5% of the agricultural output in the European Union in 2006. *Flavescence doree* has been found in France, Spain, Italy, and Portugal which are home to roughly 92% of the vineyard acres in the EU in 2006 (European and Mediterranean Plant Protection Organization 2006) and is, therefore, a threat to the grape industry in the US. '*Ca*. P. vitis' is an obligate intracellular parasite that is found in the phloem of infected plants (Agrios 2005).

The signs and symptoms of '*Ca* P. vitis' vary by cultivar, with "crispy, brittle, downward rolling, and reddening (orange to purple) in red varieties and yellowing (golden to chlorotic) in white varieties" (Delrot 2010, Angelini 2010). Depending on the variety of the rootstock and the scion, the severity of disease will vary, and American rootstocks seem to be tolerant at this time (Angelini 2010).

Vegetative propagation, grafting, and insect vectors have been found to transmit phytoplasma diseases. Confirmed insect vectors *Scaphoideus titanus* (Hemiptera: Cicadellidae) and Psyllids (Hemiptera: Psyllidae) along with potential vectors: *Dictyophara europaea* (Hemiptera: Dictyopharidae), *Orientus ishidae (*Hemiptera*:* Cicadellidae), *Oncopis alni* (Homoptera: Cicadellidae) all can harbor the pathogen but are not confirmed vectors (Weintraub and Beanland 2006). Experimental vectors include all leafhoppers from the family Cicadellidae (Hemiptera: Cicadellidae) (European and Mediterranean Plant Protection Organization 2002).

Alternate hosts for phytoplasmas include all *Vitis* species as well as *Alnus glutinosa* (Common or Black Alder), *Alnus incana* (Gray or Speckled Alder), and *Clematis species (*Clematis varieties). Possible alternative hosts include *Chrysanthemum carinatum (*Painted Daisy), *Trifolium repens (*White Clover), and *Vicia faba* (Broad bean) (European and Mediterranean Plant Protection Organization 2012) (as possible carriers as seeds). The movement of plant material and seeds continues to be a concern with a pathway of Phytoplasma diseases to the US, since grapevines are shared worldwide (USDA APHIS CAPS, 2018). This pathogen is economically important in grapes, and without control of the vector, infected vines increase at a rate of about 10 times yearly, and in a few years, may have an infection rate of 80-100% (Centre for Agriculture and Bioscience International 2013).

PCR is a versatile, specific, and sensitive method for detection of phytoplasmas (European and Mediterranean Plant Protection Organization 2007). Clair et al (2003) developed a multiplex nested-PCR to distinguish between 2 closely-related *'Ca*. Phytoplasma spp', causing similar symptoms in grapevines. This procedure was validated on 2,525 grapevine samples from the field in a French survey in 2002. ELISA was a standard tool; however, antibodies were difficult to find commercially (European and Mediterranean Plant Protection Organization 2002).

Hren et al and al (2007) and Angelini et al (2007) developed methods for real-time PCR. Pelletier et al (2009) developed an end-point PCR procedure which is now the official method in France. Since France's Plant Protection Services validated it, this method is used in Croatia, Spain, and Hungary. Kogovsek et al (2014) developed loop-mediated isothermal amplification (LAMP) for detection of FD. The approved method in CAPS for *'Ca*. Phytoplasma spp', is the nested PCR, which uses PCR primers from Lee et al (2004) and Deng and Hiruki (1991).

Grapevine Leafroll-associated Virus (GLRaV 1, 3 and 4 strains)

Grapevine Leafroll-associated Viruses (GLRaV) are ubiquitous pathogens that threaten the grape industry. Nine different viruses within the *Closteroviridae* family cause Grapevine Leafroll Disease (GLD). This disease does not kill the vines but causes a decrease in yields and reduction in quality by the uneven ripening of both wine and table grapes. (Naidu 2014). Each strain of leafroll viruses exhibits similar symptoms that can differ between cultivars and especially between white- and red-berried vines (Naidu 2014). In red-berried cultivars, symptoms can express as red or red/purple interveinal blotches while the vein remains green. White-berried cultivars show minor chlorosis and sometimes have patches in the leaf that are translucent. Later in the season, both red- and white-berried cultivars show signs of cupping leaves and eventually some leaves will completely roll, decreasing the area for photosynthesis and allowing areas for pests to hide (Naidu 2014).

Genetically, each GLRaV is a double-stranded (ds) RNA virus which is phloem-limited (Donda 2016; Martelli 2012) with different vectors and methods of transmission. The GLRaVs show notable differences in their arrangement and number of open reading frames (ORF) which makes each virus distinct (Naidu 2014). Therefore, it is impossible to identify the correct virus visually within Grapevine Leafroll disease (GLD), to determine the vector for management decisions. Sometimes, the vector can harbor more than one virus simultaneously, and grapevines often show mixed infections (Fan 2015). GLD has several modes of transmission including grafting, pruning, sharing infected plant material and insect vectors (Mahfoudhi et al 2009; Naidu et al 2014) including mealybugs and soft scale as shown in Tables 1 and 2.

The sensitivity of the double antibody sandwich, enzyme-linked immunosorbent assay (DAS-ELISA) is effective for testing symptomatic plant tissue, however, for asymptomatic or propagation materials, uneven distribution in the plant with seasonal concentrations can limit its

accuracy (Kumar et al 2015). PCR requires RNA extraction, which is difficult with grapes because grapevines are rich in inhibitory compounds; therefore, Kumar et al (2015) developed an Immunocapture Real-Time PCR (IC-RT-PCR) modified extraction technique. PCR is not routinely used in the diagnostic laboratory because positive controls for each virus strain are not readily available and require USDA APHIS PPQ permits (J. Olson, personal communication). Since symptomatic leaves were sampled, DAS-ELISA was a suitable testing method in this survey. Positive control materials for serological tests are readily available.

Since many grape diseases look similar, inspection by highly trained professionals is necessary to inspect the vines visually. A new method of inspection uses drones to acquire hyperspectral images as an efficient tool to map diseased vines (McDonald et al 2016). Diseased vines can be identified to managers and staff can remove infected vines to prevent further spread by insect vectors.

Rotbrenner

Rotbrenner is an endemic fungal pathogen in Europe that causes reddening and necrosis of the leaves of grapevines (Konig 2009). The causal agent is *Pseudopezicula tracheiphila* (Korf 1986) which is an ascomycete in the Helotiaceae family within the order Helotiales and the class Leotiomycetes. Another genus and species with similar symptoms was found in New York and Pennsylvania in 1985 (Pearson et al 1988). However, the New York and Pennsylvania samples were caused by *Pseudopezicula tetraspora* (Korf et al 1986). At this time, no occurrences of Rotbrenner or *P. tracheiphila* have been reported in the USA, and *P. tetraspora* has not been detected in Europe (Korf et al 1986).

Grapes are typically infected with *P. tracheiphila* in April and May during wet periods in Europe. Stress of the plant through nutritional deficiencies or lack of water can increase susceptibility (Korf & Zhang 1986) and apothecia develop profusely (95-125/cm^{-2}) on the lower side of the leaf (Korf & Zhang 1986). Spring inoculum can overwinter from infected leaves or

fruit lying on the ground (Korf & Zhang 1986) since *P. tracheiphila* is saprotrophic. These first spring infections typically affect the leaves, however, later infections can cause defoliation, sunscald, and loss of fruit (Pearson et al 1991). The infected plant material that resides on the ground serves as inoculum during the next rain resulting in polycyclic infections. This fungus can spread through splash, wind, and overwinter in/on the soil; symptoms typically appear 21-28 days after long periods of leaf wetness (Korf et al 1986).

Symptoms include small, faint, yellowing spots that grow into necrotic areas between the primary and secondary veins on the leaf or leaf edge (Plant Health Australia 2009). These necrotic areas of the leaves can have a yellow margin and the infected tissue turns reddish- brown and dies. Nearby fruit can get infections on pedicels which causes the fruit to dry and rot (Costanzo and Sullivan 2013, Pearson et al 1991).

Choosing resistant varieties is recommended in Europe. In high-risk areas, where Rotbrenner has been a problem, the preventative application of fungicides to foliage is recommended (Holz 2000). Forecast models based on computer technology and weather data can help growers to determine if conditions warrant preventative sprays. However, once the infection is established, there is no treatment. Removal of the infected leaves and tilling of the leaves into the soil after leaf fall is encouraged (Holz 2000).

Alternate hosts of *Pseudopezicula* include *Parthenocissus quinquefolia* (Virginia creeper), *Parthenocissus tricuspidata* (Boston ivy), *Vitis labrusca* (fox grape), *V. riparia* (riverbank grape), and *V. vinifera* (grapevine) (Korf et al. 1986).

At this time, Rotbrenner has not been detected in the USA. The recommended detection method is to boil 0.5 cm of petiole tissue in a 2% Potassium chloride solution and visualize the xylem through the microscope according to the Australia Fact Sheet on Rotbrenner provided by CAPS standards (Costanzo and Sullivan, 2013).

TABLES AND FIGURES

Table 1: Diseases included in the survey and their modes of transmission

Disease	Modes of Transmission
Grapevine Red Blotch Disease	3-cornered alfalfa treehopper (Spissistilus festinus)
Grapevine Leafroll-associated Disease	Mealybugs (Pseudococcidiae) Soft scale (Coccoidae)
Pierce's disease (*Xylella fastidiosa*)	Sharpshooters (Cicadellidae) Spittle bugs (Cercopodidae) Cicadas (Cicadoidae)
Australian Grapevine Yellows (AGY) and Flavescence Doree (FD) caused by '*Candidatus* Phytoplasma spp'	Planthoppers (Fulgoroidae) Leafhoppers (Cicadellidae) Psyllids (Psyllidae)
Rotbrenner	rain splash, water movement, dust
*All diseases can be shared through infected plant material	

Table 2: Modes of transmission for Grapevine Leafroll-associated Virus Strains

Strain	Genus	Modes of Transmission	Countries found:
GLRaV-1	Ampelovirus	Mealybugs (Hemiptera: Pseudococcidae) Soft Scale (Hemiptera: Coccidae) and Infected plant material	China, India, USA
GLRaV-2	Closterovirus	Grafting, Infected plant material	USA, Morocco, China, South Africa
GLRaV-3	Ampelovirus	Mealybugs (Hemiptera: Pseudococcidae) Soft Scale (Hemiptera: Coccidae) Infected plant material	Spain, Italy, Brazil, Tasmania, USA (WA & CA), New Zealand, Portugal, Taiwan, Iran, Australia, South Africa
GLRaV-4, 5, 6, 9	Ampelovirus	Mealybugs (Hemiptera: Pseudococcidae) Infected plant material	Turkey, Chile, Greece, USA (CA), Tunisia, Argentina, Western Australia

CHAPTER III

MATERIALS AND METHODS

Approved methods to sample for Phytoplasma diseases and rotbrenner are outlined in the CAPS Manual (CAPS Manual, 2017). The same sampling methods were applied to the other diseases since symptoms are similar. Agricultural inspectors from the Oklahoma Department of Agriculture, Food and Forestry (ODAFF) visited cooperative vineyards from July to October in 2016 and 2017 to scout for symptomatic grape plants. The selected vineyards were distributed across the state as shown in Figure 1, and large and small producers were represented. Inspectors were instructed to choose symptomatic leaves with abnormal red or yellow color, leaf curl, leaf roll, or other symptoms described for Phytoplasma diseases, Grapevine Leafroll-associated Viruses and Grapevine Red Blotch Virus. The suggested number of samples per vineyard was 8 in 2016 and 6 in 2017. However, ODAFF inspectors may have chosen greater or fewer samples per vineyard depending on the size of vineyard and severity of the problem. As shown in Tables 3, 26 vineyards in 22 counties were sampled in 2016-2017. Grape (*Vitis vinifera*) samples were sent by mail or dropped off at the Plant Disease and Insect Diagnostic Lab (PDIDL) at Oklahoma State University where they were kept at 4˚C for up to a week for processing. Samples were logged into the Plant Diagnostic Information System and assigned a sample identification number. In preparation for laboratory testing, the samples were examined and symptoms were recorded digitally.

Sample Detection

Samples were prepared as outlined below for each testing method. Following preparation, remaining sample material was stored at -20˚C for further use.

