

## Pierce's Disease Overview & Management Guide A Resource for Grape Growers in Texas and Other Eastern U.S. Growing Areas

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# Preface

With the outbreak of Pierce's disease across the Hill Country and the West Cross Timbers Area in the late 1990's grape growers across Texas were wondering what had changed. Why was Pierce's disease now a problem where it had never been before? Did the weather influence this shift? Were growers involved in moving the disease with cuttings or other plant material? As vine death became problematic for many more growers, the future of the Texas grape industry seemed to have a threatening shadow over it. Many speculated that all grape production would shift to the High Plains where "there was no Pierce's disease".

While the approach for some was to simply plant resistant or tolerant grape varieties, for many, the modern wine market place demanded that traditional vinifera varieties continue to have a place in the industry. It quickly became apparent that Texas was years behind California in understanding the dynamics of the disease <u>here</u>. These were very basic questions that only local applied research could answer.

The funding that followed through the cooperative agreement with USDA/APHIS provided the opportunity to conduct that research. Many of these questions have been answered, but as is commonplace in research, each answer begs many more questions. This work has been a joint effort from a group of dedicated research and extension investigators and much of this could not have been done without selfless collaborative attitude. Our work is not yet done, but the significant work that has been conducted over the past eight years has resulted in huge strides in cultural practices that for much of Texas, makes PD a manageable disease.

- Jim Kamas, Texas PD Program Outreach Coordinator



## **INTRODUCTION**

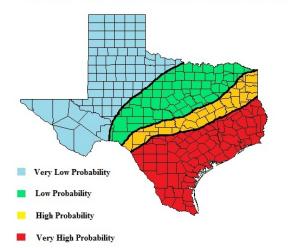
### History of Pierce's Disease in Texas - Jim Kamas

The history of grape growing in Texas predates that of California by nearly a century. In the 1680's, Franciscan monks brought grape cuttings from Mexico and established vineyards for wine production at the mission at Ysleta on the Rio Grande near El Paso. The success of these vineyards was probably due to the inherent Pierce's disease tolerance of the Mexican nursery stock. Historical accounts indicate that the wave of European settlers from wine producing countries in the mid to late nineteenth century brought vinifera grape cuttings from the Old World, and there are records of attempts to establish vineyards near Bellville, New Braunfels and Fredericksburg. There are no reports of notable production from these vineyards, and by all accounts, these vineyards soon failed. These settlers soon learned that by adding sugar to the juice of one or more native Texas grape species, stable wine could be produced. In 1883, Italian immigrants established Val Verde Winery in Del Rio where they grew 'Mission' grapes for wine production. Apparently around 1890, vines started dying and by 1910, only tolerant varieties remained. Operating for well over a century, Val Verde Winery is the only winery in Texas that survived prohibition and the Qualia family still successfully operates the winery today. They too bear testimony that during that time, the long-term survival and success of vineyards even in this part of the state depend on grape varieties being resistant to Pierce's disease.

In addition to being credited for saving the European wine industry from phylloxera, from 1880-1910, Thomas Volney Munson collected, catalogued and bred grapes from native southern species and developed hundreds of cultivars that were adapted to various areas of Texas and the southeastern United States. In addition to being cold hardy and resistant to fungal pathogens, many, but not all of Munson's varieties were tolerant to Pierce's disease. About 1900, agricultural reports and bulletins showed interest in experimental grape plantings across the state. By 1900, Texas had more than 25 wineries, but prohibition brought an end to industry expansion. Munson established a grape nursery and sold nursery stock with the profits going to further his grape exploration and breeding efforts. When the Munson & Sons Nursery closed in Denison, the collection was moved to the Winter Garden Experiment Station at Winter Haven, Texas. These varieties became a part of the extensive grape evaluations of Ernest Mortensen which were begun in 1931 and were terminated when the station closed in 1952. Grape evaluations were also conducted from 1939 to 1963 by Uriel A. Randolph at the experiment station near Montague. In addition to variety evaluations, work was conducted on fertilization, pest management and rootstock trials.

Beginning in the late 1960's- through the 1970's, Texas experienced a resurgence in grape growing. Seeking higher wine quality, the choices in variety selection changed from American varieties to French-American hybrids. In 1974, Dr. Ron Perry published Texas Agricultural

Experiment Station Report 74-3 entitled "A Feasibility Study for Grape Production in the Texas". In that study, Dr. Perry identified Pierce's disease as the #1 limiting factor to the



**Expected Probability of Pierce's Disease in Texas** 

Expected Probability of Pierce's Disease as Published in 1974 Feasibility Study production of grapes in Texas and published a map outlining the probability of disease incidence across the state. At that point, there was a rudimentary understanding that the distribution of the pathogen was limited by cold winter temperatures. It was thought that disease development was limited to areas receiving less than 800 hours of winter chilling per year. It was also thought that the range of vectors was limited to humid areas of the state.

While the industry was going through its renaissance in the early 70's, Dr. George Ray McEachern worked with grape growers to

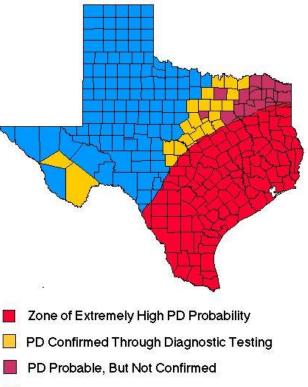
evaluate variety adaptation across the state. Drs. Hollis Bowen and Ron Perry also carried out variety and rootstock trials in the Brazos River Bottom west of College Station and at a second evaluation site near the town of London, Texas. At College Station, over 60

varieties and breeding selections were grown for evaluation and all but a small number died from Pierce's disease. At that time, PD was not seen at the London planting. Dr. McEachern also continued variety evaluation and placed sets of American, French Hybrid and *V. vinifera* grapevines in at least ten locations including Tyler, Seguin, Pleasanton, New Branufels, College Station, DeLeon, Tow, Laredo and Fort Stockton. The American Varieties in this trial included 'Black Spanish', 'Favorite', 'Herbemont', and 'Champanel'. Hybrids included S.V. 12-375, Siebel 9110,' Baco Noir', 'Aurelia' and 'Carolina Black Rose'. Based on the advice of Dr. H.P. Olmo, Dr. McEachern chose the *V. vinifera* varieties 'Ruby Cabernet', 'Chenin Blanc' and 'French Columbard'. At all locations except one, all of the French American Hybrids and *V. vinifera* varieties ultimately died of Pierce's disease and only 'Black Spanish', 'Herbemont' and 'Champanel' survived. The one sole site where all varieties survived was Fort Stockton- an area where Pierce's disease was not thought to occur.

Throughout the 1980's and 90's the Texas grape industry continued to expand. The practical rule of thumb during that time was that Pierce's disease was generally confined to areas south of Interstate 10 and east of Interstate 35. Even with this knowledge, many grape growers across the state continued to plant *V. vinifera* varieties in pursuit of high quality wines. In the early 90's, Dr. Larry Stein conducted an evaluation of 36 table grape varieties at the Stephenville Experiment Station. Of the varieties tested, Ark 1475 (now released as 'Victoria Red') and Ark 1400 (also thought to be PD tolerant) were the most vigorous and productive. In that same time

period, numerous vineyards were established in the Texas Hill Country and near the Dallas/Fort

Worth areas thought to be at minimal risk to Pierce's disease. Possibly as a result of a series of warm winters in the early 1990's, numerous accounts of vine decline were reported and investigated. Laboratory diagnostic techniques during that time were somewhat insensitive and at times faulty giving rise to several false negative results. By the late 90's it became increasingly obvious that Pierce's disease had become widely established in vineyards north and west of the area of expected occurrence. In addition to these central Texas findings, Pierce's disease was diagnosed and confirmed at an experimental vineyard in Alpine and a commercial vineyard near Ft. Davis, at elevations exceeding 5000 ft. where PD was not believed to be able to survive. Something appeared to be changing and the entire grape industry south of the High Plains appeared to be in peril. In 1999 the **Texas Grape Growers Association** approached the Dean of Agriculture at Texas A&M University to help chart a course of action. Dean Ed Heiler appointed a group of research and extension personnel as well as representatives from



No Known PD

Throughout the Late '90's to 2002, Laboratory Diagnosis Continued to Confirm PD Well Outside of its "Probable Range"

the grape growing community and the Texas Pierce's Disease Task Force was established. Growers contributed start-up funding as did A&M administration and a few initial objectives were established. The majority of the resources were aimed at developing a more sensitive diagnostic tool that could specifically single out the strain of *Xylella fastidiosa* that infected grapevines. This tool was needed to identify supplemental hosts of the disease in and around the vineyards and to confirm the specific insect species responsible for disease movement. In addition to this work, a preliminary insect survey work was done to begin to understand the diversity, seasonality and distribution of insect vectors of Pierce's disease. By 2000, all funding was exhausted and ongoing efforts came to a halt pending other sources of funds. Texas PD Task force members submitted several proposals to the California Pierce's Disease Research Grants Program, but none were funded. It was clear from the reviewers' comments that they did not understand nor want to fund efforts to understand Pierce's disease in Texas.

After a series of meetings outlining the needs and goals of the Texas group, Dr. Lloyd Wendel designated \$150,000 of his Glassy-winged Sharpshooter Program budget for fiscal year 2003 to begin the work on Texas objectives. The initial questions the group sought to answer were:

\* What is the definitive range of Pierce's disease in Texas and what vineyard attributes favor disease occurrence?

\* What insect species vector Pierce's disease in Texas? What is their relative abundance, range and seasonality?

\* What plants harbor the strain of Xylella that causes Pierce's disease outside of vineyards in Texas?

From this modest beginning, the Texas Pierce's Disease Research & Education Program has grown and continued to conduct applied research and educational programming focused on the prevention and management of the disease. This overview and management guide is the product of that work and represents a collective gain of knowledge and management techniques since 2003.

### Pierce's Disease in California: History, Discovery and then Ancient History

### The 1800s "Anaheim Disease"- Lisa Morano

Anaheim, California is the quintessential Southern California tourist destination. A region densely packed with golf courses, an enormous convention center, automobiles and amusements. Today Anaheim's foremost tourist spot, Disneyland, gets tens of millions of visitors to its Magic Kingdom annually, but back in the 1800s Anaheim was home to cattle, oil and agriculture. In the 1892 bulletin of the U.S. Bureau of Agriculture, an agency pathologist named Newton Pierce outlined the expansion of viticulture in Southern California and the crash of the grape industry from 'Anaheim Disease' over the previous decade. In his bulletin he actually refers to 'Anaheim Disease' of grapes as 'California Vine Disease' (Pierce 1892), which we can assume was not only more polite but more logical as the disease moved beyond Anaheim's borders.

In the early 1800s vineyards sprang up in the Los Angeles basin and were planted largely with the Mission grape variety. A mission north of "Pueblo de los Angeles" (the largest town in California with thousands of people) had a large vineyard of Mission grapes and was known to be a large supplier of good wines (Pierce 1892). In addition to Mission vines in Southern California, Pierce lists additional plantings of Zinfandel, Muscat and other varieties put in by European immigrants. He documents a few cases of vine disease in 1884 and then the first grape disease outbreak in Anaheim, California in 1885. By the summer of 1886 Pierce describes how the summer heat seemed to have triggered wide spread disease in many vineyards and multiple varieties. The color drawings of infected leaves at the end of his bulletin so accurately document leaf symptoms they could be photos from an infected vineyard in the twenty-first century. Symptomatic leaves and dramatic crop losses in 1886 were followed by thousands of acres of dead vines by 1887 (Pierce 1892).

Gardner and Hewitt (1974) published a historical account for the search for the Anaheim Disease and the California Vine Disease. This detailed review covers the 1800s in detail, the experts called in and the multiple meetings and commissions created to determine the cause of the disease. In 1888, a commissioned specialist named Dowlen reported that this disease was fungal and he advocated use of fungicide. By 1889 Dr. Harkness, President of the Academy of Sciences, was arguing that the disease was not fungal and the feud was apparently played out in agricultural bulletins and newspapers. Ironically, Pierce suggested the disease may be bacterial in 1889, but he could not grow a bacterial culture or find a preventative remedy (Gardner and Hewitt 1974). Over the following decades, dead vineyards in Southern California were replaced with other crops including citrus and avocados.

#### The Early 1900s "Pierce's Disease Virus"

The grape disease appeared again in the San Joaquin Valley in 1917 with additional cases in Tulare County of central California (between Bakersfield and Fresno) in years 1921, 1927 and 1931. By 1938 five vineyards of Chowchilla (north of Fresno) were dead within two seasons (Hewitt et al. 1949). During the 1930s William Hewitt, a UC Davis graduate student, began studying the disease and named it Pierce's Disease (PD) after Newton Pierce (Purcell 1993). Hewitt made several significant contributions throughout the 1940s including grafting experiments showing the disease could be spread across graft unions from an infected plant to a clean plant and that infected plants seemed to react to the virus by forming "tyloses" or growths inside the xylem (Hewitt et al. 1949). Hewitt also collaborated with entomologists who had shown that multiple species of xylem-feeding insects including leafhoppers and spittlebugs could transmit the disease (Frazier and Freitag 1946, Severin 1947). Based on observations of distribution, life history, host range, feeding habits and movement patterns of these insects Hewitt and colleagues concluded there were three leafhopper species most significant in spreading of the disease in California - the green sharpshooter, the redheaded sharpshooter and the blue sharpshooter (1949). He also showed a positive correlation between number of sharpshooters in plots and percentage of vines dying from PD.

Although most of Hewitt's studies were rather insightful, not all of his results are consistent with modern practices. In one experiment he found that mowing vineyards and roguing diseased vines did not seem to reduce disease spread. (These experimental blocks were too highly infected for these practices to have worked.) He also reported that although the use of insecticides such as DDT and cyanide killed the leafhoppers on contact (not surprisingly) it did not seem to give seasonal control of the insects (Hewitt 1949).

Hewitt also hypothesized about the etiology and the origin of Pierce's Disease. He logically assumed the disease was viral because he could never grow a culture of the disease-causing bacteria (a small comfort to many a new student working with the slow growing PD organism). To better understand the evolutionary origin of the disease Hewitt collected extensive historical information about the wild *Vitis* species of the US Gulf Coast noting that these vines appeared to be resistant to the disease. He describes historical plantings of these wild *Vitis* species in California at various locations in the 1800s "introduced for the purpose of testing their resistance to Phylloxera." He suggests that resistance to the virus among most wild *Vitis* in the Gulf Coastal Plain and their introduction into California with the start of the California outbreaks point to the Gulf Coast as the probable home of the disease (Hewitt 1958).

#### Mid to Late 1900s "Xylella fastidiosa bacterium"

In 1973 Donald Hopkins and Hilton Mollenhauer of the University of Florida published an article in *Science* reporting a *Rickettsia*-like bacteria associated with PD (Hopkins and

Mollenhaer 1973). The same year Goheen, Nyland and Lowe reported a similar organism associated with PD in grape and alfalfa dwarf in the journal *Phytopahtology* (Goheen et al. 1973). The genus *Rickettsia* is a group of bacteria responsible for diseases like Typhus and Rocky Mountain Spotted Fever. Like *Rickettsia*, the new PD bacteria were Gram-negative (have an outer membrane that prevents staining), pleomorphic (variable shape from round to long rods) and they were associated with insect vectors - PD bacteria being vectored by sharpshooters and Rickettsial diseases by ticks, fleas or lice (depending on disease). What makes *Rickettsia* unique is they are obligate intracellular parasites (must divide in host cells) and this turned out not to be the case with the PD bacteria.

The late 1970s and early 1980s brought the confirmation of the causative agent of disease and its taxonomic identification. In 1978 Koch's postulates was satisfactorily performed with the PD bacterium by Davis, Purcell and Thomson (Davis et al. 1978). Koch's postulates are a set of microbiological steps performed which confirm a particular pathogen as the causative agent of a disease. In the case of PD, cultures were isolated from PD infected grapevines and these cultures were then inoculated into healthy grapevines. The inoculated vines subsequently developed PD and the same bacterium was re-isolated (Davis et al. 1978). In 1987 Wells and colleagues described this pathogen as the new species *Xylella fastidiosa* - a Gram-negative, xylem-limited, fastidious plant pathogen related to *Xanthomonas* (another group of plant pathogens).

X. fastidiosa went from a somewhat obscure pathogen to an agricultural menace in the 1990s. In Brazil the citrus variegated chlorosis (CVC) strain of X. fastidiosa caused an epidemic killing millions of citrus trees (Hopkins and Purcell 2002). The introduction of a new insect vector into California increased the spread of PD dramatically and was correlated with outbreaks of a new X. fastidiosa disease, oleander leaf scorch (Purcell et al. 1999). The USDA has concluded that the Glassy Winged Sharpshooter (GWSS) (Homalodisca vitripennis) was introduced to California from the southeastern US in 1989 (USDA National Agricultural Library 2011). GWSS were first observed in Orange and Ventura counties (Sorensen and Gill 1996) before their numbers exploded in the Temecula region in the 1990s. The story of this expansion of PD is well reviewed by the two PD experts Hopkins and Purcell (2002). An abundance of GWSS vectors in Temecula corresponded with laboratory confirmed cases of PD in dying vineyards. Additionally, vineyards in Temecula did not get PD at the edge of a vineyard but crashed uniformly and swiftly – suggesting the larger GWSS vector was a more effective vector. Eventually population genetics studies of GWSS from multiple states of the southeast concluded that the population of GWSS introduced into California was likely from Texas (de Leon et al. 2004).

#### The New Century

Epidemics of *X. fastidiosa* diseases in Brazil and California fueled the funding of extensive research in both countries. In California the PD/GWSS Board was developed in 2001 to support scientific research using funds assessed by the California winegrape growers. The CDFA and

USDA began funding PD research and an annual PD Research Symposium was organized. Research has focused on questions of vector biology and ecology, vector management, pathogen biology and ecology, pathogen and disease management, crop biology, disease epidemiology and economics. In the last decade we have made enormous scientific strides in our understanding of the disease and in our prevention of its spread. The discoveries are too numerous for this brief historical review; however, we now have better tools for fighting PD in California and in Texas.

We have more sensitive detection methodologies. Our understanding of sharpshooter life cycles and biology has led to more precise timing of insecticide application and the use of a insecticide specific for xylem-feeders (a vast improvement over DDT and cyanide). We have bred new PD resistant plant material after identifying resistance genes in wild plant material. We have performed population genetics studies of the insect and bacteria to understand disease epidemiology, modeled insect behavior and bacterial movement in the plant, run gene expression studies to determine which pathogenicity factors are turned when plants are infected. Finally, we have invested in the cell biology studies to understand the bacterial metabolic pathways – essential for any future pharmacological intervention.

#### **Ancient History**

It is said that history must be written of, by and for the survivors. This is particularly true in matters of biology where ever-changing selection pressures leave their mark through time and death. To understand the history of Pierce's Disease in North America we must understand the history of *X. fastidiosa* in North America. If we understand how this bacterium has shaped those organisms it has interacted with we can understand its potential and its weaknesses. Modern cellular biology can describe the characteristics that have been victorious, but molecular studies of the DNA in organisms allow us describe the spatial and temporal scale of the evolutionary battles.

In 1958 Hewitt argued that there was no clear evidence of Pierce's Disease in California prior to 1884 and that high disease resistance along the southern grape species suggests *X. fastidiosa* grape strain came from the south or southeastern states. There is certainly a great deal of data to support the idea. The selection pressure for *X. fastidiosa* is very high along the Gulf Coast. In a preliminary evaluation of over 100 plant species in 40 different plant families in the Houston area about 20% of the plants were infected with *X. fastidiosa* and were non-symptomatic (McGaha et al. 2007). Disease resistance in such a large percentage of native plants did not evolve overnight and suggests a regional history with *X. fastidiosa* that goes back thousands of years. Analysis of resistance of North American wild *Vitis* also supports this idea. Wild vine species with the greatest resistance (defined as lowest bacterial levels after inoculation with *X. fastidiosa*) have originated from the southern regions (Lin et al. 2008).