Nucleic Acid Based Assays

A portion of the petioles of symptomatic leaves were cut into small pieces (3-4mm) with sterilized scissors. The pieces were mixed and 0.7-1.0 grams of material were placed in a 2mL microcentrifuge tube containing 8-10 beads measuring 2.7-3.5mm. The tubes were stored at -20˚C until DNA was extracted. Upon removing samples from the freezer, the tubes were immersed in liquid nitrogen and beat with a mini-Beadbeater-1 (BioSpec Products, Bartlesville, OK) for 20 seconds. The immersion and beadbeating was repeated once. Total DNA was extracted using the Qiagen DNeasy Plant Tissue Mini Kit (Quiagen, Germany) according to the manufacturer's instructions. Total extracted DNA was diluted 1:100 in PCR grade water. This dilution was suitable based on preliminary tests (Olson, data unpublished). Total and diluted DNA was stored at -20˚C.

Samples were tested for GRBV by amplifying a portion of the coat protein (CP) gene specific for the virus (CP for and CP rev). This reaction was in a multiplex with an internal control based on the 16S rDNA gene (Krenz et al 2014). The multiplex reaction was carried out using EconoTaq DNA Polymerase (Lucigen Corporation, Middleton, WI; product #30031) following the manufacturer's instructions with primer molar rations of 5:1:1. Individual PCR samples (25uL) contained 12.5uL Econotaq, 2.5uL 5uM CPfor, 2.5uL 5uM CPrev, 2.5uL 5uM 16Sfor, 2.5uL of 5uM 16Srev and 2.5uL of sample DNA diluted 1:100; a 22.5uL mastermix was combined with 2.5uL of 1:100 DNA to total 25uL. The thermocycling conditions were as follows: 3 min at 95˚C, 30 cycles of 15s at 95˚C, 15s at 55˚C, 30s at 72˚C, followed by one final

minute at 72˚C. The expected band size for the CP gene is 257 bp and the 16S is 105 bp. Products were visualized on 1.5% agarose gel.

Samples were tested by endpoint PCR for *Xylella fastidiosa* using *X. fastidiosa* specific primers by Minsavage et al (1994). The reactions were carried out using EconoTaq DNA Polymerase (Lucigen Corporation, Middleton, WI) following the manufacturer's instructions and with primer molar rations of 5:1:1. Individual PCR samples (25uL) contained 12.5uL Econotaq, 2.5uL 5uM RST31, 2.5uL of 5uM RST33, 5uL of PCR water and 2.5uL of sample DNA diluted 1:100; or a 22.5uL mastermix combined with 2.5uL of 1:100 DNA to total 25uL. The thermocycling conditions were as follows: 1 min at 95˚C, 40 cycles of 30s at 95˚C, 30s at 55˚C, 45s at 72˚C, followed by 5 minutes at 72˚C before cooling to 4˚C. The expected band size for the diagnostic primers is 733 bp (Minsavage 1994) and were visualized on a 1.5% agarose gel.

Samples were tested for '*Candidatus* Phytoplasma spp' using the method of Davis et al, (2015) as described in the CAPS manual and at Advanced Diagnostic Workshops in Beltsville, MD that are sponsored by the National Plant Diagnostic Network. The method used was semi-nested, endpoint PCR (Davis et al 2015). Each reaction utilized EconoTaq DNA Polymerase (Lucigen Corporation, Middleton, WI) following the manufacturer's instructions with primer molar rations of 5:1:1. For the first round of PCR, individual PCR samples (25uL) contained 12.5uL Econotaq, 2.5uL 5uM primer P1, 2.5uL 5uM primer 16S-SR, 5.0uL PCR water and 2.5uL of sample DNA diluted; a 22.5uL mastermix combined with 2.5uL of 1:100 DNA to total 25uL. The thermocycling conditions were as follows: 3 min at 95˚C, 30 cycles of 15s at 95˚C, 15s at 55˚C, 30s at 72˚C, followed by one minute at 72˚C. PCR products were diluted 1:30 with PCR grade water and a second reaction was prepared. Individual second round tubes (25uL) contained 12.5uL Econotaq, 2.5.uL 5uM primer P1A, 2.5uL 5uM primer 16S-SR, 5.0uL PCR water and 2.5uL of sample DNA diluted from previous round; a 22.5uL mastermix combined with 2.5uL of 1:30 diluted product of round 1 of the PCR to total 25uL. The thermal cycling conditions were

the same as the first round. The expected product size was 1525 bp and was visualized on a 1.5% agarose gel.

Serological Assays

Pieces of symptomatic leaves were removed with sterilized scissors and mixed. For each sample, 0.4 g (+/- 0.02 g) was placed in a sample extraction bag in between layers of plastic mesh (Agdia, Inc., Elkhart, Indiana). Extraction bags were stored at 4˚C in a plastic bag with a moist paper towel for up to 7 days. The double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) from Eurofins (Eurofins, BioReba, Colorado) was selected for testing for leafroll viruses. One kit was used to detect strains 1 and 3. The second kit was described to test for strains 4 through 9 in 2016. In 2017, the kit was renamed to reflect taxonomy changes and detected 4+ strains of GLRaV (Eurofins/BioReba 2018). Samples were tested with the two DAS-ELISA kits for GLRaV 1+3 and 4+ according to the manufacturer's instructions with the following exceptions. Rather than testing samples in single test wells with 200ul volume, two test wells containing 100ul each were utilized per sample. The change allowed for each sample to be tested in replicate. At the completion of the test, sample absorbance at 405nm was measured with a BioTek microplate reader (BioTek, Vermont). In all tests, healthy and buffer controls were colorless at 60 and 120 minutes, while the positive control was visibly colored and had an absorbance at least 2.5 times the healthy control. Samples with an absorbance 2.5 times greater than the healthy control were recorded as positive.

Staining and Microscopy

Petioles from symptomatic leaves were cut into pieces measuring 0.5 cm using sterile scissors. Two to three petiole pieces were placed in a 2ml microcentrifuge tube and stored at 4˚C until processed. A 2% aqueous potassium hydroxide solution was prepared by adding 98mL RO water to a sterile glass bottle and adding 2 grams of potassium hydroxide. Once dissolved, 1 mL of

potassium hydroxide was added to the tube containing the petiole pieces. The tubes were then boiled in water on a hot plate for 2-3 minutes.

Once cooled, each sample was prepared in the hood for microscopy. Each petiole piece was sliced vertically, to retrieve a thin center piece which was placed on a microscope slide (1 microscope slide per sample). Each slide with 2-3 center slices was viewed under the microscope at 400x magnification to visualize the xylem structure. In rotbrenner infected grapevines, the hyphae of *P. tracheiphila* grow in a sine wave in the xylem tissue (Plant Health Australia 2009, Costanzo and Sullivan 2013). At least one xylem tissue (sometimes 2-3)was visualized per sample as shown in Figure 1.

TABLES AND FIGURES

Table 3: List of vineyards sampled in 2016 and 2017

Year	County	Vineyard
2016	Payne	Vineyard 1
2016	Muskogee	Vineyard 1
2016	Delaware	Vineyard 1
2016	Pontotoc	Vineyard 1
2016	Lincoln	Vineyard 1
2016	Lincoln	Vineyard 2
2016	Garvin	Vineyard 1
2016	Major	Vineyard 1
2016	Creek	Vineyard 1
2016	Cleveland	Vineyard 1
2016	Cleveland	Vineyard 2
2016	Okmulgee	Vineyard 1
2016	Logan	Vineyard 1
2016	Oklahoma	Vineyard 1
2016	Caddo	Vineyard 1
2017*	*Creek	Vineyard 1
2017	Osage	Vineyard 1
2017	Cherokee	Vineyard 1
2017	Lincoln	Vineyard 2
2017	Lincoln	Vineyard 3
2017	Muskogee	Vineyard 1
2017	Atoka	Vineyard 1
2017	Dewey	Vineyard 1
2017	Blaine	Vineyard 1
2017	Mayes	Vineyard 1
2017	Craig	Vineyard 1
2017	Delaware	Vineyard 1
2017	Oklahoma	Vineyard 1
2017	Oklahoma	Vineyard 2
2017	Oklahoma	Vineyard 3
2017	McCurtain	Vineyard 1
2017	Pushmataha	Vineyard 1

*If pathogen was detected in 2016, a different vineyard was selected the next year.

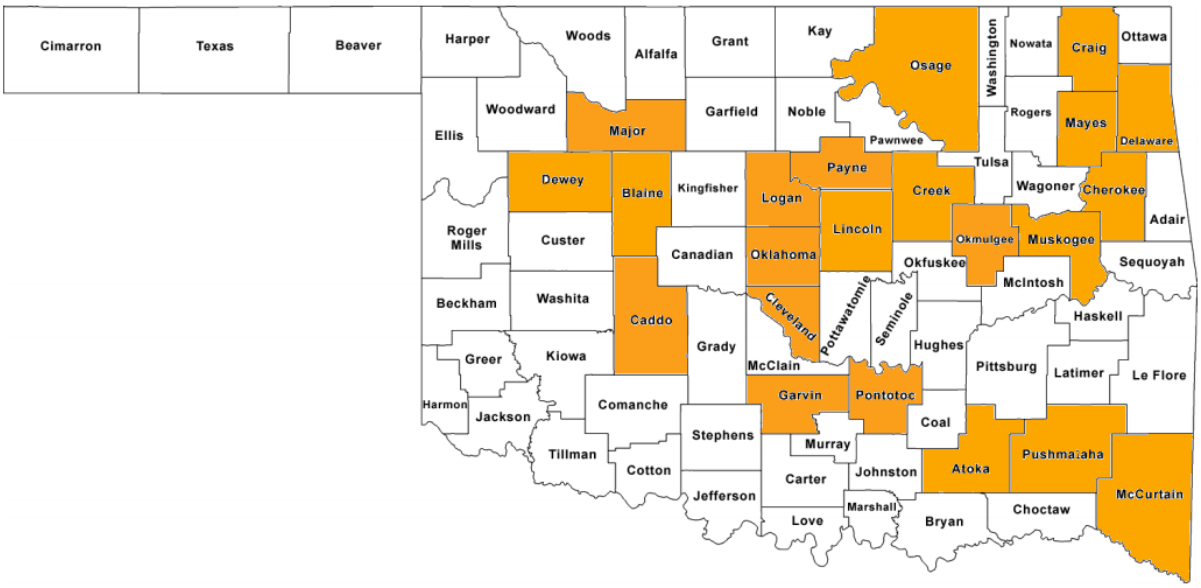


Figure 1. Counties with vineyards included in the 2016-17 Cooperative Agricultural Pest Survey for red leaf diseases

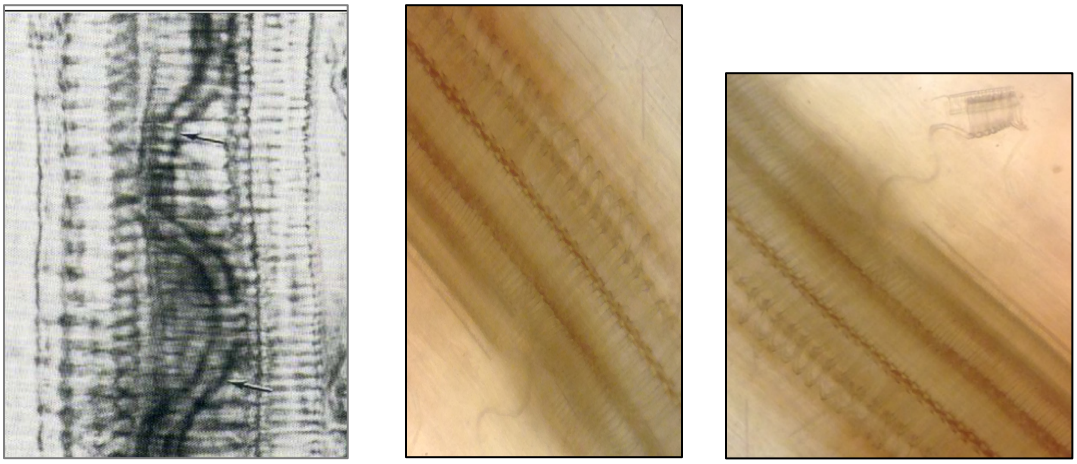


Figure 2. Infected Rotbrenner xylem from Costanzo et al 2013; uninfected xylem from 2017 samples: G35 and G74

CHAPTER IV

RESULTS AND DISCUSSION

Oklahoma Department of Agriculture, Food and Forestry (ODAFF) and the Plant Disease and Insect Diagnostic Lab (PDIDL) initiated a Cooperative Agricultural Pest Survey (CAPS) to determine the cause of red leaf diseases and increased mortality of grapes in the state. This 2-year survey (2016-2017) provided information about the location of specific grape diseases in Oklahoma vineyards; 22 counties and 26 vineyards were tested across the state as shown in Figure 1. Summary of detections of the surveyed pathogens are shown in Table 4 and Table 5. (See appendix for complete data set).

This survey included testing for exotic pathogens not currently in the US: '*Candidatus* phytoplasma vitis', '*Candidatus* phytoplasma australiense' and *Pseudopezicula tracheiphila* (rotbrenner). '*Candidatus* phytoplasma spp' were not detected in 2016 or 2017 and rotbrenner was not detected in 2017. However, two new diseases, Grapevine Red Blotch and Grapevine Leafroll, caused by viruses were detected in Oklahoma. *Xylella fastidiosa* was confirmed in new counties as an ongoing problem in grape production.

Detection of Grapevine Red Blotch Virus

Grapevine Red Blotch Virus was the most prevalent virus in 2016, where 38% of samples (n=121) were positive (Table 4). In 2017, 34% (n=96) were positive as shown in Table 5. Although GRBV was found in 2014, this is the first official report of Red Blotch in the state of Oklahoma. It is notable that GRBV was more common in the drier parts of the state since

Oklahoma has an increasing rainfall gradient from west to east as shown in Figures 3 and 4. Weather patterns may affect insect vectors or the climate may dictate the cultivars of grapes chosen for planting. Growers in Oklahoma face many environmental challenges to grow grapes including hot, dry summers, early spring frosts, and variable temperatures through the winter including extreme cold temperatures. *Vitis vinifera* varieties, that lack cold tolerance are not recommended to be planted north of Interstate-40. Growers continue to choose these cultivars based on the cultivars name and reputation and increased value (Stafne 2007). *Vitis vinifera* varieties are more common south of Interstate-40. Since the disease is found above and below Interstate-40, as shown in Figure 3, data suggest that cultivar may be unimportant as shown in Table 6.

The positive samples of GRBV varied across the state and within cultivars. With small sample sizes within the cultivars, there were 2 cultivars with 100% confirmed: American 3/3 and Cabernet Franc 2/2. Sixty-seven percent (12/18) were confirmed for Cynthiana (Norton), 58% (7/12) for Cabernet Sauvignon, 50% (1/2) detected in Chardonnay and Vertole, 35% (5/14) for Merlot, and 25% (1/4) for Chambourcin. It should be noted that GRBV was not detected in Reisling 0/7, Noble 0/3, Carlos 0/3, Plymouth 0/3, Val John 0/2 or Enchantment Red 0/2. Since the sample sizes are small, additional sampling may be needed to determine relevancy. However, these data indicate that both European and American hybrids grown in Oklahoma are susceptible to Grapevine Red Blotch disease.

As of 2016, the only known insect vector is the three-cornered alfalfa treehopper *Spissistilus festinus* Say (Hemiptera: Membracidae) (Bahder 2016) which is a common insect in Oklahoma, but has not been considered a grapevine pest. It is unknown if the distribution of GRBV is consistent with the distribution of this insect in Oklahoma. Other unknown vectors may

be present causing the skewed distribution. Perhaps rainfall or other crop management may be affecting the movement of the three-cornered alfalfa treehopper into the vineyard. With alfalfa fields scattered throughout Oklahoma with 2-3 cuttings a year, when alfalfa is harvested, potentially the three-cornered alfalfa treehopper could move to vineyards. Timing of alfalfa cuttings, location in proximity to vineyards, and long distant movement could all be assessed to see if this insect is the insect vector spreading GRBV in Oklahoma. Sampling for insects at vineyards with GRBV is suggested to learn more about potential spread.

With the continual growth of the grape industry, Oklahoma growers need a clean source for plant material. Many growers share plant material or purchase from nurseries where grapes are not tested for pathogens. Movement of plant material is the likely source of long-distance movement across Oklahoma. Extension educators have noted the lack of available certified virus-free plant material and the lack of knowledge surrounding contamination issues with shared plant material. Since the GRBV was detected in the national germplasm in California, this virus may have been latent and propagated for years, mimicking other viruses like Grapevine Leafroll disease (Al Rwahnih et al 2016). Grapes are often found to harbor multiple diseases at a time, which is confirmed with our findings in Table 6.

Management of grapevine pathogens is intensive and laborious. Although OSU has offered the Grape Management class for 10+ years, there is still a lack of understanding of preventative maintenance and Integrative Pest Management. In this survey, there were 3 vineyards with no detection of surveyed pathogens both years, another 11 vineyards where Grapevine Red Blotch was not detected. Management differs across the state from hobby growers to commercial growers, and within these groups, there is a wide spectrum of management. A schedule for the grape growers to set out sticky traps, scouting for insect pests, and insecticide recommendations are available in a factsheet (EPP-1091). A year of monthly maintenance, incorporating the work of Rebek and Overall (2017) on vineyard insects combined

with recommended fungicide spray applications (like Texas A&M) for Oklahoma would be helpful as a current report (Kamas & Scheiner 2018). Some of this information is available in the Handbook of Oklahoma Vineyard Establishment and Management E-1015 and E-832, the Extension Agents Handbook, but it is not complete and there have not been recent updates for disease management.

Detection of Grapevine Leafroll-associated Virus

Grapevine Leafroll-associated viruses had not been detected in Oklahoma, prior to this survey. In the 2016-17 survey, Grapevine leafroll disease was found throughout the state, and the distribution is random (Figure 5). GLR 1 and 3 are most common in the US (Naidu 2014) and our results were the same as GLR 1 and 3 were detected in 13 counties. The combination of GLR 1 and 3 and 4+ (both) were detected in 5 counties and GLF 4+ alone was detected in one county. The random pattern is consistent with the primary transmission of this virus: infected plant material as shown in Figure 5. As growers have purchased or shared infected grapevines, it has led to the sporadic distribution of strains in the vineyards.

Modes of transmission for GLR 1 and 3 include mealybugs (Hemiptera: Pseudococcidae), soft scale *Coccus herperidium* Linnaeus (Hemiptera: Coccidae), and shared (infected) plant material as shown in Tables 1 and 2. In conversations with growers, these pests are minor and perhaps an occasional pest. More research is needed on what insect pests are found in Oklahoma vineyards to see if these insects could be vectors for GLRaVs. However, we know from extension and conversations with growers that growers in Oklahoma regularly share plant material and are inadvertently sharing plant diseases. Many diseases display symptoms in stressed plants or older plant material only, which is why grapevines are quarantined for 2 years for testing in order to bring into the country. An economic analysis starting with clean plant material

and proper management versus free material plus possible disease could demonstrate the economic impact.

In 2016, 15.7% of samples had GLR 1 & 3, and in 2017 that increased to 22.9% of the samples. For GLR 4+, detected in 2.5% of the samples in 2016, increased to 10.4% in 2017. Weather conditions or later sampling dates may have influenced symptom expression and led to the increase in detections in 2016 and 2017. In two of the vineyards, there was no disease detected in 2016, but they tested positive for one vine out of 6 tested for GLR 1 and 3 in 2017. Perhaps this is a new section of grapes or younger vines that are just now showing symptoms, however later sampling date may also play a role. The average sampling date in 2016 was August 21 and in 2017 the average sampling date was September 7.

Detection of *Xylella fastidiosa* (Pierce's disease)

Pierce's Disease was detected in 1 new county (Cleveland) in 2016 and the first time since 2010 in Oklahoma county. In 2017, *Xylella fastidiosa* was detected in 2 new counties, Atoka and McCurtain. The concern is that the pathogen continues to spread in Oklahoma vineyards as shown in Figure 6. Growers are recommended to scout for arthropod vectors that transmit *X. fastidiosa* in the OSU Factsheet – Insect Vectors of Pierce's Disease in Oklahoma Vineyards (EPP-1091). Until growers implement scouting and management practices, *X. fastidiosa* is likely to spread in Oklahoma vineyards. Pruning tools may also be a method of spreading *X. fastidiosa* in vineyards. It is recommended to clean pruners with a disinfectant (Lysol or dip in 10% Bleach solution) between plants. Existing vineyards with *X. fastidiosa* may want to install resistant cultivars such as Black Spanish or utilize new bio-controls being developed by Dr. Hopkins in Florida and California (Hopkins 2005). Management of *X. fastidiosa* vectors is likely to be similar management for Grapevine Red Blotch, so increased management for one may reduce spread of the other.

The long distance spread of Pierce's disease is the same as GRBV and GLRaVs with infected plant material. Education and economic analysis may show the sustainability of the industry rests on planting clean, healthy plant material and increased vector management to prevent diseases as shown in Clean Plant Factsheet fact sheet as shown in Figure 9 (appendix) (National Clean Plant Network 2017).

The grape industry in Texas is also increasing and there are confirmed *X. fastidiosa* vineyards and the insect vector the Glassy Winged Sharpshooter, *Homalodisca vitripeninis* Germar (Hemiptera: Cicadellidae) (Hail et al 2010). With annual temperatures rising, perhaps the insect vector has moved north as habitat becomes available (J. Olson, personal communication).

TABLES AND FIGURES

Table 4: Summary of detection of Grapevine Leafroll-associated Viruses, Red Blotch Virus, and Xylella fastidiosa from 2016 Cooperative Agricultural Pest Survey in Oklahoma

County	Vineyard *	GLRaV 1 & 3 **	GLRaV 4+	GRBV	*Xylella fastidiosa*
Caddo	Vineyard 1	0/6	0/6	2/6	0/6
Cleveland	Vineyard 1	4/8	0/8	8/8	0/8
Cleveland	Vineyard 2	0/8	0/8	2/8	2/8
Creek	Vineyard 1	0/8	0/8	0/8	0/8
Delaware	Vineyard 1	0/8	0/8	0/8	0/8
Garvin	Vineyard 1	4/8	0/8	7/8	0/8
Lincoln	Vineyard 1	0/8	0/8	0/8	0/8
Lincoln	Vineyard 2	0/8	0/8	0/8	0/8
Logan	Vineyard 1	2/11	1/11	8/11	0/11
Major	Vineyard 1	5/10	0/10	10/10	0/10
Muskogee	Vineyard 1	0/10	0/10	0/10	0/10
Oklahoma	Vineyard 1	1/5	2/5	0/5	1/5
Okmulgee	Vineyard 1	0/7	0/7	0/7	0/7
Payne	Vineyard 1	1/8	0/8	4/8	0/8
Pontotoc	Vineyard 1	0/8	0/8	5/8	0/8

*If pathogen was detected in 2016, a different vineyard was selected the next year.

** Total detections out of the number of samples collected for each pathogen.

Table 5: Summary of detection of Grapevine Leafroll-associated Viruses, Red Blotch Virus, and Xylella fastidiosa from 2017 Cooperative Agricultural Pest Survey in Oklahoma

County	Vineyard	GLRaV 1 & 3	GLRaV 4+	GRBV	*Xylella fastidiosa*
Atoka	Vineyard 1	3/6 **	0/6	4/6	2/6
Blaine	Vineyard 1	1/6	0/6	5/6	0/6
Cherokee	Vineyard 1	0/6	0/6	0/6	0/6
Craig	Vineyard 1	0/6	0/6	5/6	0/6
* Creek	Vineyard 1	0/6	0/6	0/6	0/6
*Delaware	Vineyard 1	0/6	0/6	0/6	0/6
Dewey	Vineyard 1	3/6	2/6	2/6	0/6
*Lincoln	Vineyard 2	0/6	0/6	0/6	0/6
Lincoln	Vineyard 3	0/6	0/6	4/6	0/6
Mayes	Vineyard 1	1/6	0/6	0/6	0/6
McCurtain	Vineyard 1	3/4	3/4	0/4	1/4
Muskogee	Vineyard 1	1/6	0/6	0/6	0/6
Oklahoma	Vineyard 1	2/5	0/5	0/5	0/5
Oklahoma	Vineyard 2	0/5	0/5	4/5	0/5
Oklahoma	Vineyard 3	4/6	0/6	4/6	0/6
Osage	Vineyard 1	0/6	1/6	4/6	0/6
Pushmataha	Vineyard 1	4/4	4/4	0/4	0/4

*None of the surveyed pathogens were detected in Delaware, Creek, and Lincoln (Vineyard 2) in 2016 and 2017.