To understand evolutionary history we have evaluated the genetic similarities between the *X*. *fastidiosa* strains (or subspecies). There are several well-established subspecies of *X*. *fastidiosa* 

in North America. These are the weed strain called *X. fastidiosa* subsp. *multiplex*, the grape strain, *X. fastidiosa* subsp. *fastidiosa* and the oleander leaf scorch strain, *X. fastidiosa* subsp. *sandyi*. Randal and colleagues from New Mexico and Arizona have also argued for an additional subspecies based on a unique *X. fastidiosa* found in the ornamental chitalpa plants of the southwest (Randall et al. 2009).

What is fascinating is that the comparative genetics of conserved genes (genes critical for survival which evolve slowly at the population levels) suggests that all of the grape strains within North America are surprisingly similar. This is true if one is examining individual genes (Yuan et al. 2010) or comparing the sequenced genomes of the Temecula grape strain and the Texas grape strain GB514 (Schreiber et al. 2010). This has led Nunney and his colleagues to conclude that the grape strain actually moved into North America in the mid to late 1800s Nunney et al. 2010). The genetic evidence for this is very compelling. There is little genetic variability in grape strains collected across North America. There is genetic evidence that *X. fastidiosa* found in Central American coffee plants are more genetically diverse and there is also evidence that the grape strain evolved in a more tropical climate than the more ubiquitous North American weed strain (Nunney et al. 2010). In this 2010 work, Nunney also suggests that the grape strain came in as *X. fastidiosa* variant with in importation of tropical coffee plants into Anaheim between 1850 and 1870.

It is logical that Gulf Coast plants (weeds and shrubs) would have resistance to *X. fastidiosa* since cultures from these plants are typically infected with the weed strains. Nunney's work begs the question of why wild vines from the more southern and southeastern regions have resistance to the grape strain if the grape strain has not been here that long? It is possible that native vines have been exposed to a diversity of the weed strains over thousands of years and that this has conferred some general resistance to all *X. fastidiosa*.

We know that wild vines can pick up the grape strain, they just don't die swiftly like the European *Vitis vinifera* varieties. Perhaps the earlier exposure to the North American weed strains conferred some resistance to the 'new' grape strain that was able to grow in all *Vitis*, but kills *Vitis vinifiera*. And what of the stories going back before the 1800s of growers having trouble growing European grapes in Texas? Was that another strain of *Xylella fastidiosa* or was it a combination of diseases and environmental conditions that the European growers had not previously experienced? There are certainly a plethora of microbiological and environmental hazards to growing grapes and the intensity increase as one gets closer to the Gulf Coast.

As we continue to unlock the histories of how Pierce's Disease moved into North America, how a new Texas vector spread the disease more efficiently in California and how the interaction of plants and *Xylella* led to resistance, we will certainly refine our other discoveries within a larger historical context.

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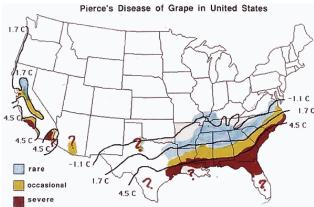
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### Pierce's Disease in Other Eastern U.S. Growing Regions

### Jim Kamas

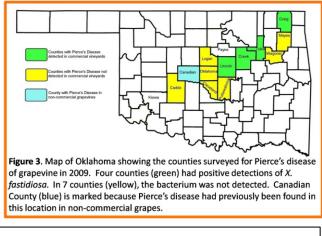
*Xylella fastidiosa* is believed to have been established in the Gulf Coast region of the United States for probably thousands of years. Native grape species along the Gulf Coast are tolerant to



the disease indicating that these species have evolved in the presence of the bacterium for quite some time. Dr. Alexander (Sandy) Purcell, perhaps the most knowledgeable researcher on Pierce's disease in the country, produced this map which represents the approximated range of PD in 2002. Repeated attempts to grow *vinifera* and French hybrid grapes across much of the Gulf Coast have ultimately resulted in vine death. Because of

our understanding of *Xylella*'s relatively sensitivity to cold temperatures, areas receiving less than 800 hours of winter chilling have been thought to have higher risk of disease incidence and severity. This map was produced from the sum of anecdotal and experimental accounts known to the author where Pierce's disease has killed plantings of susceptible varieties.

It is notable at that time that the area of unknown status includes most of Texas and parts of New Mexico and Arizona. Over the past 10 years, we have filled in many of the question marks and extended the known range of Pierce's disease to the north and the west. Through work in areas of Oklahoma and the High Plains of Texas, Pierce's disease is now thought to be widespread, but the disease is probably a chronic problem as opposed to an acute one. Competent vectors exist and overwinter as adults in these locations, but the infections seem to come

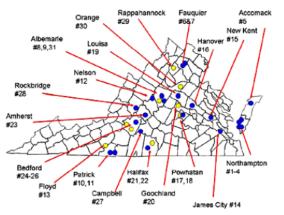


Oklahoma PD Survey Map- Damon Smith & Jennifer Olson, Oklahoma State University

and go, probably as a result of the curative effect of cold winters.

To the east, work was conducted during the 2006 growing season to survey potential PD vectors and to sample vineyards in Northwestern Arkansas and in five vineyards across Missouri.

Pierce's disease was confirmed and somewhat widespread in two Altus, Arkansas vineyards, but in 2006, no vine infection was confirmed in Missouri. Across all sampling sites, numerous Proconiini and Cicadellini species were trapped at times indicating that these species overwinter as adults, and therefore are very capable of serving as competent vectors. Pierce's disease was



Locations of Virginia vineyards surveyed for Pierce's disease (blue indicates Xylella positive, yellow indicates negative)- Anna Wallingford confirmed in one commercial Missouri vineyard in 2010.

Pierce's disease has also been found further north in the eastern seaboard than has previously been known. In her 2007 Plant Management Network publication, Anna Wallingford et.al., published the experimental findings of the distribution of Pierce's disease in commercial vineyards in Virginia. Her work cites previous studies that have confirmed Pierce's disease on the Delmarva peninsula and at

least as far north as New Jersey. Her 2007 study showed infections at 22 of 31 vineyards sampled and infection was confirmed in locations more northern and at higher altitude than previously thought possible.

With ongoing research revealing infections of Pierce's disease in more northern and western locations than had previously been know, there are several theories that growers can arrive at. One is that our climate is changing, another is that *Xylella* is becoming more cold tolerant over time, or quite possibly both are occurring concurrently. Dr. Don Hopkins, plant pathologist at the University of Florida, has been working on PD since 1968, and he has another theory. He suggests that our confirmation of additional Pierce's disease infection sites is strongly correlated with the number of people looking for it. In other words, heightened interest in Pierce's disease has given rise to work done across the country and these studies may be revealing infection sites that have been there all along.

### Other Diseases Caused by Xylella fastidiosa - Jim Kamas

The bacteria *Xylella fastidiosa* is considered to be native to the North and Central America where it appears to reside benignly in many native plants. Disease is typically of "old world" plants exposed to this "new world" endophyte. Although the subject of continuing debate, *Xylella fastidiosa* has been divided into four subspecies, each affecting specific groups of plants.

Xylella fastidiosa ssp. fastidiosa is the strain which causes Pierce's disease in grapevines, Alfalfa dwarf and almond leaf scorch. Grape species native to areas with endemic Pierce's disease pressure have evolved to be resistant or tolerant to bacterial infection. With some species, especially when exacerbated by dry conditions vines do show typical leaf scorch normally associated with Pierce's disease. These native species are believed to have a unique xylem architecture that limits spread of the pathogen more so than in susceptible species and cultivars. Wild grapevines are thought to be an important source of the bacterium which contributes to disease pressure in adjacent vineyards. The ability to tolerate *Xylella* among wild vines differs among *Vitis* species and even differs within a species depending on the severity of disease pressure in the environment from which those vines originate. Genetic basis for



Wild *Vitis berlandieri* vine exhibiting leaf scorch due to *Xylella fastidiosa* infection

resistance and tolerance also differs among wild North & Central American grapevine species.

Almond leaf scorch was first diagnosed in California almond orchards in the mid- 1930's and has subsequently become established in orchards throughout the state. Infected trees show first symptoms of scorched leaves followed by reduced productivity and a general decline in tree health. Strains of *Xylella fastidiosa* that cause Pierce's disease also cause almond scorch but not all isolates from almond cause disease in grapevines. It normally takes trees three or four years to show symptoms after infection.

Although this strain of *Xylella* infects and causes symptoms on alfalfa, Alfalfa Dwarf is not recognized to be an economic disease. Infected plants appear stunted with small leaflets and shortened stems. Infected plants eventually become chlorotic and die. Although not an economic problem in the production of alfalfa, infected hayfields can serve as an important source of the bacterium in the infection of adjacent vineyards.

*Xylella fastidiosa ssp. multiplex* is perhaps the strain with the broadest host range and is known to infect peach, plum, apricot, oak, elm, redbud, sycamore, maple, mulberry, ash, sweetgum and perhaps several other softwood and hardwood trees as well as a multitude of broadleaf weeds.



Peach Phony Photograph Courtesy of Dr. Russell Mizell

Of these hosts, economic loss has been greatest on peach. Originally named Peach Pony Disease, however, due to a typographical error in the preparation of a manuscript it became known as Peach Phony Disease. Infected trees appear dwarfed with noticeably shortened internodes. As the disease progresses, fruit on infected trees ripens a week to ten days ahead of healthy trees, are smaller and have a distinct red suture. Once a very widespread problem in Texas, a USDA survey and roguing program in the 1920's greatly reduced the incidence and severity of the disease across much of the Southeastern United States. Like the grape strain of Xylella, the range is thought to be limited to areas where the average winter minimum temperature exceeds 17° F. As with

Pierce's disease, transmission is believed to be vectored exclusively by sharpshooters or related xylem feeding insects. It remains a puzzle why Pierce's disease became an explosive disease in the Texas Hill Country in the 1990's while there were no suspected incidences of Peach Phony in

an area with over 1400 acres of commercial peach orchards in this geographic region.

*Xylella fastidiosa ssp. multiplex* also causes bacterial leaf scorch of numerous hardwood and softwood trees native across east, north and central Texas. Across the state, infected oak, elm and sycamore can be commonly seen exhibiting typical irregular leaf scorching in late summer and early autumn. The disease by itself is rarely fatal, but can weaken trees making them susceptible to infection from numerous fungal pathogens. Combined with adverse environmental conditions,

*Xylella* can indeed impact the health and shorten the life of infected native trees. Scorched limbs occur irregularly throughout the canopy and the number of limbs exhibiting symptoms increases over time. Cold winters and wet growing seasons can reverse



Bacterial Leaf Scorch of Sycamore

the trend of tree decline and mitigate the damage bacterial leaf scorch causes. The current thinking is that trees or weeds infected with *multiplex* strain do not pose a threat of serving as a supplemental source of inoculum for infection of grapevine. It is hypothesized, however that some of these trees or weeds may actually serve as a host of more than one strain of *Xylella*, and that *multiplex* outcompetes *fastidiosa* strain in culture and outnumbers it in other diagnostic tests. Some scientists further subdivide multiplex to include a recombinant type thought to infect blackberry and a type thought to be specific to mulberry.

Xylella fastidiosa spp. sandyi is specific to Oleander and causes a leaf scorch and decline of that



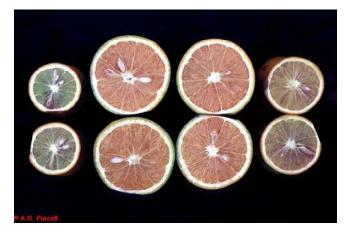
ornamental plant. As with other species and other *Xylella* subspecies, the oleander strain causes leaf scorch, dieback and decline of infected plants. As with other hosts, symptoms typically begin in summer and progress as temperatures increase and available moisture decreases. Plants eventually die, even though they may attempt to produce new shoots from the ground. The disease was first diagnosed in California in the 1990's and is believed to be a recent introduction to that state. Oleander leaf scorch is certainly not new to Texas, but the incidence appears to be increasing and the range of the disease appears to be growing. The Highland Lakes area of central Texas, as well as

the Gulf Coast, appears to be at the highest risk of plant loss. The disease is reportedly threatening the oleander collection at Moody Gardens in Galveston.

Xylella fastidiosa ssp. pauca is associated with Bacterial Scorch of coffee and a disease known

as Citrus Variegated Chlorosis (CVC). CVC was first reported in Brazil in 1987. CVC first appears as mild, then progressive chlorosis of foliage. Small lesions appear on the underside of leaves that ultimately become brown and necrotic. Fruit size is adversely affected with increased thickening of the rind. Infected trees become less productive with thinning canopy and limb dieback, but CVC is rarely fatal to the tree.

Citrus Variegated Chlorosis has become widespread in Brazil where it has been thought to be freely spread between groves by infected nursery stock and within groves from numerous xylem-feeding sharpshooters. The geographic distribution of CVC has expanded from Brazil to include





Argentina, Paraguay and most recently Costa Rica. Nearly all cultivars of sweet orange are susceptible, but there are differences in disease severity between varieties. Grapefruit, lemons, limes and mandarins are less severely affected than oranges and Rangpur limes, citron and pummelo appear to be unaffected by CVC. Trees of any age can become infected, but it appears that young trees exhibit symptoms more rapidly, and symptoms are normally seen nine to twelve months after infection. At this time, CVC has not yet been detected in the United States. There is a concerted effort on the part of USDA/APHIS to monitor disease movement in the America's and to curtail its introduction into economically important citrus groves in Texas, California and Florida. Further complicating the clear-cut host range of *Xylella fastidiosa* subspecies, CVC strains of *X.f.* have recently been reported to cause Pierce's disease in grape.

Coffee Bacterial Scorch was first diagnosed as a disease of coffee in 1995 and was first associated with *Xylella fastidiosa* in 1996. The disease has probably existed in South America for quite some time, but symptoms were believed to be associated with environmental stresses. Association with the causal agent, *Xylella fastidiosa* have subsequently been confirmed by ELISA and PCR analysis. Symptoms on coffee are similar to other susceptible hosts where recently matured leaves exhibit irregular scorching, stunting and dieback. As with CVC strains, *X.f.* strains associated with Coffee Bacterial Scorch have been reported to induce Pierce's disease symptoms in grapevines. Since coffee production is limited to the tropics, interest in the impact of this strain in the temperate America's is limited to its association with causing disease in citrus and grapevines.

#### Citrus and Oleander photos in this section courtesy Dr. A.H. (Sandy) Purcell

# PLANT, PATHOGEN & VECTOR INTERACTION

### Pathogen Biology and Epidemiology - David Appel

#### Spread in the Vine

Pierce's disease develops when *X. fastidiosa* bacterial cells are injected directly into the vascular system of a grapevine by a sharpshooter vector. The pathogen then multiplies and spreads through the water-conducting xylem of the host plant. A combination of pathogen effects and host responses leads to symptoms in the vine. For example, the bacterium is equipped with tiny, hair-like structures called pili that are needed for motility and adhesion in the vascular system of the infected plant. Also, *X. fastidiosa* produces substances that cause colonies of numerous individual cells to form plaques known as biofilms in the xylem. If enough of the vascular tissues become occluded by these colonies, then dieback and death may occur. The rate of multiplication and movement varies according to vine susceptibility.

There are many different strains of *X. fastidiosa*. One way to categorize these strains is according to the types of hosts they infect. The Pierce's disease strain infecting grapes, for example, belongs to the sub species *fastidiosa*. This grape subspecies is able to infect a number of other ornamentals. There are other groupings of *X. fastidiosa* that are able to infect hundreds of other known hosts, although many of these strain x host relationships do not cause any significant disease response, or symptoms, in their respective hosts. This complicated association of different pathogen strains with various combinations of different hosts leads to great difficulties in diagnosing the disease as well as detecting potential sources of the pathogen for spread into vineyards from adjacent vegetation.

Another important feature of *X. fastidiosa* is the sensitivity of the pathogen to low temperatures. This tropical nature of the pathogen has long been considered responsible for the limited northern expansion of the pathogen in the U.S., but it also has management implications in areas which are sufficiently low enough to eliminate the bacterium from colonized vines during the winter.

#### Spread in the Vineyard

The PD pathogen can infect a new vineyard in one of two ways. The first is by means of contaminated nursery stock. Source vines for rooted cuttings and other planting stock may be infected but with no symptoms. The resultant diseased material can then be transplanted into a new vineyard for subsequent spread to other vines. This same mechanism may be responsible for introducing the pathogen into older, established vineyards in replacement vines. The second means of the spread into a new vineyard, termed primary transmission, would be from infected vegetation in or near the vineyard. A long list of native vegetation has been proven capable of harboring *X. fastidiosa* (see Table 1). The list includes native, weedy hosts as well as

ornamentals susceptible to the grape strain. Primary transmission causes the familiar "edge effect", where the first diseased plants are found on the border of the vineyard.

2003 2004 2005 Accumulated Vine Mortality (Total = 2700) 18.0.0 14 0 0 х 12 0 0 10 0 0 1800 1000 100 100 100 ----- A ccu mu late d slope = xr(1-x) t 2000 2001 2002 2004 2005 2003 Year Polycyclic disease progress

If left unchecked, primary transmission will continue annually, leading to a slow, predictable

# Modeling Acute Disease Progress in Texas

### Studies of Disease Progression in Texas Consistently Shows Strong Vine to Vine Disease Spread When Infected Vines are Not Removed

increase in diseased vines. As long as the native, adjacent vegetation harbors reservoirs of the pathogen and sufficient populations of the insect vectors, the rate of disease increase will be additive with similar numbers of new infections each year originating from the direction of the source plants.

> Once introduced into the vineyard,

certain conditions may lead to spread of the pathogen by secondary transmission, or vine-to-vine spread. Species of vectors is particularly important because the transmission efficiencies of the various potential sharpshooters can vary widely. The degree of varietal susceptibility is important as well, because vines must be sufficiently colonized for the vectors to acquire the pathogen and move it to new vines. Vine-to-vine spread is particularly explosive in those warmer locations where the bacterium is able to survive during the dormant season, so that the bacterial titers will build-up from one season to the next, particularly in susceptible varieties. This sort of transmission results in the spread of the pathogen along the trellises more quickly than across the rows.

### Diagnosis of Pierce's Disease - David N. Appel

#### Introduction

The diagnosis of Pierce's Disease in grapevines requires the careful observation of multiple symptoms, usually combined with laboratory testing. The symptoms of infection by *X*. *fastidiosa* and results of lab testing may be influenced by cultivar, environmental conditions, and the quality of samples submitted to the plant clinic. Therefore, the diagnosis of PD, whether in the vineyard or in the clinic, often also relies on some degree of interpretation of how the different clues relate to other causes of disease in grapevine. Each of these facets of PD diagnosis will be discussed below in detail.

The first appearance of Pierce's Disease often occurs on grapevines growing on the edge or border of the vineyard (Figure 1). If left untreated, symptoms will then appear on grapevines in the interior of the vineyard, causing numerous "satellite" centers of infection. The pathogen thereafter usually spreads more quickly along the trellises rather than across rows due to the habits of the sharpshooter vectors. As with other symptoms, there are varietal and climatic influences on the rate with which the pathogen spreads.



Figure 1. Symptomatic vines infected with <u>Xylella fastiodosa</u> on the edge of a vineyard.

#### Symptoms

The expression of Pierce's Disease in grapevines is characterized by several well-described symptoms. These symptoms first appear in mid- to late summer, when transpirational demand in vines is highest and the onset of water stress occurs. Marginal scorching of foliage is the most familiar of the symptoms (Figures 2 and 3). The scorch appears as an irregular, necrotic margin on the edge of the leaf, usually with a reddish brown line demarcating the area of necrosis (arrow). Scorching often initiates in the oldest leaves, and depending on severity, progresses



Figure 2. Leaf scorching caused by <u>X. fastidiosa</u> on Chardonnay.



Figure 3. Leaf scorching caused by <u>X. fastidiosa</u> on Merlot.

outward on the cane until the younger leaves are involved. Once scorch has appeared, the grapevine has probably been infected for at least a month or so. However, symptoms may also be delayed into the next growing season following infection, depending on climatic conditions and cultivar. Some differences in symptom expression among cultivars have been noted, where the scorching in red varieties starts with a reddish discoloration (Figure 3) and white varieties exhibit marginal chlorosis. Eventually, the leaf blade, or laminae, will drop from the vine leaving the petiole remaining attached to the cane. These retained petioles are called "matchsticks", and are considered to be a very specific symptom of Pierce's Disease (Figure 4).

Another typical PD symptom is the formation of green "islands", resulting from irregular maturation of the periderm on canes (Figure 5). Green islands become evident later in the growing season. Also, berry bunches on severely affected vines will be dried and shriveled, a phenomenon known as "raisining".

If symptoms advance from slight expression to severely involving the entire grapevine, canes will begin to dieback, and death of the entire grapevine is possible. Some infected vines may fail to emerge in the spring following infection, giving the impression that they were may have been

affected by winter kill. Symptomatic vines have been known to recover, particularly in resistant or tolerant varieties.



Figure 3. Retained petioles on PD infected PD vines, known as matchsticks.



Figure 4. Incomplete periderm maturation on infected vines, known as green islands.



Figure 5. Grapevine in the advanced stages of infection by X. fastidiosa.

### Laboratory Diagnostic Tools - David N. Appel

#### **Clinical Testing**

Symptoms are adequate for preliminary diagnosis of Pierce's Disease, but due to the similarities of symptoms with those of other disease-causing agents, clinical testing in a plant diagnostic laboratory is helpful for definitive diagnosis of the disease. There are three approaches to clinical diagnosis of Pierce's Disease. Each of these approaches has advantages and disadvantages. The first and oldest of these is isolation of *X. fastidiosa*. Successful isolation, or culturing of the pathogen from diseased tissues, is the most definitive technique for diagnosis. It is the least expensive of the methods, but it is slow (app. 3 weeks), and is considered by most diagnosticians to be a difficult process. The slow-growing *X. fastidiosa* is easily overwhelmed by other contaminating organisms under lab conditions. It is somewhat difficult to distinguish it from other bacteria. Finally, bacterial distribution in the vine appears to be uneven, so that samples removed from a symptomatic vine may not harbor the pathogen. For these reasons, the other lab methods are often incorporated into a diagnostic process.



The second diagnostic protocol, ELISA (Enzyme Linked Immunosorbent Assay), is a biochemical technique using principles of immunology to develop a test that detects antigens (pathogen properties) in tissues from a vine. The test kit is developed to detect protein in the tissues following mixing with a number of reagents previously developed and included in the kit. ELISA is relatively fast (a few hours) and more sensitive when compared to isolation, but it has some significant reliability problems. It suffers

from the same sampling problem described for culturing, and may result in false positive reactions that require re-testing. ELISA is recommended when a vine is in advanced stages of symptom development when the pathogen is well-distributed in the vine and present in very high titer.

The third, and newest diagnostic technique, is PCR (Polymerase Chain Reaction). Usually formatted for rapid diagnosis as Real Time or QRT-PCR, this technique is also fast (a few hours) and is considered to be extremely sensitive to very low levels of the pathogen in diseased tissues. With proper sampling, QRT-PCR can even detect the pathogen prior to symptom development. Through elaborate sample preparation, the pathogen DNA in the sample is detected by the instrument in a manner allowing for determining the amount of the pathogen in the original

sample. Theoretically, QRT-PCR could detect one bacterium, living or dead, in the sample. However, higher levels are probably needed for reliable detection.

Proper sample collection and shipping of the tissues are critical to maximize clinical testing for Pierce's Disease. Bacteria do not necessarily distribute evenly throughout the vine, and there is sometimes a poor relationship between symptom development and presence of the pathogen in the vines. There have been many research projects conducted to determine how a vine can best be sampled to insure that a reliable diagnosis results from the effort.

Leaf petioles—the portion of the leaf that attaches the blade to the stem—are the best part of the plant for testing for *X. fastidiosa* in vines. Petioles on symptomatic leaves should be given first priority. The first (oldest) leaf on a cane should be targeted for sampling, particularly if that leaf is symptomatic. Petioles should be detached, wrapped in a dry paper towel, placed in a plastic bag, and shipped to the Texas Plant Disease Diagnostic Laboratory (TPDDL). Petioles should be shipped via overnight delivery to prevent deterioration of the sample. If there is concern for delay in shipping, the petioles should be kept cool (not frozen) until they are delivered to the TPDDL.

All submissions should be accompanied by completed forms supplied through the TPDDL. These forms, along with further instructions, can be found at http://plantclinic.tamu.edu. It is important to include the forms and complete them as thoroughly as possible. Clinical diagnosis can be an extremely valuable tool in the diagnosis of Pierce's Disease, but, as with all of the



Figure 1. Symptoms on a grapevine with Texas root rot.

stages of diagnosis, it requires interpretation of results and observations in the context of vineyard conditions and other factors influencing vine health. For this reason, attempts should be made to fulfill and record all of the steps outlined in this brochure.

#### Pierce's Disease Vs. Other Grape Diseases

Under some circumstances, symptoms caused by agents other than *X. fastidiosa* may resemble those of Pierce's Disease. These may include water stress, root rot pathogens, and even the effects of cankercausing pathogens. For example, the Texas

root rot pathogen, *Phymatotrichopsis omnivora*, causes mid-to late season scorching of infected grapevines with a distinct similarity to Pierce's Disease (Figure 1). There are other times where symptoms of Pierce's Disease, as described above, may be less distinct. Diseased plants may sometimes exhibit a wide range of chlorosis (yellowing) and necrosis (browning) that are typical of a variety of vine health problems and are not useful for diagnosis. The response of the vines to any stress, including Pierce's Disease, depends on the influences of variety, climate, and site related growing conditions such as nutritional deficiencies, that add to the variability in expression of poor vine health.

These similarities emphasize the need to diagnose Pierce's Disease on the complex of potential symptoms rather than just the foliage. This includes clinical testing as an important tool in definitively diagnosing the disease.

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### Further Expansion of the Pathogen into the Texas High Plains

### - Jacy Lewis and David Appel

A risk estimate of the distribution of Pierce's Disease in Texas in the early 1970's indicated that *X. fastidiosa* was most likely to occur along the Gulf Coast, with decreasing incidence moving inland to the north and west. This estimate was based, in part, on the tropical or subtropical nature of the pathogen and the distribution at that time of commercial winegrape production in the state. As grape production increased during the 1980s, however, the distribution of the PD pathogen was also found to increase. This was initially recognized during what was perceived as an explosion of the disease in the Bell Mountain growing region of the Texas Hill Country. In time, the disease continued to be identified further and further north and west. For example, Pierce's disease was found using ELISA and pathogen isolation during this period of vineyard expansion in damaging levels in West Texas, the Davis Mountains and Escandido Valley

regions. This expansion was particularly evident with the use of the highly susceptible European varietals as the preferred grape variety of choice.

The final grape growing region considered to be safe from PD in Texas was the Texas High Plains area, again due to the low wintertime temperatures and a perceived lack of suitable



vectors. However, episodes of unexplained mortality along with symptoms that were indicative of PD infection opened the question regarding a potential involvement of *X. fastidiosa*. In 2007, disease surveys of several vineyards in the High Plains resulted in positive results in 100% of the submitted samples using ELISA and QRT-PCR. These samples were chosen from vines that appeared to be symptomatic for PD. Subsequent surveys confirmed those observations. The vines confirmed with lab testing in subsequent surveys sometimes exhibited classic symptoms of PD, including foliar scorching, petiole retention, excessive winterkill, and mortality. At times, however, patterns of mortality were less clear and were not indicative of PD. It is important to note here that this is not the only region where the pathogen has been identified in vines that were not exhibiting symptoms.

In addition, comprehensive trapping for sharpshooters yielded higher populations of vector species than previously thought to exist. Approximately 40% of randomly selected insects that

were reviewed tested positive for the pathogen using PCR techniques. This was a small subsample of the total number of trapped insects representing 4 species and 6 counties. Subsequently, annual surveys of the same vineyards have continued to yield positive tests for laboratory confirmation of PD in the Texas High Plains, making the diagnosis of the disease a reasonable conclusion.

The pathogen has yet to be isolated from plants sampled in the High Plains, leaving some doubt as to the impact of *X. fastidiosa* in that region. However, given recent expansion of the pathogen into regions previously considered safe from the disease, the presence of PD on the High Plains of Texas would be eventually expected. The establishment of vineyard production for several decades consisted of sharing plant materials, some of which probably came from other vineyards where PD was well established. Also, winter temperatures are clearly decreasing on the Texas High Plains, allowing for the survival of *X. fastidiosa* at greater levels than was possible in the past. Temperatures, however, may still be sufficiently limiting to suppress secondary transmission during cold winters and obscuring the expected patterns of disease. One final consideration in assessing the threat of PD on the High Plains is the possibility that strains other than the grape strain may be involved and are obscuring the diagnostic tests being used. It may well be, that for a variety of reasons, the impact of the PD will be diminished compared to the destruction experienced in central and south Texas. All of these factors insure the High Plains of Texas will continue to be monitored for the presence of *X. fastidiosa*.

### Insect Vectors of Pierce's Disease in Texas -Jim Kamas and Jacy Lewis

#### Background

When populations of Glassy-winged sharpshooter (GWSS) became established in California during the mid-1990's, the dynamics of disease movement within vineyards changed dramatically. This addition meant that California now had four competent vectors rather than three. The ability of distant flight and feeding through woody tissue meant that vine-to-vine transmission became routine and that feeding and inoculation took place on parts of the vine that were not normally removed during dormant pruning.

One of the initial issues facing Texas grape researchers in the late 1990's was to begin to understand the diversity, abundance, distribution and seasonality of insects responsible for disease transmission across the state. We believed that the disease was endemic in at least parts of the state, but we also believed that, at least in part, movement of the disease was limited by the range of this group of sharpshooters. Xylem feeders need a diversity of abundant food sources, leading us to believe that insect diversity and abundance would be greater in parts of the state with higher rainfall. In order for Texas growers to manage these insects, they need to understand which vectors are present, what threat they represent, when they are present in the vineyard and when they most probably are carrying *Xylella*.

Insect trapping began in 2003 with intensive trapping in the Hill Country and survey trapping done as far north as St. Jo to Cat Spring in the southeastern part of the state. In 2005, USDA/APHIS assumed the routine trapping and the survey was expanded to vineyards around the state. Yellow stick traps used to monitor GWSS in and adjacent to commercial vineyards and traps were placed on bamboo poles at approximately 1.5 meters off the ground. For some sharpshooter species, including GWSS, yellow traps are actively attractive, for other sharpshooter species it acts only as a blunder trap where catching insects is purely chance. It became apparent that insect species varied significantly by location and that different species population seasonality was equally variable. In some cases, insect trap catches were greatly influenced by outside activity such as mowing of adjacent pastures or bailing of hayfields.

#### **Vector Taxonomy and Characteristics**

Sharpshooters are insects that belong to the order Hemiptera, or true bugs, and are further classified to the Family Cicadellidae and Sub-family Cicadellinae. They are differentiated from other sub-families because they feed on xylem sap of plants rather than phloem. Within the sub-family Cicadellinae, sharpshooters are broken up into Tribes that can generally be used to describe their flight patterns, feeding patterns and efficiency at vectoring the pathogen successfully.

#### Proconiini

From the tribe Proconiini, six different species were caught across northeast, central and southeast Texas. These included *Homolodisca vitripennis* (glassy-winged shapshooter), *Homalodisca insolita* (its grass-feeding relative), *Oncometopia orbona* (relatively abundant in eastern Texas), *Paraulacizes irrorata* (present, but relatively uncommon even in the eastern parts of Texas), and *Cuerna costalis*, (common feeder on many other fruit crops with occasional

feeding forays into vineyards). These insects are distant fliers capable of moving from vineyard edge to interior locations very readily. GWSS has been documented as being capable of flying over a mile in a single flight. Once in vineyards, they commonly move down the row feeding from vine to vine. This characteristic, and the lack of Proconiini in California prior to the late 1990's, explains the very different observations between California and Texas regarding vine to vine spread of the disease. Because of their ability to fly into the interior of a vineyard and feed on grapevine tissue that will not be removed during normal dormant pruning, GWSS and related species are considered a formidable insect vector of PD, but their disease transmission efficiency is relatively low.



#### Cicadellini

Within the tribe Cicadellini numerous species of the genera Graphocephela, Draeculacephala,

*Xyphon, Sibovia, and Ciminius* have been trapped across various parts of Texas. While some species prefer grasses, and others broadleaf plants, these insects make short feeding forays into vineyards from riparian areas. Because of their smaller size, they tend to feed on tender terminal tissue rather than more lignified canes. Their transmission efficiency with each feeding episode however is much higher than the larger sharpshooter genera. Initial trapping efforts in Texas showed that human activity, such as mowing an adjacent havfield, can trigger



extremely large influxes of Cicadellini into vineyards. In California, prior to the introduction of GWSS, experiments were conducted to determine if altering the riparian habitat adjacent to a



vineyard make it less conducive to blue-green sharpshooter (*Graphocephala atropunctata*). These efforts did indeed suppress blue-green sharpshooter numbers and were successful in reducing the incidence of Pierce's disease. Given the broad diversity of sharpshooters and corresponding feeding and oviposition hosts within this group in Texas, similar efforts would most probably be futile.

#### **Other Xylem-Feeding Tribes**

Three spittlebug from the tribe Clastopterini were caught in Texas surveys. The Sunflower spittlebug, *Clastoptera xanthocephala* was the most abundant within this genus, but *Clastoptera lawsoni* and *Clastoptera lineatocollis* were also trapped. These xylem feeders are not nearly as mobile as the larger sharpshooters of the Proconiini, but they are still quite capable of carrying



Clastopterini



Clastoptera xanthocephala



Photos- I. Lauziere

and transmitting disease. We are unsure which species was responsible, but spittlebugs were found in great abundance and thought to be responsible for the widespread movement of PD at Blue Mountain Vineyard at Fort Davis.

# Lepyroniini

Lepyronia quadrangularis



Pacarina puella

Fidicinini



Photos- I. Lauziere

A single species was caught of each two other tribes of xylem feeding insects in the eight year trapping survey. *Lepryonia quadrangularis* (tribe Leproniini) and *Pacarina puella* (tribe Fidicinini) were caught in small numbers. While present, the total number of these insects were approximately 0.1%, each, of

the total xylem feeders caught over the course of the survey.

While Texas is home to a broad diversity of xylem feeding PD vectors, Lauziere, Sheather and Mitchell reported that during 2004-2006 trapping in the Hill Country, *Homalodisca vitripennis*, *Graphocephala versuta*, and *Clastoptera xanthocephala* accounted for approximately 95% of the putative vectors caught on traps.

#### Vectors on the High Plains

As described in another chapter of this work, initial insect surveys in the High Plains were not given much attention because it was believed that Pierce's disease could not survive that far north. Trapping was conducted as it was in the rest of the state with yellow sticky cards placed at approximately 1.5 meters from the ground. Sharpshooters were thought to be rare or absent in this



Photo- Roman Rakitov

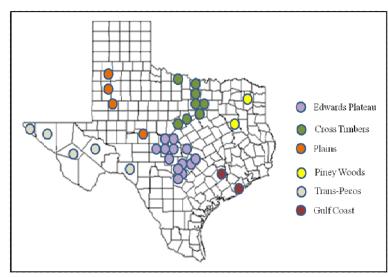
environment. In the fall of 2007, twelve different vineyards in the High Plains tested positive for *Xylella fastidiosa*. This begged the question, "What insects are moving the disease?" An independent trapping study was conducted from 2008-2009 and trapping techniques were altered to find the elusive vector species. Numerous sharpshooter species, including glassy-winged sharpshooters were indeed captured on the high plains, but the most abundant xylem feeding insects were a guild of sharpshooters from the genus *Cuerna*. These insects are relatively small members of the Proconiini tribe, but are quite capable of vectoring Pierce's disease. While many growers discount the notion of Pierce's disease in the High Plains, the pathogen appears to remain persistent in a high percentage of High Plains vineyards and vectors are present in all of them capable of moving the disease from vine to vine.

### Analysis of the Vineyard Insect Trap Project Database -

### Andrew Labay and Jim Kamas

#### **Overview of the Project**

The vineyard insect trap project was established to characterize the distribution, abundance and seasonality of various xylem-feeding insects found in Texas vineyards. The project was initiated in the spring of 2003 with 9 sites. By 2005 this number had increased to nearly 50 vineyards located throughout the state. To sample insects, 5 yellow sticky traps were placed in multiple locations within each vineyard. The traps were generally collected twice per month depending on the season, and the xylem-feeding insects were identified to the level of species. By 2011 nearly 40 thousand traps had been analyzed. Multiple journal articles have been published utilizing the



**Figure 1**: County map indicating sample locations. Over the course of the 9 year project, 51 vineyards were sampled in 34 counties (highlighted with circles) which have been categorized into 6 regions.

trap data (Lauziere et. al. 2008, Morano et. al. 2010). The objective of the current analysis is to present the general trends found in the insect trap project data on the statewide and regional level.

#### **Organization of Dataset**

Based upon both geographical location and ecological regions, the vineyard sites have been categorized into 6 regions (Figure 1). The geographical spread of the vineyard sites was designed to

represent the various grape-growing regions in the state. The number of vineyards per region and the number of traps analyzed per region however were not equal. Because of the perceived rapid change in the Pierce's disease paradigm and the proximity of vineyards to the newly formed Fredericksburg research team, the Edwards Plateau region was the heaviest region sampled and accounted for 28 of the total 51 vineyards of the project and 74% of the total traps analyzed. Due to the concentration of sample sites in this region it is likely that the statewide trends found in this analysis are biased towards the trends of the Edwards Plateau region.

#### STATEWIDE ANALYSIS

Over the course of the study, on sticky traps from all regions, 12 genera were identified belonging to 5 insect tribes (Figure 2). The dominant insect tribes found on the traps were the Proconiini (60.5%) followed by the Cicadellini (26%) and the Clastopterini (13.2%). Furthermore each of these three dominant tribes identified had a single dominant species caught: *Homolodisca vitripennis* accounted for 94% of the Proconiini, *Graphocephala versuta* accounted for 83% of the Cicadellini and *Clastoptera xanthocephala* represented 95% of the Clastopterini caught over the entire study.

There was, over time, a decrease in the average number of insects caught per day (Figure 3). This trend was commonly observed on a statewide level, on a regional level and on the individual vineyard level.

The Proconiini was the dominant insect tribe found in each year of the study and in each geographical region (Figure 4) with few exceptions. The total annual numbers of this tribe follow a decreasing trend over the course of the study and therefore this tribe, which includes *H*. *vitripennis* (the glassywinged sharpshooter), appears to be primarily responsible for the overall decrease in total insects over time.