** Total detections out of the number of samples collected for each pathogen.

Table 6: Cultivars of Grapes tested in Cooperative Agricultural Pest Survey and the pathogens detected per sample

Cultivar	GLR 1&3	GLR 4+	GRBV	*Xylella fastidiosa*
European				
Cabernet Franc 2/89	1/2	0/2	2/2	0/2
Cabernet Sauvignon 12/89	6/12	2/12	7/12	0/12
Chambourcin 4/89	0/4	0/4	1/4	0/4
Chardonay 2/89	0/2	0/2	1/2	0/2
Merlot 14/89	3/14	0/14	5/14	0/14
Reisling 7/89	1/7	0/7	0/7	0/7
American				
Noble 4/89	3/4	3/4	0/4	0/4
Carlos 3/89	3/3	3/3	0/3	1/3
American 3/89	0/3	0/3	3/3	0/3
Plymouth 3/89	0/3	0/3	0/3	0/3
Cynthiana/Norton 18/89	0/18	1/18	12/18	2/18
Val John 2/89	0/2	0/2	0/2	0/2
Vertole 2/89	2/2	0/2	1/2	0/2
Enchantment Red 2/89	0/2	0/2	0/2	0/2

Data is only shown for samples where the cultivars were known

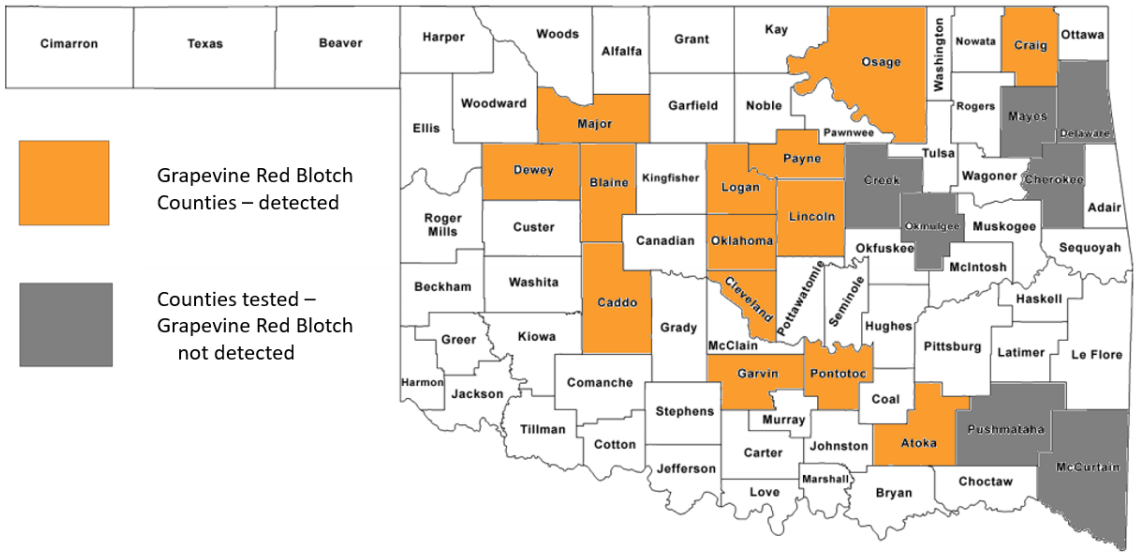


Figure 3. Distribution of Grapevine Red Blotch in Counties included in 2016-2017 CAPS. Darker color indicates counties tested but GRBV was not detected.

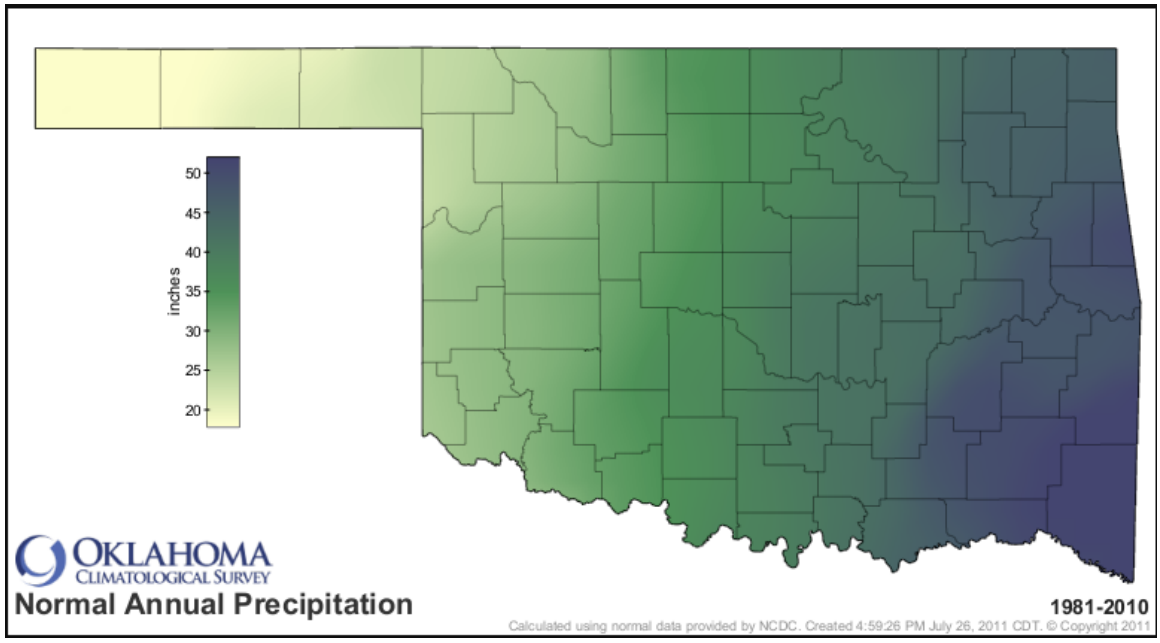


Figure 4. Normal Annual Precipitation for Oklahoma, Oklahoma Climatological Survey from http://climate.ok.gov/index.php/site/page/climate_of_oklahoma

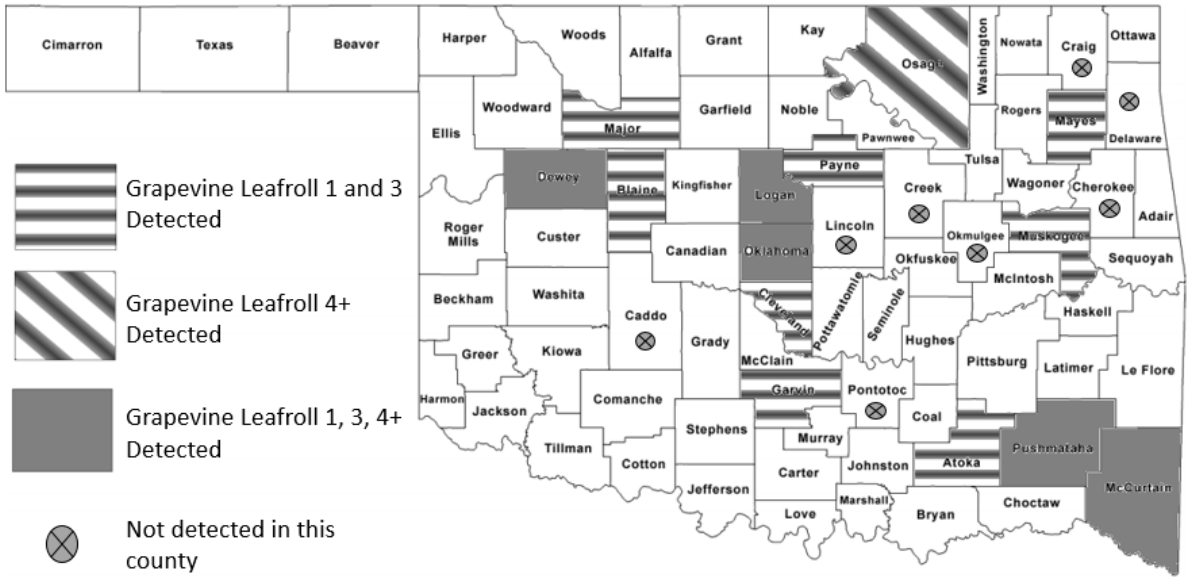


Figure 5. Distribution of Grapevine Leafoll Disease in Counties included in 2016-2017 CAPS

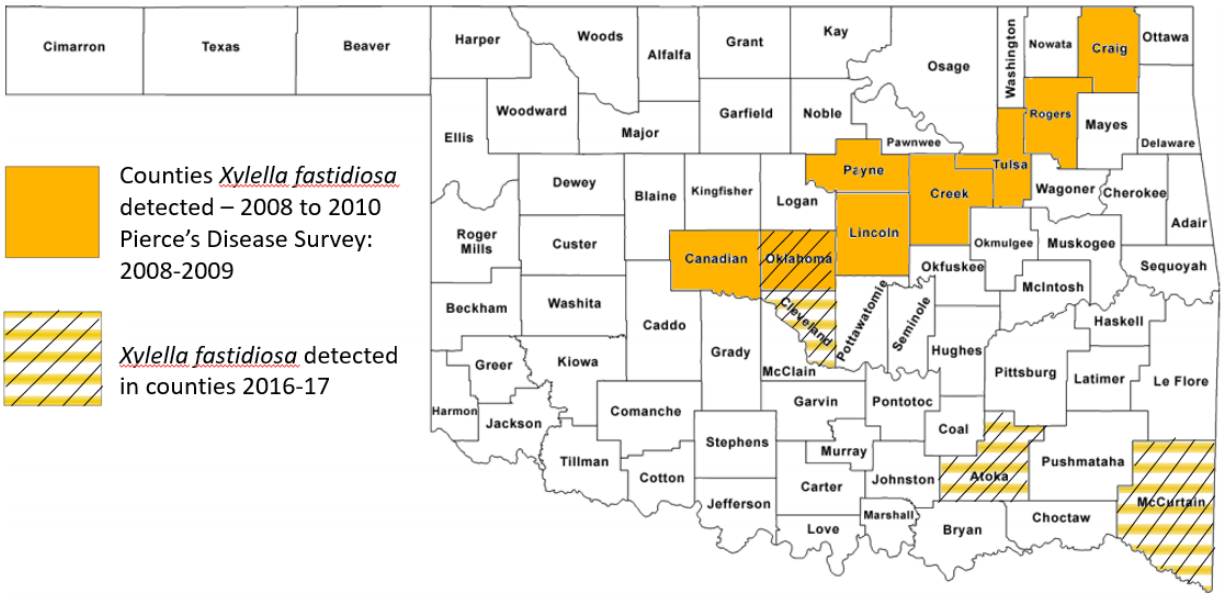


Figure 6. Distribution of Xylella fastidiosa (Pierce's disease) in Counties from 2008 to the 2016-2017 CAPS Survey

*Oklahoma county was detected in the first survey in 2008 and again in 2016

CHAPTER V

SUMMARY AND CONCLUSIONS

In this Cooperative Agricultural Pest Survey, two new viruses were detected that are detrimental to grapes: Grapevine Red Blotch Virus and Grapevine Leafroll-associated Viruses. The spread of these diseases is through shared plant material and insect vectors. However, it is also possible that the climate or ecology around the vineyards is changing (riparian buffers). Riparian buffers may be alternative host sites for both the insect pests, the viruses, and *Xylella fastidiosa*. The three-cornered alfalfa treehopper, is the only confirmed species that can spread GRBV, and is common in Oklahoma. Other unknown vectors may be present and this should be further investigated.

Overall (2013) found many sharpshooters in a survey made of Oklahoma vineyards. The sharpshooter species found include *Graphocephala versuta, G. coccinea, Oncometopia orbona,* and more. The insects are vascular feeders and are known to transmit *X. fastidiosa*. Since GRBV is also in the vascular tissue, it is possible that some of these insects may transmit both pathogens. It is critical to reduce weeds (habitat for the insects) and monitor for leafhoppers to prevent spread of *X. fastidiosa*. In this process, growers are also reducing potential vectors of GRBV and may prevent spread while researchers work to learn more about the disease. The importance

of arthropod management needs to be reiterated and continually taught through extension and the grape classes. A maintenance schedule could be created for Oklahoma vineyards to assist growers in decision making.