The Cicadellini were found at relatively low levels in all regions except in the Piney Woods where they were overwhelmingly the dominant family

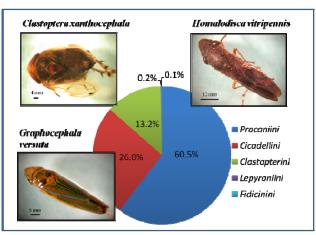


Figure 2: Percent of total insects represented by tribe with images of the dominant species found per tribe. *Lepyroniini* and *Fidicinini* represent 0.2% and 0.1% of the total insects, respectively.

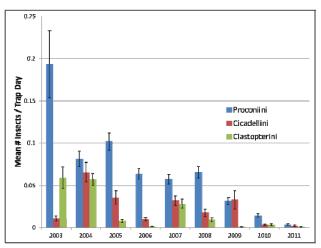


Figure 3: Mean number of insects caught per trap day for each of the dominant tribes identified in the study. Error bars represent the 95% confidence interval calculated as 2X the standard error.

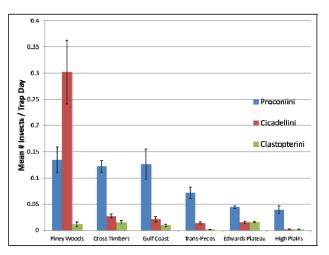


Figure 4: Mean number of insects caught per trap day for each of the dominant tribes identified in the study. Error bars represent the 95% confidence interval.

caught (Figure 4).

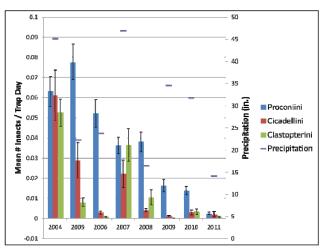
The Clastopterini were found at relatively low levels in all regions of the state. Additionally this tribe displayed relatively large peaks in 2003, 2004 and 2007 as compared to other years. These years were notable as having higher rainfall than average over the course of the study.

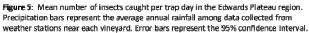
#### **REGIONAL ANALYSIS**

#### **Edwards Plateau Region**

The Edwards Plateau Region was made up of 28 vineyard sites that were collected consistently from 2004 through 2011. In 2003 there were only 6 vineyards sites. Because of the large difference in the number of locations, 2003 has been omitted from the regional analysis.

There is a downward trend of the mean numbers of all insect tribes caught in the Edwards Plateau region over time (Figure 5). This trend is most notable with the





Proconiini which was the dominant tribe caught in most years.

In the Edwards Plateau region the large spikes in the numbers of Cicadellini and Clastopterini occur in the two years with exceptionally high rainfall, 2004 and 2007. Furthermore, as discussed below the highest numbers of Cicadelllini in this region were found near river habitats (see Figure 14).

#### **Cross Timbers Region**

There was variability in the number of vineyard sites in the Cross Timbers region over the course of the study. In years 2003-2004 there were 3 vineyards, in 2005 there were 5 vineyards and from 2010-2011 there were 2 vineyards sampled. The most consistent period of widespread sampling in this region was from 2006-2009 when there were 8 vineyards sampled per year. The variability in numbers of vineyards sampled per year is reflected in increased confidence intervals of average insects caught during the years with lower numbers of sites. Furthermore site-specific variables may strongly influence mean values in years with lower numbers of

sampling sites. There is a general decrease in the average numbers of insects over time. However it is unknown, for example, whether the averages for years 2004-2005 would remain higher than years 2006-2009 if additional sites had been included.

The Proconiini was the dominant insect tribe caught in each year of the study regardless of the numbers of sites sampled. The averages of the Cicadelllini and Clastopterini were similar each year.

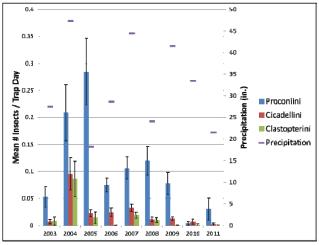


Figure 6: Mean number of insects caught per trap day in the Cross Timbers region. Precipitation bars represent the average annual rainfall among data collected from weather stations near each vineyard. Error bars represent the 95% confidence interval.

Interestingly the peak levels for these two tribes were in 2004, which had the largest annual rainfall total of the study. This trend is also present in the Edward's Plateau region and may indicate that pressure from these two tribes is highest during years of high precipitation although other variables are likely contributing to the population dynamics observed.

#### **High Plains Region**

In the High Plains region there were 4 vineyard sites sampled from 2005-2009. In 2010 and 2011 there was 1 site sampled.

The Proconiini were the dominant insect tribe caught in this region for each year of the study. There was a peak average value in year 2007 and low averages in the years 2009 and 2011. All other years of the study had similar average catch values.

The Cicadellini and Clastopterini had the lowest catch averages as compared to all other regions of the state.

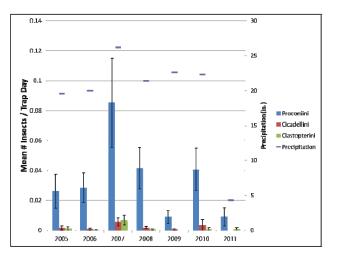
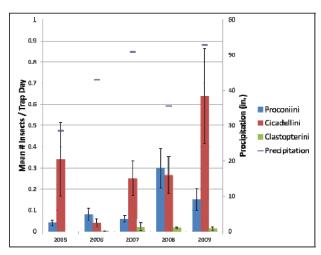
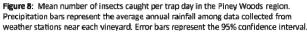


Figure 7: Mean number of insects caught per trap day in the High Plains region. Precipitation bars represent the average annual rainfall among data collected from weather stations near each vineyard. Error bars represent the 95% confidence interval.

#### **Piney Woods Region**

The Piney Woods region consisted of 2 vineyard sites which were sampled from 2005-2009. This region had the highest catch averages of Cicadellini among all vineyard sites analyzed (see Figure 4 above). This trend was consistent at both vineyards in this region.





#### 0.16 25 0.14 20 0.12 Mean # Insects / Trap Day o.1 15 🚊 Proconiini tation Cicadellini 0.08 Clastopterini 10 Precipitation 0.06 0.04 0.02 2005 2006 2007 2008 2009

#### **Trans-Pecos Region**

This region consisted of 5 vineyard sites sampled consistently from 2005-2009. The Proconiini was the dominant insect tribe caught in each year sampled, at each vineyard site.

> Figure 9: Mean number of insects caught per trap day in the Trans-Pecos region. Precipitation bars represent the average annual rainfall among data collected from weather stations near each vineyard. Error bars represent the 95% confidence interval.

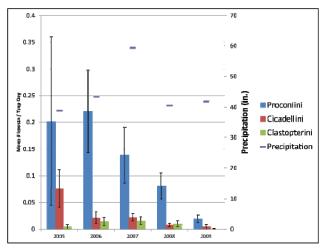


Figure 10: Mean number of insects caught per trap day in the Gulf Coast region. Precipitation bars represent the average annual rainfall among data collected from weather stations near each vineyard. Error bars represent the 95% confidence interval.

#### **Gulf Coast Region**

The Gulf Coast region consisted of 2 vineyard sites that were sampled from 2005-2009. From 2006-2009 the Proconiini was the dominant insect tribe caught. Over this period there was a decrease in the average catch of Proconiini. This decrease is similar to the trend observed in the Edward's Plateau region however in this region imidacloprid was not used. An analysis of variables that may be contributing to this downward trend in the Edwards Plateau region is found below.

#### ANALYSES OF POTENTIAL EXPLANATORY VARIABLES

The preceding graphs describe some of the general trends found in the insect study data at the statewide and the regional level. Climactic variables (e.g. rainfall and temperature), site specific variables (e.g. proximity to riparian zones) and vineyard management variables (e.g. weed management, use of insecticides) are all potentially contributing to the fluctuations of xylemfeeding insects caught per year. In order to better understand the influence of these variables on insect populations, site-specific information was collected on a subset of vineyards in the project. The focus of this assessment was upon the Edwards Plateau region due to the large number of sample sites in the region and consistency in numbers of samples per year. The Edwards Plateau region is also interesting in that it witnessed a clear decrease in the total numbers of insects caught per year over the course of the study (see Figure 5). Over 50 unique, site-specific variables were collected, many within the following categories: temperature, rainfall, weed management, insecticide use, riparian zones, geology, ecology and local vegetation. Descriptive statistical analyses were performed on each variable. The following sections represent the results from a selection of these analyses. As the insect trap project was not designed to specifically test hypotheses concerning the influence of specific variables on insect populations, the following analyses are only considered general assessments of observable trends.

#### **Soil Applied Insecticides**

Since its initial labeling in 2003, imidicloprid has been recommended for use in vineyards growing PD susceptible varieties in Texas. This product reportedly deters feeding, and if sharpshooters do feed on treated vines, they become disoriented, stop feeding and ultimately die. It was speculated that the use of this insecticide, over time, would reduce overall numbers of sharpshooters within vineyards where it was applied.

All insect traps from the Edwards Plateau region were categorized based on whether

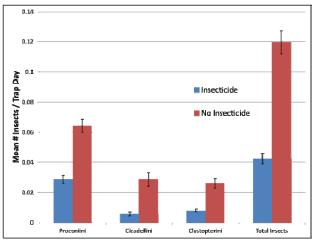


Figure 11: Mean number of insects caught per trap day for vineyards which did not use Imidacloprid insecticide versus those that did. Error bars represent the 95% confidence interval.

or not imidacloprid was used during the year of trapping. From this data, the average total insect catch was lower when imidacloprid was used as compared to when it was not used (p>0.5; Figure 11). This finding is similar for each of the three major insect tribes collected. Furthermore a significantly lower average is found in vineyards utilizing imidacloprid when each individual year of the study is analyzed (Figure 12) with the only exception being 2005.

Due to the consistency in the reduction of all insect tribes caught in vineyards that use imidacloprid, this variable is among the more significant factors affecting vineyard insect populations. However there are likely other variables leading to the downward trend in insect numbers overtime observed in the Edwards Plateau region as this downward trend was observed not only in vineyards that use imidacloprid but also in vineyards that did not (Figure 12). Consistent with this observation, there was a decrease in insect numbers, over time, in the sampled

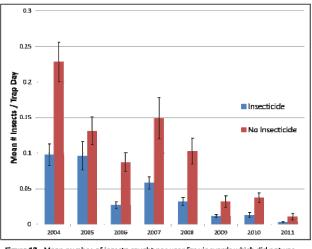


Figure 12: Mean number of insects caught per year for vineyards which did not use Imidacloprid insecticide versus those that did. Error bars represent the 95% confidence interval.

vineyards of the Gulf Coast region (see Figure 10 above) where imidacloprid is not used. Information regarding the use of imidacloprid insecticide in other regions was not recorded for this study.

#### **Influence of Proximal Riparian Habitat**

It has long been aruged that site selection plays an important role in mitigating PD risk within a geographic region. Proximity to bodies of water has been cited as a risk factor, not so much as what the risk the water itself poses, but more specifically because during the dry months of summer, relatively lush perennial plant life in close proximity of water serves as a feeding resevoir for xylem feeding insects. To assess this two separate variables were created: the distance to bodies of water (<500, 500-1000 or >2500 meters) and type of riparian habitat present (dry creek,

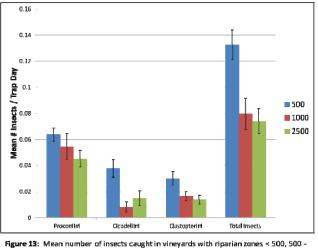


Figure 13: Mean number of insects caught in vineyards with riparian zones < 500, 500 -1000 and > 2500 meters away from the site. Error bars represent the 95% confidence interval.

pond, lake or river). For this analysis only traps that did not have imidicloprid use during the year were used to eliminate the influence of the insecticide.

There were higher average catches of Cicadellini and Clastopterini in vineyards with riparian zones less than 500 meters away as compared vineyards with riparian zones greater than 500 meters away (Figure 13). For the Proconiini, there was not a significant difference between 500

meters and 1000 meters although there was a significant difference between 500 and 2500 meters. Due to the relatively longer flight patterns of the Proconiini as compared to the Cicadellini and Clastopterini it is possible that greater riparian zone distances are necessary to have an effect on insect catches. Further analysis would be necessary to verify this hypothesis.

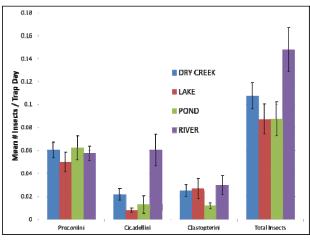


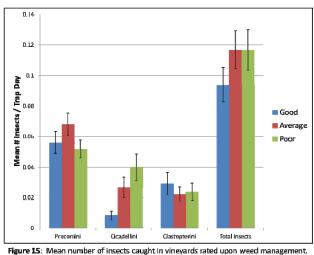
Figure 14: Mean number of insects caught in vineyards various types of riparian zones. Error bars represent the 95% confidence interval.

For the Proconiini and Clastopterini there was very little difference between the average catches present near the different riparian zone habitats (Figure 14). However in the case of the Cicadellini a different pattern emerged. For this tribe there was up to a 3-fold increase in the average catch near river habitats as compared to all others. Furthermore, the average catch was greatest in 2004, 2005 and 2007 (see Figure 5 above) which were notable as years having large rainfall totals. Proximity to perennial stream habitats and high annual rainfall could be among the more important variables for Cicadellini insect populations in the Edwards Plateau region.

#### **Vineyard Floor Management**

Our working hypothesis, based on anecdotal observations, was that managing competitive vegetation in and around susceptible grape vineyards plays an important role in reducing the risk of PD. Both grasses and broadleaved plants provide sharpshooters with an alternative feeding host and, perhaps, sites for egg ovoposition in close proximity to the plants we are trying to protect.

Vineyards were rated on vineyard floor weed management by regional viticultural



Error bars represent the 95% confidence interval

advisors based upon observation over the course of the entire study. Vineyards were scored as good, average or poor but ratings were not broken down to year-to-year management variation. Only data was used for traps that did not apply imidacloprid during the trapping year.

There was no significant difference found in Proconiini and Clastopterini averages between the different weed management ratings (Figure 15). This trend was largely consistent on a year to year basis in the study (data not shown). Considering the Cicadellini there was a trend of increasing trap averages with decreasing weed management, however it should be noted that the riparian zone habitat type variable may be confounding these results as all vineyards next to river habitats were assigned either average or poor weed management. A controlled study is warranted to further elucidate the effect of weed management on Cicadellini distributions in vineyards.

#### **Influence of Seasonal Weather**

The insect trap data, as mentioned above, shows significantly lower annual insect numbers in vineyards that use imidacloprid as compared to vineyards that do not use the insecticide. However the use of insecticide alone does not appear to be the primary factor contributing to the decreasing trend of average trap catches over the course of this study as the averages have equally decreased in vineyards that did not use the insecticide as compared to those that did. In this section we examine the influence of weather on this decreasing trend, and more specifically, as neither yearly nor seasonal rainfall were found to correlate to average insect numbers (data not shown), the focus of this section is on winter temperature.

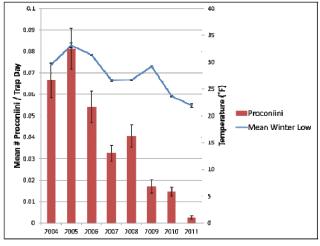


Figure 16: Mean number of Proconiini caught in vineyards per year and the mean winter low temperature calculated as the mean absolute low temperature from December - February. Error bars represent the 95% confidence interval.

Winter temperatures have been used in California to estimate the survivorship of overwintering adult sharpshooters (Proconiini) and thus estimate the size of offspring populations of sharpshooters in the spring (Johnson et. al. 2010). For the current Texas insect trap study absolute low and high monthly temperature data was collected at each trap site. A winter low temperature variable was created by calculating the average of these absolute low temperatures recorded from December through February. There is a decreasing trend of this winter low temperature variable recorded over the

course of the study (Figure 16). Furthermore there was a linear relationship observed between this variable and Proconiini numbers such that low winter temperatures were associated with low Proconiini trap averages in the following season and vice versa (Figure 17). No relationship was found between insect numbers and absolute high temperatures; nor between winter low temperatures and either the Cicadelini or Clastopterini average yearly trap values (data not shown). The year 2009 is an outlier in this correlation (see Figure 16) as a higher winter temperature average should have been associated with an increase in insect numbers. Other variables may have contributed to the low trap numbers during this year. The winter of 2008-2009 is notable in having the lowest rainfall totals of all winters in the study and 2008 had the second lowest precipitation total (16.5 inches) of all years in the study. While rainfall totals alone may not correlate with Proconiini catch numbers it could be an important variable nevertheless.

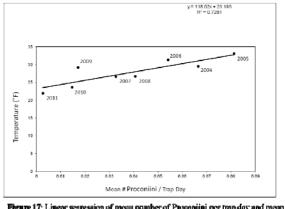


Figure 17: Linear segression of mean number of Procentini per trap day and mean winter low temperature (P=0.007).

Precisely how winter low temperatures may affect sharpshooter populations and how winter mortality may effect annual population dynamics is unclear. Work in California has focused on results which show that sharpshooter feeding is significantly decreased below 50°F and sustained periods below this temperature leads to mortality (see Johnson et. al. 2010). From this information a cooling degree-day (CDD) variable was calculated by researchers and used to estimate survival of

overwintering sharpshooter populations. Whether a similar CDD estimate correlates with sharpshooter mortality in Texas remains untested. However, if a strong association is found, this information could be usful in developing models for the estimation of local sharpshooter population sizes and evaluating year-to-year vineyard risk.

#### CONCLUSIONS AND PERSPECTIVES

The information presented here represents, firstly, an overview of the data recorded during the insect trap project in addition to an exploration of some of the variables which may affect vineyard insect populations. The database created during this project remains a valuable source of information that can be utilized for future research efforts.

To further elucidate the variables which affect xylem feeding insect populations in vineyards, and help substantiate findings in this study, addition analyses are necessary. Due to the large number of variables collected an exploratory statistical test such as a principal component analysis (PCA) could be valuable.

The data indicate that vineyards which have used imidacloprid insecticides have seen significantly lower numbers of insects in nearly all years of the study. The importance of this insecticide in controlling PD vectors is thus supported. However other variables, including environmental variables such as winter temperatures, likely have a strong influence on insect population dynamics and could be behind the overall downward trend observed in the Edwards Plateau region and beyond. Over the past few years overall insect numbers and PD have been on the decline in central Texas. The return of warm winters may lead to increases in sharpshooter

populations and increased risk of PD. Understanding vector insect population dynamics and vector management practices will be important to controlling PD on a consistent basis.

#### Acknowledgements

The authors wish to acknowledge the work of the USDA/APHIS trapping crews for their efforts in deploying and recovering insect traps in all of the statewide trapping locations and the work of the staff of the F. Mitchell and I. Lauziere laboratories for insect identification and data entry.

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# MANAGING & MONITORING VECTORS IN THE VINEYARD

# Monitoring Vectors in and around the vineyard - Jacy L. Lewis

#### Introduction

During the tenure of the Texas Pierce's Disease Program, a number of techniques have been employed in the monitoring of insects both in and around vineyards. The use of yellow sticky card traps as well as sweep netting have been the most commonly employed tactics with sticky card traps being the preferred method within the vineyard, and a preference for sweep netting outside the vineyard. While other techniques including visual inspection of plant material and vacuuming have been employed, they have not been significant in obtaining usable metrics for analysis of putative vector populations.

From the beginning of the program until the 2008 growing season, trapping for assessment of insect presence and seasonal dynamics was limited to areas where Pierces disease was currently being diagnosed and little was currently known about insect vector pressure. During this time period some growing regions were ignored or under-monitored due to long standing biases regarding the perceived lack of a potential for the disease or the occurrence of vectors in these areas. After the initial positive identification of the disease causing pathogen in conjunction with apparently symptomatic vines in two of these areas, a research study designed to address this omission was proposed. Important ecological differences in these additional areas made a new sampling protocol necessary in order to fully evaluate the presence of putative vectors in these regions. Ultimately, the High Plains region was selected for reassessment due to the large number of acreage grown in this region as well as proximity to research personnel.

As of 2008, the standard protocol for the monitoring of putative vectors in Texas vineyards was the placement of 9x5.5" yellow sticky card traps at approximately 5' to 6' heights, in a cross pattern in the vineyard representing the cardinal compass points and crossing through the center of the vineyard block. This trap height and placement was very efficient for the assessment of GWSS in regions where abundance of this species was high. Vineyards were chosen for their proximity to the Hill Country PD Center as well as cooperation of vineyard owners and previously recognized disease status of vineyards. Traps were collected on a monthly basis. While insect pressure was observed in areas outside and adjacent to vineyards, this information was collected in an anecdotal fashion.

This technique was successfully employed in a number of wine grape growing regions throughout the state from 2004 to 2010. In areas where GWSS populations are determined to be

high, concentration on other putative vectors is not necessary for adequate control measures to be initiated as the recommended control for GWSS is adequate in controlling other species which may contribute to spread of the disease.

Paucity of knowledge with regard to the occurrence of many species of insects known to have the potential to vector Pierces Disease in at least two major growing regions in the state may be attributed to the combined effect of a lack of sampling effort and poor sampling efficiency in these regions. This lack of effort resulted from the prioritization of other areas over these due to long held beliefs and biases regarding the ability of the pathogen to survive in this environment and the ability of putative vectors to successfully establish themselves in these ecosystems. Prioritizations were necessary and reasonable due to time and budgetary constraints. While hindsight may serve as a warning regarding the directing of research based on untested assumptions, the decisions were justifiable at the time given historical understanding of the pathogen.

In order to remedy this situation and increase the level of understanding regarding the suite of putative vectors in one of these areas, a novel sampling protocol was established. This protocol was established in order to address the presumed effects of minimal trapping effort in the area in previous seasons, as well as the poor efficiency of the current trapping protocol. Differences in species richness, diversity and species abundance in this region resulted in the need for a sampling protocol tailored to the specific attributes of the vector ecology of this area.

#### The importance of functional groups.

The first priority in the design of a sampling protocol is defining the species(s) to be assessed. The most common way of defining a population to be studied or sampled is by individual species. This is an appropriate approach when a single species is of some functional interest to the study. While this is often the case, it is commonly recognized that when the primary area of focus in a study is the impact the study organism may have on an environment, it may be more appropriate to group together a suite of organisms into a functional group and study that entire group as a single functioning organism in the system. In a situation where multiple organisms in a system are all capable of vectoring the same pathogen, which is the case for Pierces disease, it is appropriate to design a study with the ability to analyze the impact of these species as a single functional group. When disease epidemiology is the focus of research where functional groups exist, it is important that an understanding of the entire group is obtained in order to have a fully functional model of the potential for pathogen transmission and disease occurrence given specific environmental and ecological variables.

While the scope of this essay focuses on how the existence of these functional groups can impact sampling methodology, it is important to note that the composition of these functional groups have the potential to dramatically impact the epidemiology of the disease in regions where they occur. This affect can result from changes in level of exposure resulting from shortening or

prolonging of exposure to the pathogen over time, by increasing or decreasing the number of exposure events during a given time frame or changing the site of exposure as a result of different feeding habits. Additionally, the transmission efficiency of the various species may vary and must also be taken into account in any model used to describe the potential for disease spread.

The impact this has on the sampling methodology of the study is that now rather than tailoring the trapping protocol to a single organism, it must be adjusted to provide equal effort in trapping all of the members of this functional group. There are a number of factors that can add to the complexity of sampling in this fashion. If the individual species of this group has different habits in terms of feeding preference, mobility, seasonal occurrence and microhabitat preference; all of these factors must be taken into account when designing and placing traps for sampling purposes.

#### How differences in abundance affect sampling efficacy.

In order to recognize how differences in abundance can affect the efficacy of a given sampling protocol, it is important to first understand what is meant by the term abundance and how this metric might be specifically applied in the context of this program. In general, abundance is a measure of population density of an organism in a given area. Whether a given measure of abundance is high or low is a matter of a number of factors; size of organism, size of area measured, organism's behaviors and range etc. For a well studied organism, a range of average densities may have been established, in this case whether abundance is high or low will depend on where the density number falls when compared to that known range. When no average density has been established, it is possible to make preliminary inferences about an ideal density range based on the known ranges of other closely related species.

Another important measure is that of relative abundance. In measures of relative abundance the measure of the number of individuals of a given organism is taken as a ratio of the total number of organisms occupying the same area. This may be done in very general terms; or can be measured in more specific terms. For instance we might want to know the relative abundance of a given species of mammal as compared to all mammals in an area, or as compared to all animals in an area and finally as compared to all of the biota of an area. Obviously these numbers might be very different, and how relative abundance is measured is determined by the type of information regarding the ecology of that species that is needed for a given study.

In this particular study, when one is primarily sampling for GWSS, an important metric for determining total number of traps needed and/or rate of deployment in a given area is total abundance of GWSS. However, if there are a large number of insects that might also be attracted to the traps being utilized in this study, then relative abundance of GWSS to all insects that might be attracted to this trap could also prove important. In a situation where very few species other than GWSS will be attracted to the trap, then fewer traps might be necessary than might be the

case if a large number of species are attracted to the traps, depending on the abundance of these other species. This is especially the case if the traps can be coated with insects and then become incapable of continued capture. It is evident here how an understanding of the abundance and relative abundance of insects that might potentially be trapped with your sampling method is necessary in order to make the best decisions about sampling for a given species.

Differences in abundance affect how intensively an area must be sampled in order to make assumptions regarding the presence and abundance of a species or group. When abundance is high, fewer traps are required to sample for presence and changes in the density of the target species/group over time. When abundance is low, many more traps per given area are required in order to obtain this same information.

#### Richness vs. Diversity and how each can affect sampling efficacy.

The affects that total abundance can have on trapping efforts is generally obvious. It stands to reason that in circumstances where the total number of individuals of a given "type" in this case putative vectors of Pierce's disease is low, then the number of traps required to detect the presence of these individuals will increase. What may be less obvious is how both diversity and species richness may affect this effort. Even in situations where total abundance of individuals of a given class, in this case all individuals who may act as vectors of Pierce's disease; regardless of species, species richness and low diversity can adversely affect trapping efforts if not taken into account in research design and sampling methodology.

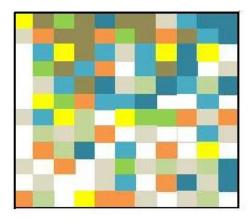
In addition to understanding something about the ecology of the species(s) that is being sampled; it is important to understand something about the general ecology of the area being sampled with regard to number, type, and distribution of species in the area. Metrics regarding organisms as well as the biotic elements of their habitat are not independent of each other, but are in fact components of one another. It is important that all of these factors are considered when designing a study aimed at an understanding of the presence, abundance and in this case feeding behaviors of an organism(s).

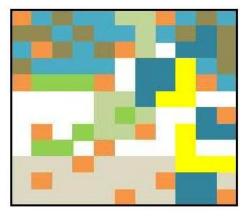
Two interrelated factors that play an important role in sampling efficiency are richness and diversity. While richness is used to describe a habitat based primarily on the number of different species or groups of species that occur, diversity goes a step further in describing how these species are distributed within a habitat.

Richness along with abundance is important in determining the number of traps that might be required in order that the sampling effort be adequate to obtain samples of a given target species or group of species. In a situation where richness is low, one can assume that if the targeted species/group occurs in the region, then a large percentage of the species in this environment will be of the type that is being targeted. Like abundance, this may have an effect on the total number of traps required to produce a strong sampling effort. Additionally, this may give insight into various types of traps required if multiple species of an ecological functional group is of interest.

The other important descriptor of the habitat being sampled is that of diversity. Diversity describes how the species within this habitat are distributed or "spread out". When diversity of a given habitat is high, species are more or less equally distributed across the landscape. When diversity is low, the same number of overall species may occur, but their distribution can be better described as clumped. Understanding the diversity of the area being monitored will aid in understanding how the given traps should be distributed in the environment. If diversity is low, and it is not known exactly where the targeted species may be found within this habitat area, then trap placement that covers the entire habitat region becomes much more important in order to obtain reliable metrics for the given species or group.

In the two below images, richness is the same. However, the image on the left demonstrates a landscape with greater diversity than that of the image on the right.





#### Novel techniques employed in previously under sampled growing areas.

While previous protocols appeared sufficient for sampling directed primarily at a single vector species that was known to occur in reasonably high numbers in the areas monitored; this method would not prove sufficient under different ecological and environmental conditions. After a preliminary evaluation of the region, shortcomings in the efficacy of previously employed sampling protocols became apparent. Rather than focus the effort on a single species, the effort was refocused to include a functional group of putative vector species. Species richness, diversity and abundance were all factors that necessarily had to be accounted for in the design of a novel sampling protocol for this region.

To address trapping effort, a large number of vineyards and areas adjacent to and some distance from vineyards were selected for sampling from June 2008 to November 2009. These sites were sampled on a bi-weekly basis. This decrease in sampling intervals resulted in an increase of both sampling effort and efficiency. To more specifically address the lack of sampling efficiency; sample blocks were standardized to approximately one acre and the number of traps per block

was increased to 20. These traps were placed randomly within the selected block. The standardization of the sample area size as well as the random placement of traps within these areas increased efficiency by reducing the effects of bias on trap placement and increasing the potential power of future analyses by providing for an equal sampling effort between areas. To further increase the power of future analyses, the region was divided into localized areas and sampling sights were selected both randomly and with a bias towards vineyards with positive pathogen tests within these areas. Because placement of vineyards within this region are both non-random and clumped, breaking this area into "site areas" or mini-regions allows for the traps within each site area to be treated as a single sample area in order to consider landscape level analyses for the region.

This study found putative vectors of Pierce's Disease in every mini-region sampled in this study of the High Plains growing region. Vectors were found to reside both inside and outside of vineyards. These insects were trapped during almost every month of the year including late into November. GWSS were found on traps in areas outside of vineyards as early as January of 2009. The continuous sampling throughout the year and the findings of many of these insects including GWSS in the same locations throughout the year is suggestive of resident rather than transient populations of these insects.

One question of common concern is how it is that large numbers of putative vectors have gone undetected for such a long period of time in this growing region. First and foremost, it can be difficult to find what you are not looking for. Most of these species are quite small, fast, and generally innocuous if not for their potential to spread disease. Additionally it is important to recall how older trapping methodologies were poorly suited to the ecological and environmental challenges sampling in this region is presents.

#### Monitoring your own vineyard.

From a practical perspective, how can this information be utilized in order to set up a monitoring program in your own vineyard? Monitoring of insect pests in the vineyard can be a valuable tool in your pest and disease management program. Not only can this information make it possible to identify the necessity of implementing control measures, but it can help you to pinpoint the best possible timing for these measures and perhaps over time determine efficacy of implemented control strategies. Collection of data over a number of seasons can be used to help make predictions about seasonal insect pressure in subsequent years.

While you may not be aware of all of the ecological factors impacting your own trapping program, you can obtain some guidance from your local viticulture specialist. In most cases implementing a trapping regime that includes 3 to 5 traps per acre should be sufficient in helping you to identify pests as they occur in your vineyard. Approximately half of these traps should be placed within six feet of the perimeter of the vineyard and the other half should be randomly placed within the vineyard interior. This placement is recommended for the entire vineyard not

per acre. In other words, each acre does not need this ratio of edge to interior traps, but it should be approximated by the total number of traps in the vineyard. The traps should be monitored on a regular basis as a "walk by" measure. What constitutes a regular basis may change from season to season; the best way to implement this is to make it a part of your regular routine for checking vine health and fruit maturity. Please see the insect vector chapters of this manual for photographs of putative vectors in order to aid in your identification. More or less traps may be required depending on specific circumstances and conditions, but this protocol should be sufficient in most cases and can serve as a starting point from which you can refine it for your particular circumstances. In vineyard blocks of greater than 5 acres, the lower number of 3 traps per acre should be sufficient, taking care that there is good coverage of the perimeter of the block as well as the interior.

#### Using this information in your management program.

How can you use what you find to make management decisions? Do lower numbers of insects mean decreased disease risk? Yes and No. In short; maybe. The situation is complex and many of the variables remain unknown.

It is important to remember that so little research has been done into the communities of vectors in some regions, that there are really not good estimates for what constitutes large vs. small absolute numbers of vectors of any given species. While data is available regarding expected numbers of insects in some heavily researched areas, comparisons to absolute numbers in other regions may or may not be relevant in a region where the environmental and community dynamics may be vastly different.

When describing the importance of abundance, an important factor that need be addressed is that of relative abundance. In other words, the exact numbers of a sampled organism or functional group may be less important than those numbers relative to some other ecological factor. In this case food sources may be that factor. Therefore, comparisons of raw numbers in a habitat of heavily abundant food sources to an environment where sources of food may be more limited would not be valid.

The safest bet in any region where PD is known to occur is to treat your vineyard as if it is always at risk. When cultural or budgetary constraints make this type of prophylactic measure prohibitive, then it would be most prudent to initiate treatment after finding known vectors of the disease in your vineyard. In a situation where there is not good data available to make interpretations about population numbers based on trap counts, nor a strong understanding of how feeding pressure may be correlated with disease transmission; a conservative strategy to management is recommended.

# Soil Applied Neonicotinoid Insecticides

### Jim Kamas

For grape growers in low, moderate and even relatively high risk areas, the single greatest tool we have to protect susceptible grapevine varieties from *Xylella* carrying sharpshooters are the group of synthetic insecticides within the nicotinoid group. This insecticide group acts on the central nervous system of insects, but was fast-tracked by EPA for registration because of the relatively low toxicity on mammals. Within this class of insecticides, there are several specific chemistries that have different persistence in plants and soil. These materials are systemic within plants and have relatively high activity against insects with a piercing/sucking feeding mechanism. Although systemic, it appears that the movement of neonicotinoids is impeded into fruit. Modern techniques now allow the level of detection in the low part per billion range and minute levels of neonicotinoid insecticides can be found. Application according to labeled rates and following posted pre-harvest intervals will result in residues well within tolerances established by EPA. At the writing of this document, there are three specific neonicotinoid products labeled for the control of vectors of Pierce's disease in the United States.

#### **Currently Labeled Soil-applied Materials**

Imidacloprid- This chemistry was the first labeled for grapes and can be applied either as a foliar spray or as a material injected through the drip system. The quality and duration of plant protection is much greater when applied through the drip system. The material is taken up by grapevine roots and is moved systemically throughout the plant. The advantage a soil application is that uptake continues well after application which means that new growth is continually supplied with a supply of newly taken-up material, protecting the vine throughout the season. Bayer chemical company first registered the injectable formulation of imidacloprid as Admire®. Considerable work was done on this product prior to and after registration by Drs. Nick Toscano and Frank Byrne, from the University of California at Riverside. He developed rates and application logistics for citrus and grape growers that provided season-long management of sharpshooters. It should be noted that all of the work done on these products was conducted specifically for management of Glassy-winged sharpshooter. We have no reason to believe otherwise and in fact we have some Texas trapping data that indicts imidacloprid provides excellent control of all of the 30+ species of Texas putative PD vectors.

Work on imidacloprid indicates that it acts first as a feeding deterrent. When sharpshooters move from riparian areas into vineyards, the scent given off by vines adequately dosed with imidacloprid inhibit the sharpshooters from probing grapevine tissue. If sharpshooters do feed, the initial ingestion of imidacloprid causes cessation of feeding. Sharpshooters then become disoriented and ultimately die. This greatly improves the defenses from vine to vine movement. Although the original Bayer label recommended a single full rate application of imidacloprid, work done by Toscano and Byrne showed that split, half-rate applications, made 30 days apart,

provided better control. This split application results in imidacloprid levels within vines high enough to deter and kill sharpshooters for at least twelve months.

Now that the patent has expired on the initial imidacloprid product Admire®, numerous generic formulations are being produced and sold with a label for application in vineyards. At this writing, there are products with 2 lbs. of active ingredient per gallon and products with 4 lbs. of active ingredient per gallon available. The labeled rate on the 2lb. products is 32 ounces per acre while 4lb. products are labeled at 16 ounces per acre. The Bayer product is currently being produced and sold as Admire Pro. which contains 4.6 pounds of active ingredient per gallon. The full labeled rate of Admire Pro is 14 ounces per acre. All evidence seems to indicate that there is no difference in the level of control between any of these products as long as the full rate is applied and all other timing and logistical recommendations are followed. Most growers make material choices between these products based on economics. Toscano also suggests that first and second leaf vineyards can be well protected with half-rate applications. Current label restrictions cite a 30 day pre-harvest interval and a 12 hour re-entry interval (unless injected and "there is no contact with treated material")

Toscano and Byrne investigated cases of reported imidacloprid product failure in Napa. Their findings indicated that on some soils, and under some circumstances, imidacloprid became chemically bound on to soil colloids and unavailable for uptake by grapevines. This is primarily because of two chemical properties of imidacloprid- its insolubility in water and its high coefficient of sorption ( $K_{oc}$ ). Its high insolubility makes imidacloprid less subject to leaching through the soil profile, but also less available to vines under low soil moisture situations. The high  $K_{oc}$  also binds the imidacloprid molecules so tightly to soil exchange sites that once again, the material is unavailable for uptake by the vines. In hindsight, it is most likely that this phenomenon occurred in Napa on sites with very high clay content that were irrigated very little or not at all. Studies on very poorly structured clay vineyard sites in Texas have shown when made as a split application, with appropriate irrigation practices, there has been adequate uptake that has resulted in more than twelve month protection.

There are also numerous formulations of imidacloprid that are labeled for soil application on ornamental plants. This allows us to continue to grow ornamental plants in or around vineyards without having the negative impact of providing additional feeding and reproductive hosts for sharpshooters. Further discussion of this topic is addressed in other sections of this guide.

<u>Thiamethoxam</u>- Currently, this product sold only under the trade name Platinum is labeled for use in vineyards when applied through the drip system. Thiamethoxam is roughly eight times more water soluble than imidacloprid, and the  $K_{oc}$  is roughly three times less than imidacloprid, which means it is held less tightly by soil particles and is much more subject to leaching. This means that on heavy soils, especially under no or minimal irrigation, this material is theoretically more available for uptake by grapevines. Currently, the labeled rate of thiamethoxam is from 8 to 17 ounces per acre with a maximum annual application rate of 17 ounces per acre. The 2011

label carries with it a 60 day pre-harvest interval with a 12 hour re-entry interval. At present, we have little experience with the length of time this material remains active within grapevines, so there are no additional application recommendations other than those that appear on the label.

<u>Dinotefuran</u>- This product approved for application in vineyard has the trade name Venom and is labeled for both soil and foliar applications. Most grape growers seeking to manage sharpshooters are making applications through the drip system because there are many other foliar products that carry shorter pre-harvest intervals. Dinotefuran is seventy-seven times more water soluble and its  $K_{oc}$  is only one fourteenth that of imidacloprid. Very loosely held by soil exchange sites and extremely water soluble, dinotefuran can be a valuable tool in some situations but may fail under high rainfall and potentially pose a problem due to leaching. Current vineyard soil application rates range from 5 to 6 ounces per acre with a 12 ounce per acre, per year maximum. Pre-harvest interval is 28 days and re-entry interval is 12 hours. Like thiamethoxam, we have little data on the persistence of effective doses of this material in grapevines.