Pierce's Disease (*Xylella fastidiosa*) has moved into a new part of the state, previously detected across the center from SW to NE (Figure 6) from Canadian county to Craig County. However, in this survey, *X. fastidiosa* was detected in Oklahoma County, which was not detected since 2010, and in Cleveland, Atoka and McCurtain in the southeast region of the state. Rebek and Overall (2017) discussed insect management in the vineyard, but it is not clear yet if this in insect management or a shared plant material caused issue. A survey of insects in southern counties with vineyards closer to the Texas border, where X. fastidiosa has been detected, could determine if this new area is from a new insect vector or shared plant material. Economic analysis could determine the costs of clean material plus management compared to free plant material plus disease management.

Grapevine Red Blotch was more common in the drier counties of the state in this survey. Th findings may be related to insect vector biology and it would be worth investigating. Future studies that determine the presence of insects in vineyards that have GRBV are warranted.

We did not detect *Candidatus* phytoplasmas or rotbrenner in Oklahoma as shown in Table 7 (appendix) which confirms we do not have these two exotic pathogens causing red leaf diseases in Oklahoma. The diseases reported are present in the US, but both Red Blotch and leaf roll are new in Oklahoma. Although there are new challenges to grape production, growers should continue to pursue viticulture in Oklahoma.

REFERENCES

Ag Marketing Resource Center. 2015. *Wine: Wine Industry Profile*. Iowa State University: Ames, Iowa.

Agrios, G. N. 2005. Plant Pathology. 5th eds. *Department of Plant Pathology.* University of Florida. United States of America.

Almeida, R. P. P., Wistrom, C., Hill, B. L., Hashim, J., & Purcell, A. H. (2005). Vector transmission of *Xylella fastidiosa* to dormant grape. *Plant disease*, *89*(4), 419-424.

Al Rwahnih, M. A., Rowhani, A., Golino, D. A., Islas, C. M., Preece, J. E., and Sudarshana, M. R. 2015. Detection and genetic diversity of Grapevine red blotch-associated virus isolates in table grape accessions in the National Clonal Germplasm Repository in California. *Canadian Journal of Plant Pathology*, *37*(1), 130-135.

Al Rwahnih, M., Dolja, V. V., Daubert, S., Koonin, E. V., and Rowhani, A. 2012. Genomic and biological analysis of Grapevine leafroll-associated virus 7 reveals a possible new genus within the family Closteroviridae. *Virus research*, *163*(1), 302-309.

Angelini, E. 2010. Field Assessment and Diagnostic Methods for Detection of Grapevine Phytoplasmas. In S. Delrot et al. (eds.), *Methodologies and Results in Grapevine Research.* (pp. 5-6). New York, NY, USA:: Springer.

Annual Rainfall Map of Oklahoma 1981-2010. Oklahoma Climatological Survey. 2018. http://climate.ok.gov/images/site/avg_prec_1981-2010.png

Appel, D. Black, M. Labay, A. Lewis, J. Kamas, J. Morano, L. 2010. Pierce's Disease Overview and Management Guide. Texas AgriLife. Texas A&M: College Station, Texas. https://aggie-horticulture.tamu.edu/fruit-nut/files/2010/10/Texas-Grape-Growers-PD-Management-Guide.pdf

Bahder, B. W., Zalom, F. G., Jayanth, M., and Sudarshana, M. R. 2016. Phylogeny of Geminivirus Coat Protein Sequences and Digital PCR Aid in Identifying *Spissistilus festinus* as a Vector of Grapevine red blotch-associated virus. *Phytopathology*, *106*(10), 1223-1230.

Bahder, B. W., Zalom, F. G., and Sudarshana, M. R. 2016. An evaluation of the flora adjacent to wine grape vineyards for the presence of alternative host plants of Grapevine red blotch-associated virus. *Plant Disease*, *100*(8), 1571-1574.

Banks, D., Albibi, R., Chen, J., Lamikanra, O., Jarret, R. L., and Smith, B. J. 1999. Specific detection of *Xylella fastidiosa* Pierce's disease strains. *Current microbiology*, *39*(2), 85-88.

Beanland, L., Noble, R., and Wolf, T. K2006. Spatial and temporal distribution of North American grapevine yellows disease and of potential vectors of the causal phytoplasmas in Virginia. *Environmental Entomology*, *35*(2), 332-344.

Bextine, B. R., and Miller, T. A. 2004. Comparison of whole-tissue and xylem fluid collection techniques to detect *Xylella fastidiosa* in grapevine and oleander. *Plant disease*, *88*(6), 600-604.

Cabaleiro, C., and Segura, A. 1997. Field transmission of grapevine leafroll associated virus 3 (GLRaV-3) by the mealybug Planococcus citri. *Plant Disease*, *81*(3), 283-287.

Centre for Agriculture and Bioscience International. 2013. Factsheet: Grapevine flavescence doree phytoplasma. Wallingford, UK.

Calvi, B. L. 2011. *Effects of red-leaf disease on Cabernet Sauvignon at the Oakville experimental vineyard and mitigation by harvest delay and crop adjustment*. University of California: Davis, California.

CAPS Survey Manuals. 2017. *Grape Survey Reference – 2017*. https://caps.ceris.purdue.edu/survey-manuals?list=grapeandyear=2017

Cieniewicz, E. J., Pethybridge, S. J., Loeb, G., Perry, K., and Fuchs, M. 2017. Insights Into the Ecology of Grapevine red blotch virus in a Diseased Vineyard. *Phytopathology*, *108*(1), 94-102.

Clair, D., Larrue, J., Aubert, G., Gillet, J., Cloquemin, G., and Boudon-Padieu, E. 2003. A multiplex nested-PCR assay for sensitive and simultaneous detection and direct identification of phytoplasma in the Elm yellows group and Stolbur group and its use in survey of grapevine yellows in France. *Vitis*, *42*(3), 151-157.

Costanzo, T. and Sullivan, M. 2013. CPHST Pest Datasheet for *Pseudopezicula tracheiphila*. USDA-APHIS-PPQ-CPHST.

Cornara, D., Sicard, A., Zeilinger, A. R., Porcelli, F., Purcell, A. H., and Almeida, R. P. P. 2016. Transmission of *Xylella fastidiosa* to Grapevine by the Meadow Spittlebug. *Phytopathology*, *106*(11), 1285-1290.

Davis, R. E., Dally, E. L., Gundersen, D. E., Lee, I. M., and Habili, N. 1997. "Candidatus Phytoplasma australiense," a new phytoplasma taxon associated with Australian grapevine yellows. *International Journal of Systematic and Evolutionary Microbiology*, *47*(2), 262-269.

Davis, R. E., Dally, E. L., Zhao, Y., Lee, I. M., Wei, W., Wolf, T. K., ... and Walter-Peterson, H. 2015. Unraveling the Etiology of North American Grapevine Yellows (NAGY): Novel NAGY Phytoplasma Sequevars Related to 'Candidatus Phytoplasma pruni'. *Plant Disease*, *99*(8), 1087-1097.

Davis, R. E., Jomantiene, R., Dally, E. L., and Wolf, T. K. 1998. Phytoplasmas associated with grapevine yellows in Virginia belong to group 16SrI, subgroup A (tomato big bud phytoplasma subgroup), and group 16SrIII, new subgroup I. *Vitis*, *37*(3), 131-137.

Delrot, S., Medrano, H., Or, E., Bavaresco, L., and Grando, S. (Eds.). 2010. *Methodologies and results in grapevine research* (pp. 5-6). New York, NY, USA: Springer.

Deng, S., & Hiruki, C. (1991). Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. *Journal of microbiological methods*, *14*(1), 53-61.

Donda, B. P. 2016. *Molecular biology and epidemiology of grapevine leafroll-associated viruses* (Doctoral Dissertation). Retrieved from research.libraries.wsu.edu.

Duncan, Cindy. 2018. Personal Communication.

European and Mediterranean Plant Protection Organization. 2006. Factsheet: Phytoplasma australiense. Wallingford, UK.

European and Mediterranean Plant Protection Organization. 2002. Factsheet: Phytoplasma vitis. Wallingford, UK.

Extension Agent Handbook. 2018. Oklahoma Cooperative Extension Service. Oklahoma State University: Stillwater, Oklahoma.

Fan, X., Hong, N., Dong, Y., Ma, Y., Zhang, Z. P., Ren, F., … and Wang, G. 2015. Genetic diversity and recombination analysis of grapevine leafroll-associated virus 1 from China. *Archives of virology*, *160*(7), 1669.

Firrao, G., Andersen, M., Bertaccini, A., Boudon, E., Bove, J. M., Daire, X., ... & Gundersen-Rindal, D. E. 2004. Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *International Journal of Systematic and Evolutionary Microbiology*, *54*(4), 1243-1255.

Frank, Rimerman and Co. LLP. 2010. *The Economic Impact of Oklahoma Wine, Grape Juice and Vineyards – 2010*. St. Helena, California.

Gardi, C., Koufakis, I., Tramontini, S., Andueza, M., Pautasso, M., Stancanelli, G., … and Bragard, C. 2016. Update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. *EFSA Journal*, *14*(2), 1-40pp.

Gibb, K. S., Constable, F. E., Moran, J. R., and Padovan, A. C. 1999. Phytoplasmas in Australian grapevines-detection, differentiation and associated diseases. *Vitis*, *38*(3), 107-114.

Goldsmith, Earl. 2015. Prohibition Repealed in Oklahoma. *The Henryettan*. https://thehenryettan.com/index.php?option=com_contentandview=articleandid=979:prohibition-repealed-in-oklahomaandcatid=13andItemid=128andlang=en

Hail, D., Mitchell, F., Lauzière, I., Marshall, P., Brady, J., & Bextine, B. 2010. Detection and analysis of the bacterium, Xylella fastidiosa, in glassy-winged sharpshooter, Homalodisca vitripennis, populations in Texas. *Journal of Insect Science*, *10*(1).

Herrick, Christina. 2017. "Understanding the Economics of Red Blotch of Grapes." www.growingproduce.com. Posted March 29,2017. Accessed June 25, 2017.

Hill, B. L., & Purcell, A. H. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, Graphocephala atropunctata. *Phytopathology*, *85*(2), 209-212.

Holz, B. 2000. Integrated control of Rotbrenner (Pseudopezicula tracheiphila (Mull.-Thurg.) Korf & Zhuang (Discomycetes Helothiales) in the wine growing region Moselle-Saar-Ruwer. *IOBC WPRS BULLETIN*, *23*(4), 59-62.

Hopkins, D. L. 2005. Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. *Plant Disease*, *89*(12), 1348-1352.

Hren, M., Boben, J., Rotter, A., Kralj, P., Gruden, K., and Ravnikar, M. 2007. Real-time PCR detection systems for Flavescence dorée and Bois noir phytoplasmas in grapevine: comparison with conventional PCR detection and application in diagnostics. *Plant Pathology*, *56*(5), 785-796.

Janse, J. D., and Obradovic, A. 2010. *Xylella fastidiosa*: its biology, diagnosis, control and risks. *Journal of Plant Pathology*, S35-S48.

Kamas, J and Scheiner, J. 2018. *2018 Grape Pest Management Guide*. Texas A&M AgriLife Extension. Texas A&M: College Station, Texas.

Kamas, J., Stein, L., & Nesbitt, M. 2010. Pierce's Disease-Tolerant Grapes. *Texas Fruit and Nut Production.* Texas AgriLife Extension. Texas A&M University: College Station, Texas*. http://aggiehorticulture. tamu. edu/fruit-nut/files/2010/10/pd-grapes. pdf*.

Kogovšek, P., Hodgetts, J., Hall, J., Prezelj, N., Nikolić, P., Mehle, N., ... & Dermastia, M. (2015). LAMP assay and rapid sample preparation method for on-site detection of flavescence dorée phytoplasma in grapevine. *Plant pathology*, *64*(2), 286-296.

König, H., Unden, G., & Fröhlich, J. (Eds.). 2009. *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 3-30). Heidelberg: Springer.

Korf, R.P. and Zhang, W.Y. "4.3.3 *Pseudopezicula tracheiphila* (Mull.-Thurg) Helotiales*:* Rotbrenner" in *Biology of Microoganisms on Grapes, in Must and in Wine:* editied by Konig, H., Unden, G., and Frohlich, J. 2009. pp117-118.

Korf, R. P., Pearson, R. C., Zhuang, W. Y., and Dubos, B. 1986. Pseudopezicula (Helotiales, Peziculoideae), a new discomycete genus for pathogens causing an angular leaf scorch disease of grapes (" Rotbrenner"). *Mycotaxon (USA)*.

Krenz, B., Thompson, J. R., McLane, H. L., Fuchs, M., and Perry, K. L. 2014. Grapevine red blotch-associated virus is widespread in the United States. *Phytopathology*, *104*(11), 1232-1240

Kumar, S., Rai, R., and Baranwal, V. K. 2015. Development of an immunocapture–reverse transcription–polymerase chain reaction (IC-RT-PCR) using modified viral RNA release protocol for the detection of Grapevine leafroll-associated virus 3 (GLRaV-3). *Phytoparasitica*, *43*(3), 311-316.

Kurtural, S.K. 2003. A Brief History of the Grape and Its Uses [Power Point Slides]. University of Kentucky: Lexington, Kentucky. Retrieved from https://www.uky.edu/Ag/CCD/historyanduses.pdf

Lee, M., Martini, M., Marcone, C., & Zhu, S. F. 2004. Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi'for the phytoplasma associated with elm yellows. *International Journal of Systematic and Evolutionary Microbiology*, *54*(2), 337-347.

Liefting, L. W., Veerakone, S., & Clover, G. R. 2011. New hosts of "Candidatus Phytoplasma australiense" in New Zealand. *Australasian Plant Pathology*, *40*(3), 238-245.

MacDonald, S. L., Staid, M., Staid, M., and Cooper, M. L. 2016. Remote hyperspectral imaging of grapevine leafroll-associated virus 3 in cabernet sauvignon vineyards. *Computers and Electronics in Agriculture*, *130*, 109-117.

Mahfoudhi, N., Digiaro, M., and Dhouibi, M. H. 2009. Transmission of grapevine leafroll viruses by Planococcus ficus (Hemiptera: Pseudococcidae) and Ceroplastes rusci (Hemiptera: Coccidae). *Plant Disease*, *93*(10), 999-1002.

Martelli, G. P. 2009. Grapevine virology highlights 2006–2009. In *Proceedings of 16th meeting of the international council for the study of virus and virus-like diseases of the grapevine. Dijon, France* (pp. 15-23).

Martelli, G. P. 2014. Directory of virus and virus-like diseases of the grapevine and their agents. *Journal of Plant Pathology*, *96*(1sup), 1-136.

Martelli, G. P., Ghanem-Sabanadzovic, N. A., Agranovsky, A. A., Al Rwahnih, M., Dolja, V. V., Dovas, C. I., … and Katis, N. I. 2012. Taxonomic revision of the family *Closteroviridae* with special reference to the grapevine leafroll-associated members of the genus Ampelovirus and the putative species unassigned to the family. *Journal of Plant Pathology*, *94*(1), 7-19.

MFK Research. 2010. *The Impact of Wine, Grapes and Grape Products on the American Economy 2007: Family Businesses Building Value.* MFK Research LLC, St.Helena, CA. Accessed on June 30, 2017 at http://www.wineinstitute.org/files/mfk_us_econ_report07.pdf

Minsavage, G. V., Thompson, C. M., Hopkins, D. L., Leite, R. M. V. B. C., and Stall, R. E. 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. *Phytopathology*, *84*(5), 456-461.

Mirchenari, S. M., Massah, A., and Zirak, L. 2015. 'Bois noir': new phytoplasma disease of grapevine in Iran. *Journal of Plant Protection Research*, *55*(1), 88-93.

Naidu, R., Rowhani, A., Fuchs, M., Golino, D., and Martelli, G. P. 2014. Grapevine leafroll: A complex viral disease affecting a high-value fruit crop. *Plant Disease*, *98*(9), 1172-1185.

National Agricultural Statistics Service. *Fruits, Tree Nuts, and Berries: Non-citrus Fruits and Nuts 2015 Summary*. USDA: Washington, DC.

National Agricultural Statistics Service. *2012 Census of Agriculture Highlights: Fruits, Tree Nuts, and Berries.* ACH12-30. USDA: Washington, DC.

National Agricultural Statistics Service. *2012 Census of Agriculture*. State Data. Table 6. Income from Farm-Related Sources: 2012 and 2007. USDA: Washington, DC.

National Clean Plant Network. 2017. Factsheet: Grapevine Red Blotch Disease.

Nome, S. F., Raju, B. C., Goheen, A. C., Nyland, G., and Docampo, D. 1980. Enzyme-linked immunosorbent assay for Pierce's disease bacteria in plant tissues. *Phytopathology*, *70*(8), 746-749.

Oklahoma Agritourism. Oklahoma Agritourism. 2017. Oklahoma Department of Agriculture, Food and Forestry Agritourism program. http://www.oklahomaagritourism.com/trails/wine-trails

Oklahoma Climatological Survey. 2018. Board of Regents of the University of Oklahoma. http://climate.ok.gov/index.php/site/page/climate_of_oklahoma. Accessed on July 6, 2018.

Oklahoma Farm Report. 2017. *Agricultural News: Wine Made of Oklahoma-Grown Grapes Remains Competitive International Stage with Big Wins* (20 Jan 2017).

Oklahoma Farm Report. 2012. *Agricultural News: State Department of Agriculture Grant Proves Grape Industry Impact* (28 Aug 2012).

Oklahoma History Society. 2009. *The Encyclopedia of Oklahoma History and Culture*: *Grape and Wine Industry.* http://www.okhistory.org/publications/enc/entry.php?entry=GR037

Olmos, A., Bertolini, E., Ruiz-García, A. B., Martínez, C., Peiró, R., & Vidal, E. 2016. Modeling the accuracy of three detection methods of Grapevine leafroll-associated virus 3 during the dormant period using a Bayesian approach. *Phytopathology*, *106*(5), 510-518.

Olson, Jen. 2018. Personal Interview.

Osman, F., Leutenegger, C., Golino, D., and Rowhani, A. 2007. Real-time RT-PCR (TaqMan®) assays for the detection of Grapevine Leafroll associated viruses 1–5 and 9. *Journal of virological methods*, *141*(1), 22-29.

Overall, Lisa. 2013. Thesis Dissertation: Incidence of *Xylella fastidiosa* in Oklahoma, a survey of potential insect vectors, and identification of potential plant reservoir hosts. Oklahoma State University. https://search.proquest.com/pqdtglobal/docview/1428424339/426F751157BD4A5CPQ/1?accountid=4117

Overall, L. M., and Rebek, E. J. 2015. Seasonal abundance and natural inoculativity of insect vectors of *Xylella fastidiosa* in Oklahoma tree nurseries and vineyards. *Journal of economic entomology*, *108*(6), 2536-2545.

Pearson, R. C., Siegfried, W., Bodmer, M., and Schüepp, H. 1991. Ascospore discharge and survival in Pseudopezicula tracheiphila, causal agent of Rotbrenner of grape. *Journal of Phytopathology*, *132*(3), 177-185.

Pearson, R. C., and Smith, F. D. 1988. Angular leaf scorch, a new disease of grapevines in North America caused by Pseudopezicula tetraspora. *Plant disease*, *72*(9), 796-800.

Pelletier, C., Salar, P., Gillet, J., Cloquemin, G., Very, P., Foissac, X., and Malembic-Maher, S. 2009. Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas of the 16SrV and 16SrXII-A groups with an endogenous analytical control. *Vitis*, *48*(2), 87-95.

Perry, K. L., McLane, H., Hyder, M. Z., Dangl, G. S., Thompson, J. R., and Fuchs, M. F. 2016. Grapevine red blotch-associated virus is Present in Free-Living Vitis spp. Proximal to Cultivated Grapevines. *Phytopathology*, *106*(6), 663-670.

Pinney, T. 2007. A History of Wine in America, Volume 1: From Beginnings to Prohibiotion volume 1,2 edn: University of California Press. Accessed through the Boss 10/28/17.

Plant Health Australia. 2009. Pest Risk Review: Rotbrenner. Viticulture Industry Biosecurity Plan.

Poojari, S., Alabi, O. J., Fofanov, V. Y., & Naidu, R. A. (2013). A leafhopper-transmissible DNA virus with novel evolutionary lineage in the family geminiviridae implicated in grapevine redleaf disease by next-generation sequencing. *PloS one*, *8*(6), e64194.

Purcell, A. H. 1997. *Xylella fastidiosa*, a regional problem or global threat? *Journal of Plant Pathology*, 99-105.

Rebek, E.J. and Overall, L.M. 2017. Factsheet: Insect Vectors of Pierce's Disease in Oklahoma Vineyards. Oklahoma Cooperative Extension Service. Oklahoma State University: Stillwater, OK.

Rodrigues, J. L., Silva-Stenico, M. E., Gomes, J. E., Lopes, J. R. S., and Tsai, S. M. 2003. Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and gyrB sequences. *Applied and Environmental Microbiology*, *69*(7), 4249-4255.

Schaad, N. W., Opgenorth, D., & Gaush, P. 2002. Real-time polymerase chain reaction for one-hour on-site diagnosis of Pierce's disease of grape in early season asymptomatic vines. *Phytopathology*, *92*(7), 721-728.

Schaad, N. W., Postnikova, E., Lacy, G., Fatmi, M. B., & Chang, C. J. 2004. *Xylella fastidiosa* subspecies: X. fastidiosa subsp piercei, subsp. nov., X. fastidiosa subsp. multiplex subsp. nov., and X. fastidiosa subsp. pauca subsp. nov. *Systematic and applied microbiology*, *27*(3), 290-300.

Sharma, A. M., Baraff, B., Hutchins, J. T., Wong, M. K., Blaisdell, G. K., Cooper, M. L., … and Almeida, R. P. 2015. Relative prevalence of grapevine leafroll-associated virus species in wine grape-growing regions of California. *PloS one*, *10*(11), e0142120.

Smith, D. L., Dominiak-Olson, J., and Sharber, C. D. 2009. First report of Pierce's disease of grape caused by *Xylella fastidiosa* in Oklahoma. *Plant Disease*, *93*(7), 762-762.

Stafford, Jim. Q and A with Abby Cash. *NewsOk.com.* 27 September 2007.

Stafne, E. T. 2006. 'Rubaiyat' and Oklahoma's wine grape legacy. *Journal of American Pomological Society*.

Stafne, E.T. 2007. "Profile and Challenges of the Emerging Oklahoma Grape Industry," Oklahoma Cooperative Extension Service Publication E-999 (2007) E-999.

Stafne, E.T. 2010. "Past and Present Industry Profile," in *Handbook of Oklahoma Vineyard Establishment and Management*, ed. Eric T. Stafne. Oklahoma Cooperative Extension Service: Stillwater, Oklahoma.

Stafne, E. Ed. 2010. E-1015: Handbook of Oklahoma Vineyard Establishment and Management. Oklahoma Cooperative Extension Service. Oklahoma State University: Stillwater, Oklahoma.

Stafne, E.T. 2013. *Overview of the Oklahoma Grape and Wine Industry*. Oklahoma Cooperative Extension Articles: Oklahoma State University, Stillwater, Oklahoma.

Suarez, S. and Cevallos, F. 2017. Presesentation Phytobacteriology Class. Pierce's Disease: Examination of a High-Impact Bacteria & Vector.

Sudarshana, M. R., Perry, K. L., and Fuchs, M. F. 2015. Grapevine red blotch-associated virus, an emerging threat to the grapevine industry. *Phytopathology*, *105*(7), 1026-1032.

Sudarshana, M., and Fuchs, M. 2015. Grapevine red blotch. *Compendium of Grape Diseases, Disorders, and Pests. W. Wilcox, WD Gubler, and JK Uyemoto, eds. The American Phytopathological Society, St. Paul, MN*, 122-123.

Von Broembsen, S.L. and Olson, B. 2005. Poster: Surveys of Oklahoma Grapevines for Xylella fastidiosa.

Tew, C., and Barbieri, C. 2012. The perceived benefits of agritourism: The provider's perspective. *Tourism Management*, *33*(1), 215-224.

Website, USDA APHIS CAPS. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/pest-detection

Website. "Wine Trails." http://oklahomaagritourism.com/trails/wine-trails

Weintraub, P. G., & Beanland, L. (2006). Insect vectors of phytoplasmas. *Annu. Rev. Entomol.*, *51*, 91-111.

Widener, J. M. 2010. A political quagmire within the Oklahoma wine industry. *Oklahoma Politics*, *20*, 113-126.

Widener, J. 2010. The sooner state has winescapes: The development and culture of winemaking in Oklahoma. *Material Culture*, 50-74.

Wilkerson, April. 2009. *Growing an industry: Legislation may boost Agritourism in Oklahoma*. Journal Record. Oklahoma City, Oklahoma 20 October 2009.