#### **Timing of Soil Applied Neonicotinoid Applications**

In California, sharpshooter feeding in vineyards begins very shortly after bud-break, but in Texas, we typically see very little sharpshooter activity in vineyards (except the High Plains) in Texas vineyards until approximately the end of March. Because of this timing, our goal is to have the full rate of neonicotinoid insecticide applied at least two weeks ahead of anticipated movement. Timings in far south Texas may be ahead of this schedule, but for most low, moderate, and relatively high risk locations in Texas, we suggest that the first application of imidacloprid be made by approximately April 15th, with the second half of the dosage made one month later, approximately May 15th. Uptake in grapevines takes place usually within 48 hours, but may take as long as 7 days. We believe that the split application peaks insecticide concentration shortly before anticipated insect entry into vineyards. Splitting this application also extends the window of effective insecticide concentration in vines for at least 12 months. Annual applications with this protocol provide continual season-to-season levels of imidacloprid capable of deterring and killing feeding sharpshooters.

If either dinotefuran or thiamethoxam are used, it is suggested that single application of these materials occur shortly before the end of May. If allowed by label, subsequent applications can be made later in the season, but no later than the pre-harvest interval will allow. We do not know how long these materials will stay active, and yes, these materials can be applied concurrently. However, the economics of insecticide choices at this time highly favor using some formulation imidacloprid rather than either of these other two options.

#### **Irrigation, Timing & Application Logistics**

April 15th and May 15 are general target dates for half-rate imidacloprid applications in most

Texas Vineyards. For uptake to be effective, we suggest that vines have at least five inches of shoot growth to create a sufficient transpirational stream. In most areas of Texas, It is highly recommended that vines be well watered at least a week prior to the first application. Remember, when soil is dry, neonicotinoid insecticides are more readily subject to being bound by soil particles. Keeping the irrigation zone at or near field capacity will optimize material availability and uptake by roots. In the High Plains, we have less empirical data on when sharpshooters routinely move from riparian areas into the vineyards. Especially because of the lack of other vegetative growth for sharpshooters to feed on, High Plains growers should consider making these applications



In the "Closed Position", Mechanical Injection Pumps Can Simplify Accurate Injection Through Drip Systems earlier in order to protect grapevines earlier in the season

Uniform distribution of water through a drip irrigation system is essential for the accurate placement

for the accurate placement and dosage of neonicotinoid insecticides in the vineyard. Make sure lines have been thoroughly flushed and that emitters are all flowing to specifications. Injecting acid through drip lines is commonly used to remove calcium and other mineral buildup. There are several mechanical methods of injecting neonicotinoid insecticides. Simple siphon mechanisms can be constructed in irrigation head houses or within individual blocks within the vineyard. Bypass units can be installed in irrigation main lines that allow for siphon hoses to be attached and material injected from open containers. Normally, valves restrict the flow of irrigation water through the injection device. When growers want to inject materials, either insecticides or nutrients, the main

line is closed and the injection loop is opened diverting water into the loop. Tubing attached to a "T" will draw material into the irrigation system. While not as precise as mechanical dosing units, with attention to detail, careful monitoring of irrigation timing and logistics, these simple mechanisms can accurately deliver an effective application of insecticides. Mechanical or



With Careful Attention to Detail and Application Logistics, Simple Irrigation Loops Can Accurately Deliver Reliable Rates of Injectable Insecticides

electrical injection devices are also available that can simplify material injection through the drip system.

Before injecting insecticides through a drip system, make sure a backflow preventer has been installed and is working effectively. This will prevent potential backflow of insecticide into a water tank or well. When starting an injection application, start the irrigation process and time how long it takes water to begin flowing at a sustained rate at the most distant emitter. Begin the injection process and make sure chemical containers are completely drained, then add some water to rinse the holding tank. Once that is also drained, continue to run the irrigation system for at least as long as it took from the start-up of the system to the time injection began. Some growers add special agricultural dyes to the insecticide mix tank to enhance the ability to know when insecticide is in the system and when it is thoroughly flushed from irrigation lines.



Agricultural Dyes Can Help Determine When Insecticides are Flowing Within An Irrigation System.

While the timing of insecticide injections may vary because of weather or logistical complications, growers should be acutely aware of the prescribed Pre-Harvest Interval (PHI). These restrictions have been developed to ensure that potential chemical residues are well within EPA's established tolerance levels.

# Using Contact Insecticides - Jim Kamas

Before the most recent research on Pierce's disease was started, the vineyards in higher risk parts of Texas depended on contact insecticides to manage insect vectors. There was no understanding of what the vectors were, when they were active and which insecticides worked best, it was simply guess work. Effectiveness was limited by weather and economics, but the cost of no control action was not considered an option. The use of neonicotinoid insecticides applied through the drip system has dramatically altered our approach to sharpshooter management. These insecticides are only active against insects directly feeding on treated grapevines and have little or no impact on beneficial insects and arachnids living in the vineyard. Although many experts think there is minimal risk for resistance to this class of chemistry, the possibility does exist. Some growers choose to also apply contact insecticides to control vectors and potentially manage resistance. More commonly, growers may need to apply an insecticide for another insect pest and want to select a product that will also be active against sharpshooters. We do not recommend that growers rely on contact insecticides alone to manage insect vectors of Pierce's disease. This brief overview of currently labeled insecticides is intended to only offer guidance on the effectiveness and potential side effects of specific material choices.

#### **Early GWSS Insecticide Trials**

Po	Post-application exposure (days)†			
Treatment	1	2	6	
Endosulfan	81 c‡	85 bc	95 b	
Fenpropathrin	100 c	100 c	100 b	
Phosmet	78 c	87 bc	100 b	
Dimethoate	54 b	77 b	85 b	
Thiamethoxam	97 c	100 c	97 b	
Control	0 a	0 a	0 a	

\*Mortality expressed as % efficacy (percentage reduction from control).

†Twenty-five GWSS placed in cages before insecticide application with exposure given in postapplication days. Data based on mean of three replicates of each treatment. A replicate equals one caged grapevine with 25 GWSS.

‡Percents in columns with different letters are significantly different by ANOVA and LSD at P  $\leq$  0.05; analysis is based on mean-transformed data,  $\sqrt{(x + \frac{1}{2})}$ .

In 2000, after Glassy-winged sharpshooter became established in southern California, Akey, Henneberry and Toscano screened products labeled in vineyards at the time and experimental insecticides for efficacy against GWSS in Temecula, California. Although there were differences in how quickly each of these materials killed sharpshooter populations, after six days, all had statistically similar results. The most rapid GWSS efficacy came from fenpropathrin (Danito®). Danitol® is labeled for a number of insect pests of grapevines, has excellent efficacy against grape berry moth, but is considerably more expensive than other material options. Endosulfan (Thionex®) labeled for control of phylloxera had good results, but this product is being phased out by EPA and

registration is expected to expire in vineyards by 2012

Imidan® (phosmet), like Danitol®, had 100% GWSS efficacy after six days. Imidan® is thought to be relatively easy on beneficial insects, but has only average activity against grape berry moth. Its 14 day pre-harvest interval makes it less valuable in managing fruit feeding insects near harvest. The material tested with the slowest, and numerically lowest efficacy was

dimethoate. Previously labeled and used for sharpshooter management in riparian habitat, it is no longer labeled for use in vineyards.

#### Foliar Nicotinoid Insecticides

There are currently two other nicotinoid insecticides labeled for foliar application on grape. These products have the advantage of being locally systemic, making them resistant to removal by rainfall. Provado® is the formulation of imidaclorid labeled for foliar application in vineyards. It is relatively weak against grape berry moth, but has won favor in management of metallic June beetle because it has a 0 day pre-harvest interval. Research by Nick Toscano in California indicates that applications of Provado® persist for two to three weeks in grapevines. It should go without saying that using this product does nothing to aid in management of resistance to soil-applied imidacloprid. Assail® (acetamiprid) is another nicotinoid insecticide labeled for use in vineyards against a number of insect pests including sharpshooters. Acetamiprid most probably is not helpful in managing insect resistance to imidacloprid.

#### **Other Insecticide Options**

Carbaryl (Sevin®) has long been a cost effective insecticide option employed to manage 30+ insect pests of grapevine foliage and fruit. It has good activity against grape berry moth, pholoem feeding leafhoppers and numerous other insect pests of grapevines. Repeated application of carbaryl is known to trigger mite outbreaks, which while uncommon in Texas vineyards, is common in many other annual and perennial crops. Carbaryl has a seven day preharvest interval in grape.

Numerous other insecticides are labeled for use in vineyards, but specific activity against sharpshooters are unknown. Pyrethroid insecticides such as Evergreen® and bifenthrin products such as Brigade® are broad spectrum insecticides but are considered harsh on natural predators, bees and aquatic organisms. Pyrethroids are also known to trigger mite outbreaks. Diazanon® as well as other organophosphate insecticides are labeled for use in vineyards, but there appear to be better material choices for management of PD vectors as well as other important insect pests.

It needs to be restated that foliar insecticides are not the recommended way of managing insect vectors of Pierce's disease. Soil applied nicotinoids are more effective, more economical and have far less negative impact on natural predators and the environment. At times foliar insecticides are called for. Growers should make their choices based on efficacy against targeted insect pests, economics, and collateral consequences on the environment and subsequent secondary insect pest outbreaks.

# VITICULTURAL CONSIDERATIONS FOR MANAGING PIERCE'S DISEASE

# Vineyard Site Selection & Risk Factors Associated with Pierce's Disease - *Jim Kamas and Jacy Lewis*

In addition to selecting a vineyard site with appropriate soils, and a topography that will

minimize frost and freeze injury, we now know that one of the most important decisions prospective growers in Texas and other Gulf coastal states can make is to choose sites with reduced risk from Pierce's disease. The key to minimizing this risk is understanding the disease triangle. For disease to become rampant, there must be a source of the disease (either infected vines within the vineyard or plants harboring grape-strain *Xylella* outside of the vineyard), a susceptible host (non-tolerant grapevines), and vectors to move the disease. Choosing a site less conducive to supporting vectors can greatly decrease the probability of incidence and severity of Pierce's disease.

Sharpshooters feed entirely on xylem fluid of plants. Because this liquid is primarily water, with



While Sites Adjacent to Creeks and Rivers May Be Ascetically Appealing, They Pose a Significant Risk of Pierce's Disease

a small amount of amino acids and minerals, sharpshooters need to change feeding hosts frequently in order to satisfy their dietary requirement. Different sharpshooter species have differing feeding and oviposition preferences. They also choose plant hosts suitable for feeding to lay eggs, so these areas also serve as sites for reproduction. Riparian habitats that contain an abundance of diverse plant life are ideal for supporting large populations of sharpshooters. Creek bottoms and river bottoms are especially suited to this purpose and consequently pose a significant risk of increasing the probability of PD in adjacent vineyards. These sites commonly also contain wild grapevines or other plants that can serve as a source of the strain of *Xylella* that can be passed to susceptible vines in the vineyard.

While it is possible to alter the plant species in an adjacent riparian habitat to discourage a particular species of insect vector, when there are a multitude of competent vector species, it is



Hilltop Sites Far From Perennial Vegetation Are Sites With Reduced PD Risk

impractical, if not impossible to create an plant community that will suppress all potential vectors. Consequently, for most of Texas, the recommendation is to select a vineyard site as far away from perennial vegetation as possible. We commonly also recommend that vineyards be located as far away from bodies of water (rivers, lakes, creeks) as well. It is not the water directly that poses a risk, it is the fact that plant life adjacent to bodies of water are ideal feeding hosts for sharpshooters. Most summers have at least several months with little or no rainfall. During those months, sharpshooters will migrate to plant life that is well supplied with water. There is no magic number of feet or miles that a vineyard should be away from these locations- the answer is

the further, the better. An ideal site would perhaps be a hill with existing annual or perennial grass vegetation. While there are sharpshooters that prefer to feed on grass, most are short-distance fliers and managing grasses can be accomplished with mechanical or chemical methods.

The situation in the High Plains may be entirely different. Some argue that in an environment with minimal perennial vegetation, it may be in a grower's best interest to retain the natural riparian habitat. There are distinct sharpshooter species in the High Plains and they have a unique preferred habitat. Where grapes are not native, indigenous sharpshooters may indeed prefer to stay in the vegetation they are adapted to, rather than journey into vineyards to feed or reproduce. Sharpshooters, will however, feed on what they can find. If native scrub vegetation is removed, sharpshooters may have no other choice except to move into vineyards in order to survive. There were many more sharpshooters caught outside of vineyards as opposed to inside of vineyards in the two year extensive trapping studies conducted. Further research is needed to confirm or refute this notion.

Where grapevines are not native, it hard to understand how grape-strain *Xylella fastidiosa ssp. fastidiosa* found a niche in the natural environment. It is speculated that human activity has played a significant role in the spread of Pierce's disease, and the High Plains is certainly no exception. Anecdotal evidence suggests that contaminated nursery stock had some role in the widespread distribution of PD in the High Plains. It goes without saying that the purchase and planting of certified clean nursery stock is a wise investment. For more reasons than just Pierce's disease, settling for uncertified nursery stock is risky at best. Managing vegetation within the vineyard is critical in minimizing the incidence of Pierce's disease. Creating a weed-free zone underneath the trellis is not only important to minimizing competition with grapevines, but also is important to discourage sharpshooters from staying within vineyards to feed or reproduce. Vineyard row centers should be tightly mowed to keep the stature of the vegetation so short it deters sharpshooters from feeding. The goal is to create an environment that is wholly hostile to sharpshooters. Using neonicotinoid insecticides makes grapevines undesirable hosts and managing other vegetation as described leaves the vineyard devoid of desirable feeding and reproduction



Closely Mowed Vegetation in Vineyard Row Centers Deters Sharpshooters, Prevents Erosion and Supports Equipment Movement

opportunities. Dormant season cover crops, however appear to pose no additional risk of PD infection. Oats or annual rye grass are preferred species, and it is recommended that they be routinely mowed during the dormant season.

# Grapevine Susceptibility & Variety Selection- Jim Kamas

Grapevine species and varieties vary widely in their susceptibility to Pierce's disease. There are different mechanisms among grapevines that do not die from PD. Some varieties such as 'Black Spanish' and 'Blanc du Bois' are capable of being heavily infected, supporting very high concentrations of the bacterium, while still growing and producing acceptable crop loads. Especially under drought or heavy crop loads tolerant varieties may scorch and exhibit typical symptoms of Pierce's disease, but they recover and grow normally the next year. Native species are likewise tolerant or in some cases, resistant. There is a distinction because with resistance, a plant has the ability to suppress the bacterial titer or concentration levels within the xylem tissue. Morphological studies suggest that differences in xylem architecture are at least one mechanism whereby plants can keep the bacterial numbers down by inhibiting movement between xylem vessels. Dr. Andy Walker, grape breeder at U.C. Davis, has conducted studies on the level of bacterial titer seedling populations of Vitis girdiana and Muscadinia rotundifolia collected from areas where Pierce's disease is rare. He found that these plant populations support from 20 to 100 times more bacteria than from seedlings collected from areas with very high PD pressure. His findings seem to support the hypothesis that PD resistance has evolved in response to disease pressure. The important point here for those attempting to grow susceptible grape cultivars is that tolerant or even resistant grapevines often carry the disease and are capable as serving as sources of the disease for further spread by sharpshooters. Our strong recommendation is that susceptible cultivars be grown completely isolated from wild vines or plantings of resistant/tolerant grape cultivars.

#### PD Tolerant & Resistant Varieties

Although the cause of vine death was not known to him, T.V. Munson realized that utilizing grape parents that survived local conditions was important in creating new, improved adapted grape varieties. Many, but not all of Munson's varieties are indeed tolerant of Pierce's disease. While some of these varieties are commonly utilized in home winemaking, they may not produce a wine of commercial acceptability by today's standards. The most widely planted of these include 'Lomanto', 'Wine King', 'Beacon', 'Edna', 'Ellen Scott' and 'Carman'. 'Champanel' is commonly used for jelly and is perhaps the most widely propagated of all of Munson's varieties. Some of Munson's highest quality wine varieties are being included in ongoing evaluation trials and will be evaluated for wine quality relative to other tolerant varieties. In the 1930's, grape variety trials in the Winter Garden area of Texas identified 'Black Spanish' and 'Herbemont' as resistant to "vine disease" which we now know as Pierce's disease.

Across the Gulf Coast, commercial wineries have been established using new resistant/tolerant varieties produced by numerous public and private breeding programs. In many southeastern states, some wineries make wine exclusively out of muscadine grapes. High in antioxidants, muscadine wines are generally finished with relatively high residual sugar. While many consumers enjoy and appreciate the distinct flavor of these wines, the market for muscadine wines is generally limited on premises sales and local distribution. The most widely planted PD tolerant varieties in today's commercial setting are 'Blanc du Bois' and 'Black Spanish'.

<u>'Blanc du Bois'</u>- Released in 1988 by the University of Florida, this variety is currently perhaps the highest quality named winegrape cultivar that has resistance to Pierce's disease. This grape is



the result of a cross made in 1968 by Dr. John Mortensen which was selected as H18-37 for further evaluation in 1974. It has a complex lineage which includes *Vitis vinifera*, *smalliana*, *simpsoni*, *labrusca* and an unknown open-pollinated selection thought to be *V. lincecumi*. In addition to being resistant to Pierce's disease, 'Blanc du Bois' has reported resistance to downy mildew, Isariopsis leaf blight and grape leaf folder. 'Blanc du Bois' averaged approximately 5.5 tons per acre under initial evaluations and ripened in hot climates with good acid retention.

Clusters average 133 grams with 45-55 berries per cluster which average 2.9 grams each. Berries are round, light green, slipskin, with a pleasant muscat flavor. While 'Blanc du Bois' is susceptible to other fungal pathogens, the loose cluster architecture makes it less prone to sour rot complex than more tight clustered varieties. 'Blanc du Bois' typically ripens in early July along the Texas Gulf Coast.

<u>'Black Spanish'</u>- Also known as 'Lenoir' and 'Jacquez', 'Black Spanish' is considered the current highest quality red wine grape variety that is tolerant to Pierce's disease. The parentage and history of 'Black Spanish' are a subject of debate and some believe its history goes back several hundred years. We do know that 'Black Spanish' has produced high yields under severe PD pressure in South Texas since 1889. Vines of 'Black Spanish' are moderately vigorous, and clusters are large and compact with small berries. Juice from 'Black Spanish' is very highly pigmented, high in tannins and acidity lending some wine makers to use juice for production of high quality port style wines. 'Black Spanish is also used for red wine production, but enologists are



working to come up with winery techniques to deal with the overpowering acidity. While 'Black Spanish' is typically grown successfully on its own roots, it is subject to iron chlorosis in alkaline soils. 'Black Spanish' typically ripens in mid to late July in Texas coastal regions. 'Favorite' is another variety very similar to, and reportedly is an open pollinated seedling of 'Black Spanish'. Some consider the fruit to be of superior quality, but commercial availability of 'Favorite' is quite limited.

#### 'Victoria Red'

A recent joint release by the University of Arkansas, Tarkington Vineyards and Texas AgriLife,



Victoria Red is a Pierce's disease tolerant, seeded table grape that produces good yields of high quality attractive fruit. Evaluated as Arkansas 1475, 'Victoria Red' was bred in 1971 and is the result of a cross between Ark 1123 X 'Exotic'. Although its paternal parent ('Exotic') is purely *Vitis vinifera*, the female parent is a derivation of largely French-American Hybrids produced in France in the late 1800's. While neither of the parents exhibit resistance or tolerance to Pierce's

disease, there are several ancestors within the complex lineage of Ark 1123 that have repeatedly been shown to exhibit sustained field tolerance to *Xylella fastidiosa*. Tolerant ancestors include

'Villard Blanc' (S.V. 12-375), 'Jacquez' ('Black Spanish', 'Lenoir'), 'Herbemont', as well as the native Texas species *Vitis berlandieri*.