The Wine Institute. 2015. Table 4.1: *World Wine Consumption by Country.* San Francisco, California: The Wine Institute. http://www.wineinstitute.org/files/World_Wine_Consumption_by_Country_2015.pdf

The Wine Institute. 2015. Table 1: *World Wine Production by Country.* San Francisco, California: The Wine Institute. http://www.wineinstitute.org/files/World_Wine_Production_by_Country_2015.pdf

The Wine Institute. 2017. *Number of California Wineries*. San Francisco, California: The Wine Institute. Available at: https://www.wineinstitute.org/resources/statistics/article124

Yepes, L. M., Cieniewicz, E., Krenz, B., McLane, H., Thompson, J. R., Perry, K. L., & Fuchs, M. 2018. Causative Role of Grapevine Red Blotch Virus in Red Blotch Disease. *Phytopathology*, PHYTO-12.

APPENDIX A. Complete data for 2016–17 CAPS

Year	County	Vineyard	Cultivar	GLRaV 1+	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	Rotbrenner
16	Caddo	1	Unknown	not detected	not detected	not detected	not detected	not detected	Not tested in 2016
16	Caddo	1	Unknown	not detected	not detected	not detected	not detected	Not detected	
16	Caddo	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Caddo	1	Unknown	not detected	not detected	not detected	not detected	Not detected	
16	Caddo	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Caddo	1	Unknown	not detected	not detected	Confirmed	not detected	Not detected	
16	Cleveland	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Cleveland	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Cleveland	2	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Cleveland	2	Unknown	not detected	not detected	Confirmed	Confirmed	not detected	
16	Cleveland	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Cleveland	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Cleveland	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Cleveland	2	Unknown	not detected	not detected	not detected	Confirmed	not detected	
16	Cleveland	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Cleveland	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	

Year	County	Vineyard	Cultivar	GLRaV 1+	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	Not tested in 2016
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Delaware	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Garvin	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Garvin	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Garvin	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Garvin	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Garvin	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Garvin	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Garvin	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Garvin	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Lincoln	2	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Logan	1	Unknown	Confirmed	not detected	not detected	not detected	not detected	
16	Logan	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Logan	1	Unknown	not detected	Confirmed	Confirmed	not detected	not detected	

Year	County	Vineyard	Cultivar	GLRaV 1+	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	
16	Logan	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Logan	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Logan	1	Unknown	not detected	not detected	not detected	not detected		Not tested in 2016
16	Logan	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Logan	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Logan	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Logan	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Logan	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Major	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Muskogee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Oklahoma	1	Unknown	not detected	Confirmed	not detected	not detected	not detected	not detected
16	Oklahoma	1	Unknown	not detected	not detected	not detected	Confirmed	not detected	not detected
16	Oklahoma	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Oklahoma	1	Unknown	Confirmed	Confirmed	not detected	not detected	not detected	not detected
16	Oklahoma	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected

Year	County	Vineyard	Cultivar	GLRaV 1 & 3	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	Rotbrenner
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Okmulgee	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Payne	1	Unknown	Confirmed	not detected	Confirmed	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Payne	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	Confirmed	not detected	not detected	not detected
16	Pontotoc	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Atoka	1	Cynthiana	not detected	not detected	Confirmed	Confirmed	not detected	not detected
17	Atoka	1	Cynthiana	not detected	not detected	not detected	not detected	not detected	not detected
17	Atoka	1	Cynthiana	not detected	not detected	Confirmed	Confirmed	not detected	not detected
17	Atoka	1	Merlot	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Atoka	1	Vertole	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Atoka	1	Vertole	Confirmed	not detected	not detected	not detected	not detected	not detected
17	Blaine	1	Cab Sav	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Blaine	1	Cab Sav	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Blaine	1	Tempranillo	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Blaine	1	Cab Franc	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Blaine	1	Merlot	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Blaine	1	Merlot	not detected	not detected	not detected	not detected	not detected	not detected

Year	County	Vineyard	Cultivar	GLRaV 1 & 3	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	Rotbrenner
17	Cherokee	1	Cynthiana	not detected	not detected	not detected	not detected	not detected	not detected
17	Cherokee	1	Cynthiana	not detected	not detected	not detected	not detected	not detected	not detected
17	Cherokee	1	Cynthiana	not detected	not detected	not detected	not detected	not detected	not detected
17	Cherokee	1	Old Oklahoma	not detected	not detected	not detected	not detected	not detected	not detected
17	Cherokee	1	Sunbelt	not detected	not detected	not detected	not detected	not detected	not detected
17	Cherokee	1	Cynthiana	not detected	not detected	not detected	not detected	not detected	not detected
17	Craig	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Craig	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Craig	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Craig	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Craig	1	Cynthiana	not detected	not detected	not detected	not detected	not detected	not detected
17	Craig	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
Repeat	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Creek	1	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
Repeat	Delaware	1	Riesling	not detected	not detected	not detected	not detected	not detected	not detected
17	Delaware	1	Riesling	not detected	not detected	not detected	not detected	not detected	not detected
17	Delaware	1	Riesling	not detected	not detected	not detected	not detected	not detected	not detected
17	Delaware	1	Riesling	not detected	not detected	not detected	not detected	not detected	not detected
17	Delaware	1	Riesling	not detected	not detected	not detected	not detected	not detected	not detected
17	Delaware	1	Riesling	not detected	not detected	not detected	not detected	not detected	not detected
17	Dewey	1	Cab Sav	Confirmed	Confirmed	Confirmed	not detected	not detected	not detected
17	Dewey	1	Merlot	Confirmed	not detected	not detected	not detected	not detected	not detected
17	Dewey	1	Merlot	not detected	not detected	not detected	not detected	not detected	not detected
17	Dewey	1	Cab Sav	Confirmed	Confirmed	not detected	not detected	not detected	not detected
17	Dewey	1	Cab Franc	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Dewey	1	Cab Sav	not detected	not detected	not detected	not detected	not detected	not detected
Repeat	Lincoln	2	Chardonnay	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	2	Merlot	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	2	Merlot	not detected	not detected	not detected	not detected	not detected	not detected

Year	County	Vineyard	Cultivar	GLRaV 1 & 3	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	Rotbrenner
17	Lincoln	2	Merlot	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	2	Merlot	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	2	Merlot	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	3	Merlot	not detected	Confirmed	not detected	not detected	not detected	not detected
17	Lincoln	3	Merlot	not detected	Confirmed	not detected	not detected	not detected	not detected
17	Lincoln	3	Merlot	not detected	Confirmed	not detected	not detected	not detected	not detected
17	Lincoln	3	Cab Sav	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	3	Cab Sav	not detected	not detected	not detected	not detected	not detected	not detected
17	Lincoln	3	Cab Sav	not detected	Confirmed	not detected	not detected	not detected	not detected
17	Mayes	1	Chambourcin crossed with Native – Ozark Jack	not detected	not detected	not detected	not detected	not detected	not detected
17	Mayes	1	Enchantment Red	not detected	not detected	not detected	not detected	not detected	not detected
17	Mayes	1	Enchantment Red	not detected	not detected	not detected	not detected	not detected	not detected
17	Mayes	1	Vidol Blanc	Confirmed	not detected	not detected	not detected	not detected	not detected
17	Mayes	1	Marquette	not detected	not detected	not detected	not detected	not detected	not detected
17	Mayes	1	Northern 'Fochs'	not detected	not detected	not detected	not detected	not detected	not detected
17	McCurtain	1	Noble	not detected	not detected	not detected	not detected	not detected	not detected
17	McCurtain	1	Noble	Confirmed	Confirmed	not detected	not detected	not detected	not detected
17	McCurtain	1	Carlos	Confirmed	Confirmed	not detected	not detected	not detected	not detected
17	McCurtain	1	Carlos	Confirmed	Confirmed	not detected	Confirmed	not detected	not detected
Repeat	Muskogee	1	Plymouth	not detected	not detected	not detected	not detected	not detected	not detected
17	Muskogee	1	Plymouth	not detected	not detected	not detected	not detected	not detected	not detected
17	Muskogee	1	Plymouth	not detected	not detected	not detected	not detected	not detected	not detected
17	Muskogee	1	Val John	not detected	not detected	not detected	not detected	not detected	not detected
17	Muskogee	1	Val John	not detected	not detected	not detected	not detected	not detected	not detected
17	Muskogee	1	Vignoles	Confirmed	not detected	not detected	not detected	not detected	not detected
Repeat	Oklahoma	1	Chambourcin	not detected	not detected	not detected	not detected	not detected	not detected
17	Oklahoma	1	Seyval blanc	not detected	not detected	not detected	not detected	not detected	not detected

Year	County	Vineyard	Cultivar	GLRaV 1 & 3	GLRaV 4+	GRBV	Pierce's Disease	*Ca.* Phytoplasma species	Rotbrenner
17	Oklahoma	1	Orange Muscat	not detected	not detected	not detected	not detected	not detected	not detected
17	Oklahoma	1	Riesling	Confirmed	not detected	not detected	not detected	not detected	not detected
17	Oklahoma	1	Cab Sav	Confirmed	not detected	not detected	not detected	not detected	not detected
17	Oklahoma	2	Unknown	not detected	not detected	not detected	not detected	not detected	not detected
17	Oklahoma	2	Chardonnay	not detected	not detected	not detected	not detected	not detected	not detected
17	Oklahoma	2	Cab Sav	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	2	Cab Sav	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	2	Merlot	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	2	Cab Sav & Pinot Gris	Confirmed	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	3	American	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	3	American	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	3	American	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	3	Norton	-	-	-	-	not detected	not detected
17	Oklahoma	3	Norton	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Oklahoma	3	Norton	not detected	not detected	not detected	not detected	not detected	not detected
17	Osage	1	Chambourcin	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Osage	1	Chambourcin	not detected	not detected	not detected	not detected	not detected	not detected
17	Osage	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Osage	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Osage	1	Cynthiana	not detected	Confirmed	Confirmed	not detected	not detected	not detected
17	Osage	1	Cynthiana	not detected	not detected	Confirmed	not detected	not detected	not detected
17	Pushmataha	1	Isons	Confirmed	Confirmed	not detected	not detected	not detected	not detected
17	Pushmataha	1	Carlos	Confirmed	Confirmed	not detected	not detected	not detected	not detected
17	Pushmataha	1	Noble	Confirmed	Confirmed	not detected	not detected	not detected	not detected
17	Pushmataha	1	Noble	Confirmed	Confirmed	not detected	not detected	not detected	not detected

Appendix B. ELISA absorbance data sample 2017 samples for GLRaV 4+

Sample	Sample 1	Sample 2	Healthy Control (AVG)	>2.5 x Healthy	Diagnosis
G26	0.110	0.103	0.108	0.270	Negative
G27	0.115	0.117	0.108	0.270	Negative
G28	0.118	0.130	0.108	0.270	Negative
G29	0.113	0.120	0.108	0.270	Negative
G30	0.112	0.111	0.108	0.270	Negative
G31	0.121	0.121	0.108	0.270	Negative
G32	0.117	0.118	0.108	0.270	Negative
G33	0.125	0.123	0.108	0.270	Negative
G34	0.130	0.136	0.108	0.270	Negative
G35	0.124	0.134	0.108	0.270	Negative
G36	0.106	0.118	0.108	0.270	Negative
G37	0.111	0.140	0.108	0.270	Negative
G38	0.113	0.117	0.108	0.270	Negative
G39	0.115	0.114	0.108	0.270	Negative
G40	0.116	0.114	0.108	0.270	Negative
G41	0.139	0.124	0.108	0.270	Negative
G42	0.131	0.144	0.108	0.270	Negative
G43	0.142	0.145	0.108	0.270	Negative
G44	0.277	0.289	0.108	0.270	Positive
G45	0.109	0.110	0.108	0.270	Negative
G46	0.108	0.105	0.108	0.270	Negative
G47	OUT*	OUT*	0.108	0.270	Positive
G48	0.118	0.116	0.108	0.270	Negative
G49	0.163	0.134	0.108	0.270	Negative
G50	0.129	0.117	0.108	0.270	Negative
G51	0.128	0.140	0.108	0.270	Negative
G52	0.106	0.150	0.108	0.270	Negative
G53	0.114	0.110	0.108	0.270	Negative
G54	0.249	0.125	0.108	0.270	Negative *
G55	0.137	0.135	0.108	0.270	Negative
G56	0.109	0.115	0.108	0.270	Negative
G57	0.117	0.120	0.108	0.270	Negative
G58	0.121	0.122	0.108	0.270	Negative
G59	0.116	0.115	0.108	0.270	Negative
G60	0.113	0.110	0.108	0.270	Negative
G61	0.115	0.113	0.108	0.270	Negative
G62	0.113	0.120	0.108	0.270	Negative
G63	0.120	0.120	0.108	0.270	Negative
G64	0.116	0.118	0.108	0.270	Negative
G65	0.114	0.123	0.108	0.270	Negative
G66	0.138	0.135	0.108	0.270	Negative
G67	0.116	0.128	0.108	0.270	Negative
G68	0.114	0.116	0.108	0.270	Negative
G69	0.141	0.147	0.108	0.270	Negative
G70	0.116	0.119	0.108	0.270	Negative
Healthy	0.101	0.115	0.108	0.270	Negative
Buffer	0.133	0.153	0.108	0.270	Negative
Positive	OUT	OUT	0.108	0.270	Positive

*Initial result was inconclusive. Sample was retested and determined to be negative for GLRaV 4+

Appendix C. Figure of mixed infection with GLRaV 1, 3, 4+

Symptoms on the leaves (browning, red color) are due to a mixed infection with both GLRaV-1, -3, and 4+

Appendix D. Primer design project

The Polymerase Chain Reaction (PCR) method is becoming a basic tool in diagnostic and research labs. It is imperative that students learn how to design primers for use in PCR methods. Next Generation Sequencing provides molecular data that can be accessed from public forums like the National Center for Biotechnology Information (NCBI) and used to design primers for new organisms and to test primers for existing primers. The evolution of microbial organisms brings the need to diagnose if this is a strain or a new organism. The software for primer design is easily accessed and available on the internet; when primer sets are listed in publications, these primers can also be tested by software to predict their sensitivity and specificity prior to ordering. Primer design is a skill that involves knowledge and access to available software on the internet and is very simple once understood.

For this project, primers were developed for a common disease problem of Fig (*Ficus* spp.) at the Oklahoma State's PDIDL. *Fig Mosaic Virus* is an *Emaravirus*, a negative sense single stranded (ss) RNA virus responsible for causing Fig Mosaic Disease which is found broadly in fig producing areas in the world. No species-specific primers are published, but primers for *Emaravirus* group are available. Primers were developed for this project, the melting temperature assessed, (Olwedo et al unpublished) and primers were validated against a positive control.

The first step in primer design is learning about the organism of interest. NCBI was searched for coat protein (CP) and RNA dependent RNA polymerase (RdRp) genes. Routinely,

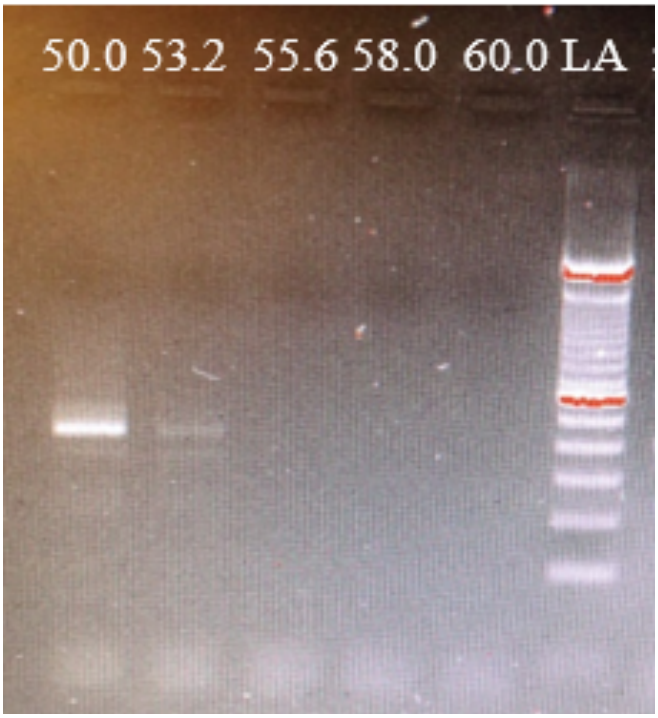
accessions listed are aligned to find the conserved region and compare. However, for *Fig Mosaic Virus*, only one accession was found and the sequence of the partial genome was used to design primers to fit the parameters set for primer design within various software.

The process of primer design is not completed by designing and ordering primers. Once primers were ordered, concentrations were prepared, and optimizing of the primers began as the primers must be tested *in vitro*. Healthy plant material (negative control) and material infected with the target organism (positive control) must be obtained. The RNA must be extracted from the plant material and cDNA must be generated. Then, the actual PCR test to validate the primers can be run.

In order to determine the annealing temperature, a gradient test is performed. In this test, a gradient from 50-60˚C was used in the assay. The correct annealing temperature of 50˚C was determined by running the PCR products on a 1.5% agarose gel as shown in Figure 8 (appendix). Interestingly, the computer software indicated a higher recommended annealing temperature than the one determined in the gradient.

The validation of the primers in incomplete. It is necessary to test these primers *in vitro* against near relatives to confirm specificity. This would include near relatives, *Rose Rosette virus* and *High Plains Virus*, two other common *Emaraviruses* in Oklahoma. This future work will be done by another student, to ensure the viability of these primers to be used in the PDIDL.

Appendix E. Gel confirmation of temperature gradient for primer design



Temperature gradient for Fig Mosaic Primers at 5uM concentration

Appendix F. Fact Sheet: Grapevine Red Blotch Disease

Figure 9. Fact Sheet: Grapevine Red Blotch Disease, National Clean Plant Network

FACT SHEET

National Clean Plant Network

Start clean, stay clean.

Grapevine Red Blotch Disease

What is red blotch?

Grapevine red blotch-associated virus (GRBaV), is the latest addition to the list of more than 75 graft-transmissible agents that have been identified in grapevines. This recently reported virus is associated with the emerging red blotch disease that was described for the first time on Cabernet Sauvignon in Napa Valley in 2008. There is a very good correlation between the presence of GRBaV and red blotch symptoms, but this correlation does not prove causality.

What are the symptoms of red blotch?

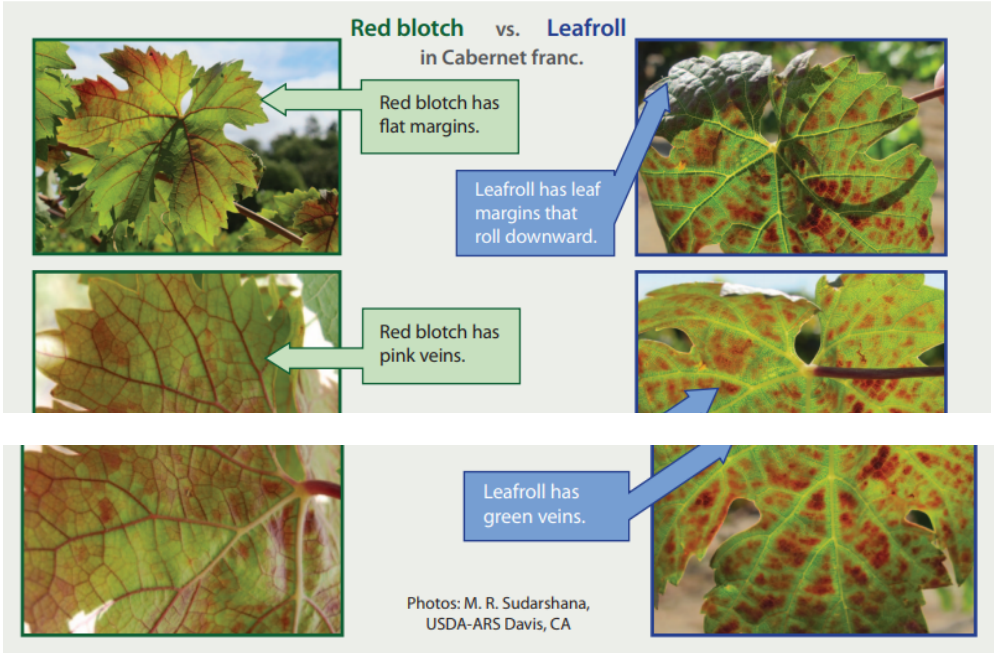
Vines with red blotch disease show symptoms much like leafroll disease. Like leafroll, leaves turn red in early fall primarily at the base of the shoots. Unlike leafroll, vines with red blotch disease show pink/red veins on the leaf undersides and no rolling.

How serious is it?

Red blotch disease can result in a significant reduction in sugar accumulation - up to 5 °Brix. Much is still unknown about effect on yield and possible differences in cultivars and rootstocks.

Where has it been found?

Findings suggest a wide geographic distribution, as well as a widespread occurrence in red and white vinifera cultivars. Infected vines have been identified in California, New York, Virginia, Maryland, Pennsylvania, Texas and Washington. GRBaV was found both in young (first leaf) and mature (5-20-yr old) vineyards. The sequence of a virus nearly identical to GRBaV was also obtained in Canada. GRBaV has been detected in Cabernet franc, Cabernet Sauvignon, Chardonnay, Malbec, Merlot, Mourvèdre, Petite Syrah, Petit Verdot, Pinot noir, Riesling and Zinfandel.



When was it found?

Investigations into what appeared to be a new disease began in 2009. Grapevine red blotch-associated virus was reported in independent studies in California and New York in 2012.

How does it spread?

Based on the wide host and geographic distribution of GRBaV and the fact that the virus is transmitted by grafting, it is likely that spread primarily occurs through propagation material. Also, an increased incidence of GRBaV over time in young, healthy vineyards that are adjacent to old, infected vineyards suggests the existence of a vector.

How is it treated?

Like other viruses, once it is present in a vineyard there is no cure. However, evidence suggests that GRBaV can be eliminated using microshoot tip culture, the same method used to eliminate other viruses, to establish clean Foundation vines.

What kind of virus is it?

Analysis of the genomic nucleotide sequence indicates a new circular, monopartite DNA virus that is tentatively assigned to the family *Geminiviridae*.

How is it detected? How can I get my vines tested?

GRBaV can be detected by a PCR test. Several labs offer a test for GRBaV.

What is the status of vines at Foundation Plant Services at Davis?

All of the vines planted at the new Russell Ranch Foundation vineyard have been tested for red blotch and none of them are infected. The Classic Foundation Vineyard has been partially tested and the incidence of GRBaV is very low. Test records are available on the Foundation Plant Services website http://fps.ucdavis.edu

What is being done?

Studies are ongoing to investigate the role of GRBaV in red blotch disease, monitor incidence and spread, improve detection techniques, and evaluate the efficacy of microshoot tip culture for virus elimination.

For the latest information see:

http://iv.ucdavis.edu

References:

Al Rwahnih, M., Dave, A., Anderson, M., Uyemoto, J. K., and Sudarshana, M. R. 2012. Association of a circular DNA virus in grapevines affected by red blotch disease in California. Proc. 17th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG), Davis, California, USA, October 7-14 2012, pp. 104-105.

Krenz, B., Thompson, J., Fuchs, M. and Perry, P. 2012. Complete genome sequence of a new circular DNA virus from grapevine. Journal of Virology 86:7715.

Red blotch in Cabernet franc.

Photo: Marc Fuchs, Cornell University, Geneva, NY

Leafroll in Cabernet franc.

Photo: Marc Fuchs, Cornell University, Geneva, NY

VITA

Sara Elizabeth Wallace

Candidate for the Degree of

Master of Science

Thesis: DETECTION AND DIAGNOSIS OF RED LEAF DISEASES OF GRAPES (*VITIS SPP*) IN OKLAHOMA

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Biographical:

Education:

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in July, 2018.

Completed the requirements for the Bachelor of Science in Horticulture at Oklahoma State University, Stillwater, Oklahoma in 2016.

Experience:

Internship at USDA APHIS PPQ CPHIST PERAL – May – June, 2018
Red Team Event – Biosecurity with Johns Hopkins University, Spring, 2018
Teaching – public, volunteers, students; plants to grow in Oklahoma
Youtube videos from Oklahoma Gardening: Nutsedge, Fire Blight, Cytospera Canker on plum
Managed vegetable and fruit gardens, tree fruit at The Botanic Garden at OSU
Landscape Designer with edibles, horticulturalist, landscaping
Community Garden teacher and volunteer (YMCA, Tulsa)
Community Supported Agriculture – (CSA) Entrepreneur (Virginia)

Professional Memberships:

American Phytopathological Society 08/2017 – present
American Society for Horticulture Science (ASHS) 08/2014 - present