'Victoria Red' has survived PD for over 25 years with extremely high PD pressure at Tarkington Vineyards near Victoria and has produced reliable crops of high quality fruit. While the primary value of this variety is for home fresh fruit production, this variety may well have a place as a neutral blending wine grape. With soluble solids up to 25° brix, Victoria Red may help Gulf Coast wineries source a higher portion of their fruit from local vineyards.

#### 'Herbemont'

Bred and propagated by Nicholas Herbemont (1771-1839) of South Carolina and France, 'Herbemont' is purportedly a hybrid of *Vitis vinifera, borquiniana*, and *aestivalis*. 'Herbemont' has long been valued as a reliable producer of wine grapes and the vines are resistant to Pierce's disease, phylloxera and several fungal pathogens. Along with 'Black Spanish' this variety has been heavily relied upon by Val Verde winery for the production of port wines and is also used at the Maderia winery at Parras, Coahuila and at the Ferrino Winery at Cuatro Cienegas, Coahuila. Herbemont has also been referred to as the "brown



grape" throughout the southeast and produces clear juice for white wine. While this variety has been widely used for perhaps 200 years, there is little written record comparing wine quality to that of modern cultivated varieties.

#### Other Resistant/Tolerant Varieties

Other PD resistant or tolerant vines are available such as 'Miss Blanc', 'Miss Blue', 'Mid-South', 'Orlando Seedless', 'Roucaneuf', 'Daytona', 'Conquistador', 'Stover' and 'Lake Emerald'. While these can be grown without fear of loss due to PD, the ability of these varieties to successfully

compete in the commercial marketplace is questionable. There are however at least two ongoing breeding programs using classical techniques that are producing tolerant winegrape seedlings that are under evaluation in Texas. Of special note is the important finding of Dr. Andy Walker, grape breeder at



U.C. Davis, that all of the genes for PD resistance in the wild species *Vitis arizonica* are all located on a single locus. This means that by using marker assisted selection, the time needed to produce and screen seedlings that are resistant has been greatly reduced. Dr. Walker has produced a number of breeding lines currently under evaluation in California that have 87%, 94% and 97% *vinifera* parentage. Seven 87% *vinifera* lines are currently under evaluation in Texas and the hope and expectation is that these resistant selections will produce wines that do not possess the color and flavor flaws associated with wines made from American varieties.

#### Susceptible Varieties

Although all *Vitis vinifera*, *Vitis labrusca* and most French-American Hybrids are susceptible to Pierce's disease, they vary in their longevity and productivity after infection. Varieties such as 'Chardonnay' and 'Sangiovese' are extremely sensitive, show symptoms soon after infection and

commonly die that same year. In contrast, varieties such as 'Cabernet Sauvignon' and 'Chenin Blanc' may take a number of years to show first symptoms and may live and be productive for quite some time before the ultimately die. Most other varieties are intermediate in their susceptibility. It should be noted that while some believe 'Norton' ('Cynthiana') is resistant to PD, it is indeed not. Dr. Lisa Morano's early work with 'Black Spanish', 'Blanc du Bois' and 'Norton' showed that over the course of a season, the bacterial titer of the first two varieties climbed, then leveled off in late summer. By contrast, *Xylella* concentrations in 'Norton' climbed, but never leveled off. There are indications that under low or moderate disease pressure, 'Norton' may be able to sustain growth and productivity, but under high Pierce's disease pressure, 'Norton' slowly decreases in vine size and productivity, and ultimately dies.

There are different ways of looking at the relative susceptibility or field longevity of susceptible varieties. While varieties such as 'Cabernet Sauvignon' may continue to produce quite a while after infection, the lack of symptoms for perhaps years makes it an unseen source of inoculum in the vineyard that may exacerbate further disease spread. Rouging infected vines is an important part of our recommended management strategy, but if a grower cannot see symptoms on an infected vine, there is no way to know which if any vines need to be pulled. Conversely, while very sensitive varieties die soon after infection, the fact that they show symptoms very rapidly may be seen as a management advantage. With rapid symptom expression a grower can act quickly and remove symptomatic vines before sharpshooters can spread the disease to adjacent vines.

## The Use of Rootstocks in the Management of Pierce's Disease in Texas - Andrew Labay

#### Introduction

Rootstocks have long been used in viticulture as a means of disease management, adaption to local soil conditions and improving scion performance. The use of rootstocks as a tool to manage Pierce's Disease (PD) in Texas remains largely unexplored although recent and ongoing research projects address this topic. From these and other studies, it does not appear that PD tolerant rootstocks alone have the ability to rescue a susceptible scion from the disease. However, there are reports of an influence of the rootstock on the severity of the disease, and in a typical vineyard setting where recommended viticultural practices are in place, the use of tolerant rootstocks could be greatly beneficial. A thorough understanding of the impact of rootstocks on the performance of the scion in addition to continued trials of the common and newly developed rootstocks should provide additional approaches to viticulture in locations that experience high PD pressure.

#### The Use of Rootstocks in Viticulture

The first major application of rootstocks in viticulture was intended as a means of managing an insect pest imported to Europe from the new world. The need for resistance to the root-feeding aphid, phylloxera, was the primary impetus for the selection and breeding of many of the rootstocks that remain in use today. During the late 19<sup>th</sup> and early 20<sup>th</sup> century when this aphid caused widespread destruction of vineyards, researchers discovered that while the European grapevine, *Vitis vinifera*, is highly susceptible to phylloxera, certain American *Vitis* species display various degrees of tolerance. This led to intense breeding and evaluation of rootstocks which are either pure selections or hybrids of different American species including *V. riparia*, *V. rupestris*, *V. berlandieri* and *V. champini* (Lider, 1995). Incorporation of the natural disease tolerance to phylloxera found in these species remains a criterion for a good rootstock.

A major focus in rootstock breeding beyond phylloxera management has been focused on nematode resistance. Work conducted in California has similarly identified sources of tolerance to nematodes in various American *Vitis* species (see Snyder 1936, Walker 1994). However, the difficulty in developing sustainable nematode resistance has been due to the various species of nematodes that may be pathogenic: rootknot nematode (*Meloidogyne spp.*) and dagger nematode (*Xiphinema index*, which vectors Fanleaf virus) each has a diverse population with a wide spectrum of virulence. A single rootstock may provide high levels of tolerance to a single nematode species whereas it may be susceptible to a second species. Moreover, the rootstock varieties that have shown good nematode tolerance are often lacking in other qualities; for

example increased susceptibility to phylloxera or other pathogens (1613C, Freedom, Harmony) or excessive vigor (1613C, Dog Ridge, Salt Creek). As a response, a recent breeding program has focused on screening for multiple-disease resistance leading to newly released rootstock varieties that coincidentally have high potential for PD tolerance as well (GRN series rootstocks; Walker 2006, Covert 2008).

In addition to disease management, the ability of a rootstock to adapt to various soil conditions (e.g., compactness, drainage, salinity, alkalinity and acidity) and the effects of a rootstock on scion performance (e.g., vigor, nutritional status, timing of developmental stages, yield and fruit quality) are highly important considerations in the choice of a rootstock. There has been thorough research on this subject, notably in France, Italy, Australia and California. Excellent reviews that compile the characteristics of common rootstocks can be readily found (e.g., Wolpert 2002, Christensen 2003, Dry and Coombe 2005, and Pongracz 1983). While the information found in these reviews can serve as a guide to rootstock performance in Texas, evaluation of rootstocks in the various geographical regions of the state remains an ongoing process.

In considering rootstocks' role in viticulture, it has been noted that parentage of the stock and the soil type of the vineyard site are particularly important (Wolpert 2002). Rootstock performance is highly dependent on soil structure and knowing the characteristics of *Vitis* species in a rootstock's background can be a general guide to performance. *Vitis riparia* is shallow-rooting and has a preference for moist soils, whereas *V. rupestris* is more drought tolerant. *Vitis berlandiari* is a deep-rooting species with high phylloxera tolerance though poor rooting ability from dormant cuttings. Therefore many common rootstocks are *V. berlandieri* crossed with either *V. rupestris* or *V. riparia* in order to improve rooting ability.

In Texas, phylloxera and nematode infestations have not been significant factors in the choice of rootstock although the potential for an outbreak of either pest remains. The primary application of rootstocks has been for adaption to regions in the state with alkaline soils and propensity for drought. The rootstock 5BB Kober (*V. berlandieri* x *V. riparia*) has been commonly used and is known for moderate drought tolerance and moderate-high lime tolerance. A current trend is toward the more drought tolerant rootstocks such as 1103 Paulsen (*V. berlandieri* x *V. rupestris*).

Recent and ongoing rootstock trials in Texas, as described below, have been initiated with the focus on resistance to PD. It will be equally important to continue to characterize the adaptability of common and newly developed rootstocks with respect to Texas soils, climate, pests and pathogens.

#### Testing the Susceptibility of Un-grafted Rootstocks to Pierce's Disease

Over the course of the last century, field trials in areas of high PD pressure across the Southern U.S. have documented a wide range of responses, from susceptibility to tolerance, among American grape species and common rootstocks. Multiple trials in Mississippi found varieties of *V. champini*, including Dog Ridge, among the better performers (Magoon and Magness 1938, Loomis 1952). A 24-year field analysis in Florida of 83 rootstocks also identified Dog Ridge as a top performer in addition to Lake Emerald (*V. sampsoni*) and Tampa (*V. aestivalis* x *V. labrusca*; Mortensen 1985). More recently, a six year field trial in Florida of 10 rootstocks was conducted. All rootstocks were reported to have leaf necrosis attributed to PD. The authors however noted variability in PD symptom severity. Ramsey (*V. rupestris* x *V. candicans*) and St. George (*V. rupestris*) had the lowest PD symptoms. The *V. berlandieri* hybrids (5BB, 5C and 110R) were among the intermediate performers, whereas Freedom (1613C x *V. champini*) had the highest levels of PD and 100% death by the third year (Lu et. al. 2004).

In Texas, a three year field trial was conducted near the town of Tow, in a vineyard site with high PD pressure (Kamas et al. 2007). Thirteen rootstocks were chosen for the trial, many of which are common stocks that are in use in Texas or have high potential for the region (Table 1). By the second year of the trial all rootstock varieties displayed at least minor PD symptoms and had detectable levels of *Xyllela fastidiosa*. However over the course of the study there was a wide spectrum of response observed (Figures 1 and 2). The rootstocks Harmony, Freedom 1616C, and 1613C experienced high levels of PD symptoms and mortality. These stocks were thus classified as highly susceptible to PD. The *V. berlandieri* hybrid rootstocks had intermediate performance and are considered moderately susceptible. Among these rootstocks the *V. berlandieri* x *V. rupestris* hybrids displayed lower levels of *X. fastidiosa*,PD symptoms, and higher pruned weights (Figure 3) on average as compared to the *V. berlandieri* x *V. riparia* hybrids.

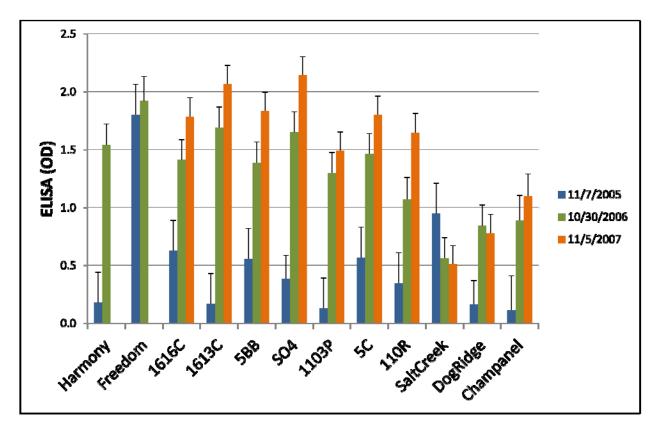
The best performers of the Tow trial were the *V. champini* rootstocks which had consistently low, although increasing, levels of *X. fastidiosa*, low levels of PD symptoms, and high pruned weights. *Vitis champini* is thought to be a naturally occurring hybrid of *V. candicans* (Mustang grape) and *V. berlandieri*, and the geographical distributions of all three species overlap in Texas. Due to the high levels of disease tolerance and the natural adaptability to regional soil and climactic conditions, rootstocks with these species in their background are of particular interest in Texas viticulture. In fact, the *V. champini* selection Dog Ridge and the *V. champini* hybrid rootstock Champanel have long been recommended for Texas due to phylloxera and *Phymatotrichum* root rot tolerance (Mortensen 1940, Perry and Bowen 1974). However, the use of pure selections of *V. champini* such as Dog Ridge and Salt Creek, and similarly the use of selections of *V. candicans* as rootstocks has certain drawbacks. These stocks are highly vigorous and readily develop suckers, both of which are characteristics that can lead to challenges in vineyard management. An alternative strategy is the use of *V. champini* hybrid rootstocks developed through breeding V. champini selections with other grape species. The goal would be to have a rootstock with the disease resistance of V. champini selections without the negative characteristics. However, field tests are needed for verification of both disease tolerance and performance. For example, Harmony, Freedom, and Champanel are all V. champini hybrids but only Champanel has been shown to be PD tolerant. Furthermore, while Champanel does not display the high levels of vigor as the pure V. champini selections, it is considered to be highly susceptible to nematode pressure and does not perform well on alkaline soils (Jim Kamas, personal comm.).

The evaluation of new and untested rootstocks will be necessary to finding stocks that possess desired characteristics. As previously mentioned five new nematode resistant rootstocks were recently released from an extensive U.C. Davis breeding program (Walker 2006). In crosses, V. champini was frequently used as a source of disease tolerance and V. riparia was used to provide good horticultural benefits. These rootstocks have been included in recently initiated grafted trials in Texas.

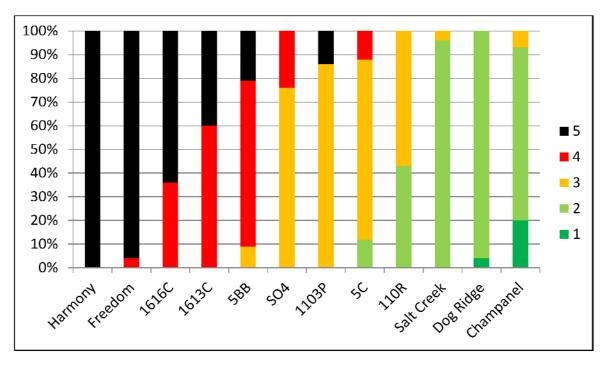
Rootstock	Species Origin	PD Category*	
Harmony	1613C x V. champini		
Freedom	1613C x V. champini		
1616C	Solonis (V. acerfolia) x V.	Highly	
	riparia		
1613C	Solonis (V. acerfolia) x	Susceptible	
	Othello (V. labrusca, V.		
	riparia, V. vinifera)		
5BB Kober	V. berlandieri x V. riparia		
SO4	V. berlandieri x V. riparia		
1103 Paulsen	V. berlandieri x V. rupestris	Moderately	
5C Teleki	V. berlandieri x V. riparia	Susceptible	
110 Richter	V. berlandieri x V. rupestris		
Dog Ridge	V. champini	Tolerant	
Salt Creek	V. champini		
Champanel	V. champini, V. labrusca, V. vinifera		

**Table 1.** List of rootstock varieties used in a 3 year field trial in Tow

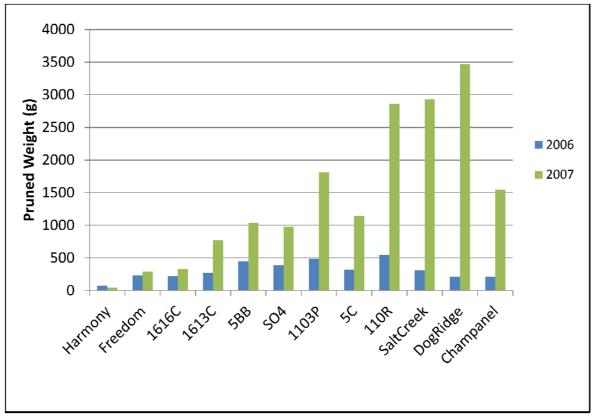
\* Mean PD symptom values from the final year of the study, 2007, were used to categorize rootstocks such that Highly susceptible = 4-5, Moderately susceptible = 2-3 and Tolerant = 1-2.



**Figure 1**: *X. fastidiosa* detection via ELISA (optical density) in a 3 year ungrafted rootstock trial in Tow, Texas.



**Figure 2**: Percentage of plants with PD symptom values 1(no symptoms) to 5(death) in the final year of a 3 year ungrafted rootstock trial at Tow, Texas.



**Figure 3**: Mean pruned weight (g) per variety in a 3 year ungrafted rootstock trial at Tow, Texas.

### Testing Rootstocks as a Tool to Manage Pierce's Disease

While progress has been made in the identification of tolerance in rootstocks, the true interest for the grape growing industry rests in how the rootstock-scion combination responds to PD. As previously discussed, rootstocks have long been used as a tool of managing disease resistance, however with the two principal cases (phylloxera and nematodes), the primary site of infection is the root zone. In the case of PD, *X. fastidiosa* is inoculated directly into the scion, and influence of the rootstock on physical and/or chemical environment of the scion would be important factors for resistance.

There is evidence that would support the idea that transmission of resistance from rootstock to scion is possible. Select grape rootstocks have been shown to reduce disease in pre-infected scions in cases of fanleaf virus and crowngall (Walker 1991; Sule and Burr 1998). Furthermore, Gould et al. (1991) found that certain *Prunus* rootstocks lead to variability in xylem chemistry, decreases in vector feeding, and reduced *X. fastidiosa* levels in peach scions. In each of these cases the mechanisms of induced tolerance are unknown. However, these studies suggest potential for induced resistance via rootstock in the case of PD.

Field trials of grafted grapevines however have yielded mixed results. N.B. Pierce noted that *V. vinifera* varieties 'Mataro' and 'Grenache' are resistant when grafted onto St. George (*V. rupestris;* Hewitt 1958). Trials in Mississippi of PD susceptible *V. labrusca* varieties found increased longevity and yield primarily from Dog Ridge, Barnes (*V. champini*), and B4-5 (*M. rotundifolia* x *V. bourquiniana* hybrid) rootstocks, although it was stated that no one rootstock was best for all varieties (Loomis 1965). More recently, a study in Florida of 'Chardonnay' grafted onto 9 rootstocks and own-roots found high levels of leaf necrosis (50-75% of leaves) on all combinations beginning in the second year of the trial and yield at or below 50% in all combinations in the third year (Cousins 2003). While nearly all own-rooted vines died by the third year of this study, all vines grafted onto 5BB were alive although the percent of fruiting vines was below 40%.

In Texas a study involving grafted combinations of three scions ('Merlot', 'Chardonnay', and 'Cabernet Sauvignon'), three rootstocks (Dog Ridge, 1103P and Freedom), and own rooted *V. vinifera* vines was initiated in 2008 at two sites, Uvalde and Stonewall, Texas (Black 2010). In 2010, 96% of plants at Uvalde tested positive for *X. fastidiosa* as compared to 2.6% of plants at Stonewall. The high level of PD pressure at the site in Uvalde has yielded preliminary information. While it shows that all combinations have developed PD rapidly there is variation with respect to the scion variety used. 'Merlot' vines displayed lower *X. fastidiosa* levels and PD symptoms compared with other combinations on average (Figure 3). Additionally, for all scions 1103P had the lowest levels of PD symptoms.

Continued analysis of PD development at these two sites should allow for verification of these initial observations and yield additional information. Furthermore, an extension of this trial was initiated in 2011. The extended rootstock trial includes the *V. vinifera* variety 'Sangiovese' grafted onto 11 different PD tolerant rootstocks at two sites and the PD tolerant hybrid variety Blanc du Bois with the same combinations at two additional sites. Ultimately, results of these trials should greatly expand our knowledge on the impact of a wide range of rootstocks on disease tolerance and performance of multiple varieties of scions at various sites across the state.

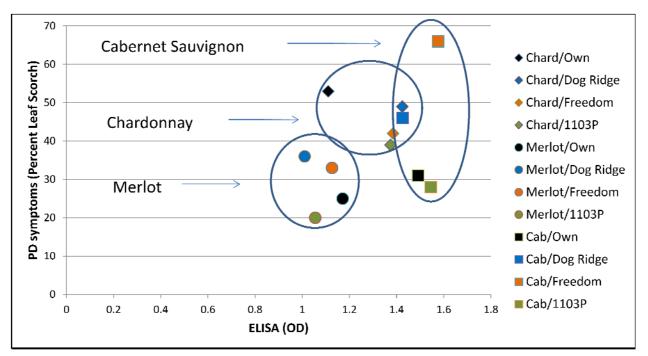


Figure 3: *X. fastidiosa* detection level (ELISA) versus percent leaf scorch in a grafted rootstock trial at Uvalde, Tx. Data collected in 2010.

### Rootstocks and Pierce's Disease: Conclusions and Perspectives

What has become evident is that there exists a wide diversity of response to PD among the American grape species and this is equally found among the common rootstock varieties which themselves are mostly hybrids of native grape selections. Rootstocks tested to date do not appear to have resistance to PD but rather varying degrees of tolerance. The molecular mechanisms that underlie this tolerance are currently not fully understood. They could involve the chemical components within the xylem, anatomical features of the xylem, or both depending upon the species. Rootstocks with parentage that includes American *Vitis* species of tolerance or even resistance. PD resistance has been reported to be found in *Muscadinia rotundifolia*, *V. candicans*, *V. shuttelworthii* and *V. arizonica* among other species (Fritschi et. al. 2006). Among the rootstocks that will be assessed in the extended rootstock trial in Texas, GRN-1 is a *M. rotundifolia* x *V. rupestris* cross. This variety along with other newly released rootstocks could serve as additional choices for the region.

From trials conducted thus far, rootstocks have not shown the ability to rescue susceptible scion varieties from PD as symptoms have developed rapidly. However, there is preliminary evidence that certain rootstock-scion combinations enhance disease tolerance, albeit slightly, in very high disease pressure environments. Further assessment is warranted and research on additional scion varieties and rootstocks would be valuable. Highly susceptible varieties such as Chardonnay have often been a choice in trials although greater benefit may be found in choosing *V. vinifera* varieties that may be more suitable to local growing conditions. The current trend in the Texas

grape growing industry is the use of varietals such as Tempranillo and Sangiovese which are commonly grown in the warmer, southern regions of Europe and could be a good match for the Texas climate.

Additionally, in many cases, trials have been carried out at sites where disease pressure is exceptionally high and spread of disease is not controlled. Perhaps the benefits of certain rootstocks are more profound in environments where disease pressure is either naturally moderate or low due to viticultural practices that limit the spread of disease (e.g., site selection, weed removal, insecticide application etc.). In such a case there could be a detectable additive effect from the choice of rootstock. Trials at multiple sites and ecoregions within the state would allow for a more thorough assessment of the impact of rootstocks.

Recently initiated rootstock trials have been put into place across the state to answer many of these questions. The use of *V. vinifera* varieties or hybrid varieties, which perform well in regional climates, grafted onto highly tolerant rootstocks that are well adapted to local soil conditions may be an important approach to increasing PD tolerance in Texas. The extent of such an increase in PD tolerance will be the focus of ongoing research.

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### Managing Adjacent Vegetation - M. C. Black

Planting *Vitis vinifera* (European winegrape) varieties is risky in about three-fourths of Texas due to Pierce's disease (PD). Goals of vegetation management adjacent to vineyards are to reduce bordering external sources of the bacterial pathogen and insect vectors, and to reduce vegetation corridors that facilitate insect vector movement into vineyards.

#### Pathogen

Bacteria known collectively as *Xylella fastidiosa* (*Xf*) cause PD of grape and similar diseases in certain other plants (Table 1). These bacteria have specialized to grow either in plant xylem where they digest streaming fluids and certain cell wall components, or in a mouthpart of an insect that feeds mostly on xylem fluids. *Xf* cells can apparently survive in xylem of nearly all plants if placed there by a probing insect, or by humans (pin prick inoculation). For most plants, these bacterial colonies remain small or die out over time, and the plant functions well.

The wrong bacterium (Table 1), placed by the wrong insect (Table 2) into the wrong plant, begins to reproduce and spread and disease develops. Several annual, perennial, and woody plant species in Texas allow significant Xf reproduction and spread through xylem (1,000 to 100,000,000 Xf cells/g of xylem-bearing stem, petiole, leaf vein) but only woody species develop obvious leaf symptoms (Table 3).

Grape strain *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*) and close relatives (Table 1) apparently can exist alone (one subspecies) or in mixtures (two or more subspecies) lining an insect vector mouthpart. After feeding, nutrition available in a new plant strongly favors reproduction of one bacterial subspecies. Consequently, *X. fastidiosa* subsp. *multiplex* (*Xfm*) numbers surge in some weeds and trees, *X. fastidiosa* subsp. *sandyi* (*Xfs*) grows best in oleander, and *Xff* develops large populations in susceptible grape varieties.

Mechanically inoculated common sunflower was colonized by more *Xf* isolates representing three subspecies than were giant ragweed, seacoast sumpweed, or grape. Some weeds are apparently reservoirs of *Xff* (grape subspecies). Eliminating or limiting nearby *Xf* host plants reduces sources of bacteria for xylem-feeding insects to acquire and move to grapevines.

#### Insects

Xylem-feeding insect vectors of the grape PD bacterium, *Xff*, probably number more than 25 species in Texas, including glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Table 2). GWSS is thought to be the most important vector of *Xff* and these vegetation management suggestions primarily focus on that insect. In general, smaller xylem-feedomg insects are efficient and less dangerour *Xf* vectors than larger xylem feeders.

All vectors of the PD bacterium also utilize non-grape plants for feeding and reproduction. For example, GWSS feeds and reproduces on a large number of host plants and thrives especially well in diverse plant communities near permanent standing water (riparian habitat). GWSS adults survive winter by alternately seeking cover in leaf litter when temperatures drop and feeding on tender woody stems, evergreen leaves, and perennials on warm days. Warm season GWSS movements occur as many short flights from one plant canopy to another.

### Site selection and preparation

Vegetation management necessity for PD control is fundamentally set long before vineyard land is acquired. In southeast Texas, PD is very intense and only PD tolerant varieties can survive long term. PD incidence and severity is low to absent in the Texas Panhandle where there are no large efficient vectors, hard freezes are therapeutic for the few infected vines, and *Xfm* and *Xfs* are largely absent from weeds, trees, and irrigated landscapes.

In a broad sense, PD risk in Texas varies from high along the Gulf Coast to very low in the northwest Panhandle because of climate (rain, temperature) and land features (elevation, latitude, bodies of water, plant communities). More days of freezing temperatures reduces *Xff* numbers in plants and reduces GWSS and other large vector insect survival. Past and current land uses strongly influence plant communities. Hard freezes and months of drought may temporarily reduce pathogen and insect vector populations in plant communities near a vineyard site because both depend on plants, but a mild winter and plentiful rains in the growing season allow plant growth and have the opposite effect.

Growers looking to buy land for new vineyards should also consider PD risk in a narrow sense for each property. Riparian habitats at standing water (river, stream, lake, drainage ditch, seasonal swale, stock tank) support diverse plant communities used by insect vectors and should be avoided. Observe whether water collects nearby after rains because seasonal wet sites promote weed growth used by vectors. Land in the vineyard vicinity should be contoured to provide adequate surface drainage from rainfall and avoid long term standing water and associated plants.

Vineyards bordering irrigated landscapes (home, business, office) and fruit and nut orchards increases PD risk by favoring GWSS overwintering sites, reproduction, and feeding. For example, crape myrtle was used extensively for adult feeding in a Florida study (R. Mizell), but holly and Bradford pear were preferred for ovipositing (egg laying in leaves). In southwest Texas, GWSS adults feed in dormant pecan, maple, walnut, and oak canopies through the winter.

### Soils

PD site risk varies with presence or absence of certain plant species associated with soil type and human activities. In controlled experiments with four central Texas soils and a commercial potting mix, PD developed similarly on susceptible Chardonnay grapevines. However, plant

surveys at high and low PD risk sites, including where those soils were collected for our experiments, consistently found certain unsafe plants at high risk sites. Higher risk was associated with slowly drained clay soils and the lower risk sites had well drained soils with more sand.

Alluvial soils near streams and rivers typically have more water holding capacity, greater nutrients, and less slope compared to most upland soils but PD risk is higher in bottom land. Increased PD risk near riparian habitats is just one more reason to plant on higher ground. Deep slowly draining soils lead to fruit quality problems during seasons with excess rainfall. Bottom lands in Texas also have greater risk for grape winter cold injury and late spring freezes because cold air is less dense than warm air.

Human activities often introduce and favor unsafe plants where soil type would otherwise have limited establishment. For example, seeds of common sunflower and giant ragweed may be introduced on shredding equipment into highway rights-of-way and drainage areas where the soil type and original contouring were not favorable for these species. Risky weeds can flourish in highway rights-of-way ditches than channel rainfall.

Ornamentals used by Xf vectors survive in almost any soil in Texas with irrigation.

### **Risk indicator and 'safe' plants**

We began compiling a list of plants that must not be tolerated anywhere near PD susceptible grape varieties. This suggestion applied to the scale of entire properties plus all public and private property where you can gain access.

Asteraceae (composite) plant family had the most species with *Xf* growing in their vascular systems in Texas (Table 3) and controlled experiments with four Asteraceae species raised our concerns. We have proposed giant ragweed, common sunflower, narroleaf sumpweed, and seacoast sumpweed as PD risk indicators based on their frequent occurrence in clayey soils and riparian habitats in central and southwest TX with PD histories, and as *Xff* host plants from field surveys and controlled experiments (Table 3, Fig. 1).

With the possible exceptions where PD risk is low in the Panhandle, sites with populations of one or more of these PD risk indicator weeds should 1) be subject to ongoing species-specific control efforts, or 2) be avoided for any European winegrape variety (*Vitis vinifera*) because all varieties to date are highly susceptible to PD. American hybrid varieties with high PD tolerance are options at such sites.

Our field surveys never detected *Xf* in any grass or other monocot plant, although work in other states has found *Xf* in some of those plants.

### Mowing

GWSS locates plants for feeding using sight, smell (including flowers), feel, and taste (nutritional needs) in that order of importance. Distance from tall weeds, ornamentals, brush and trees decreases GWSS attraction to grapevines because sight and smell stimuli decrease with distance.

Mowing or shredding discourages tall broadleaf weeds and encourages grasses and other monocots that are not good hosts of *Xff* and not highly preferred by GWSS and other vectors for reproduction and feeding.

In a 2-year study in Conservation Reserve Program (CRP) acreage (no vineyard) adjacent to the Nueces River, mowed and un-mowed grassland reduced GWSS movement out of brush and trees near the river more than it reduced johnson grass sharpshooter (JGSS) movement. However, cicadas moved freely among brush/trees, mowed areas, and un-mowed weedy grassland. *Xff* detected in cicadas is evidence of feeding on infected plants (apparently mustang grape)(F. Mitchell & J. Brady), but cicada transmission of *Xff* to susceptible grapevines has not yet been documented.

Mower clippings can create a thin temporary mulch layer that increases rainfall absorption, filters sediments from vineyard surface drainage after heavy rainfall, and reduces germination of annual weeds in perennial plantings.

Russell Mizell, University of Florida entomologist, estimated GWSS typically does not move from diverse vegetation to new plants isolated by more than 100 m (328 ft) of mowed area. Mowing 328 ft. or more around each vineyard block will not be an option for some growers.

Mow up to 328 feet between vineyard and undisturbed vegetation (trees, brush, weeds, orchards, landscape ornamentals) where GWSS and other vectors can overwinter and build large populations that move into the vineyard.

Isolation, especially with compromised distances, should not be the only PD management strategy for highly susceptible grape varieties (*V. vinifera*) grown in regions with high PD risk. Mowing should be combined with other strategies such as variety and rootstock tolerance, vineyard insecticide, trap hedge insecticide, etc.

### **Brush and vine control**

Grape is highly sensitive to the most effective non-cropland herbicides, but vegetation in areas impractical or unsafe for mowing or shredding can be managed with chemicals. Small populations of high risk weedy plants can be spot treated with a labeled herbicide using a backpack sprayer or small volume sprayer on an all-terrain vehicle (ATV). Options may include plant growth regulators (reduce growth rate), systemic herbicides (sub-lethal dosage), contact herbicides (burn-down), cut stump treatments, and mechanical stump removal.

Wild grapevines seldom develop PD symptoms, but can harbor *Xff*. Therefore, all native Texas grapes (about 14 species) should be eradicated near *V. vinifera* vineyards on a property wide scale. Other vining plants should also be removed and the stumps killed, including heart-leaf ampelopsis (Table 3), peppervine, and Virginia creeper.

### Grazing

Grazing and browsing by livestock (cattle, sheep, goats) and wildlife reduces pasture or understory vegetation near vineyards. An overgrazed pasture is not aesthetically pleasing to visitors and increases soil erosion. Interest in short-term goat access (Fig. 2) for vegetation management has increased due to wildfire losses statewide. Temporary confined goat herds near vineyards may have potential for urban tourist appeal. For non-mechanical and non-chemical vegetation management, contact local goat or sheep producers about services of their animals confined by temporary fencing.

Some vineyards are near weedy windbreaks, orchards, trees, brush, and woody ornamentals on adjacent properties. Build good relationships with your neighbors and communicate concerns about vegetation management and certain high risk plant species.

Mowing, shredding, chemical 'mowing,' grazing, and short-term intense browsing may not be feasible for all risky vegetation. Hand weeding, hoeing, or trimming may be necessary at difficult sites to control PD risky plants before they produce seeds for future years or decades.

### Cultivation

Cultivation is not an option for vegetation management in steep terrain and it's a poor option even with slight slopes. Clean cultivation can allow serious soil loss from water and wind erosion and should be avoided. Ground cover plants increase equipment mobility, reduce erosion potential, and improve aesthetic appeal of areas around the vineyard.

### Groundcover

Weeds require ongoing control efforts because of seed banks in soil and capacity for rapid growth after rains. 'Safe' plant species (not used heavily by vectors, no high *Xff* populations) should be managed to outcompete and replace 'unsafe' plants. Numerous plants will grow in disturbed soils after rainfall, so give the advantage to 'safe' plants and discourage 'unsafe' plants.

"Nature abhors a vacuum" François Rabelais, 1532

Non-crop areas nearby as well as the vineyard floor should be managed to minimize habitat for supplemental plant hosts of *Xff* and insect vectors and maximize populations of 'safe' plants. Ideally, *Xff*-safe plants adjacent to and within vineyards would be little-used by GWSS and other vectors, highly competitive with weeds, easily established at low cost, able to re-seed or re-grow

from roots or crowns, require minimal maintenance once established, senesce without mowing or herbicide treatment, provide a temporary standing mulch for erosion control, have neutral or beneficial effects on PD vectors and other pests problems, and have seasonal tourist appeal. If also used in the vineyard, cool season 'safe' plants should compete minimally with grapevines for water and nutrients due to size and season of growth.

Spring wildflowers are a phenomenon to behold when a site receives sequential fall, winter, and spring rains. Advantages of wildflowers near vineyards include ground cover for erosion control, traction for equipment on wet days, nectar for beneficial insects that prey on GWSS and other vectors, and admiring tourists enjoying outings and spending money in vineyard country (<u>http://www.gotexanwine.org/texaswinetrails/</u>). Most wildflowers are cool season annuals and perennials that mature or go dormant at the onset of hot weather. A few GWSS and other vectors may use wildflowers so plantings should be monitored periodically to fine-tune a list of species to use. However, in central and southwest Texas, GWSS populations remain low until late May, after most wildflowers have senesced. In wet years, wildflowers can bloom into June.

We propose use of *Xf*-safe wildflowers based on field surveys, greenhouse and screenhouse studies, and their bloom dates. Several species of cool season forage plants and wildflowers were aggressively challenged in controlled experiments (Table 4) using mechanical inoculation with *Xff*, *Xfm*, and *Xfs* in the absence of insect vectors. We interpreted higher risk species as those susceptible to more isolates from our collection of three subspecies including grape isolates, according to *Xf* serology (ELISA).

Cilantro, plains coreopsis, catchfly, annual ryegrass, buckwheat, and tuber vervain met our criteria for 'safe.' Where there is PD risk, forage legumes (clovers, etc.) should never be allowed to grow near vineyards. *Xf* established well in many composites (Asteraceae), so we were pleasantly surprised that plains coreopsis (*Coreopsis tinctoria*) qualified as *Xf* 'safe.'

Bluebonnet had mixed results in our tests, but has advantages of a low growth habit, nitrogenfixing capabilities, popular with tourists, fragrant, etc. We did notice several GWSS hanging on the sides of the screenhouse when the container-grown bluebonnets were in full bloom, suggesting that at least one vector is attracted to the bluebonnet flower fragrance. If planted on droughty soils, flowering would end sooner and be less likely to overlap with high GWSS populations.

Cool season annual grasses (annual ryegrass, small grains) are apparently safe, but we have not studied the small grains. Seeds are widely available and there is some income potential as forage, hay, seed contracts, and grain. Soil preparation and replanting are necessary every fall.

*Note*: some plants in the wild may not be preferred by *Xf* vectors, and though deemed 'unsafe' in greenhouse and screenhouse experiments, they may not be risky near vineyards. Likewise, some wildflower and forage species deemed 'safe' in these experiments may be highly utilized by vectors in the field and support enough *Xff* reproduction to create risk for PD.

We tested our hypothesis of 'safe' plants in 2008-09 in a vineyard with a long history of PD. In October08 we planted 14 wildflower species (Fig. 3). The vineyard cooperator irrigated more than we needed, and we weeded and sampled until July09. Even though screenhouse tests previously found that mechanically-inoculated *Xf* of three subspecies colonized some of the species, plant samples collected 5May, 3June and 15July09 were all negative with *Xf* serology (ELISA). Some GWSS were caught on nearby yellow sticky traps in the vineyard but plants in our test plots did not have *Xf* in the xylem tissues.

Our 'safe' plants list (Table 4) probably errs on the side of caution. Growers interested in wildflowers should be cautious and note any use by GWSS, other sharpshooters, large leafhoppers, spittlebugs, etc. The inevitable termination of maturing/senescing wildflower plantings could be imposed early (before mid-May) by mowing, shredding, or chemical 'mowing' without greatly increasing PD risk.

Start with a small area because weed control among wildflowers can be a challenge (think 'hand labor'). Blocks of one or two species can be more effective visually than a mixture of several species. Several Texas businesses sell wildflower seeds. Ornamental nurseries produce transplants of a few species including Texas bluebonnets. Early fall seeding and transplanting are ideal and winter freezes will kill tender weeds. The flower show will vary according to fall, winter, and spring rains unless you irrigate.

### Conclusion

Vegetation management adjacent to *V. vinifera* vineyards should be rigorously implemented in Texas where there is PD risk. This practice will help reduce *Xf* vector insects and their easy access to PD susceptible grapevines because GWSS and other vectors use plant cues to move across the landscape and locate plants for feeding and reproduction. Vegetation management includes eliminating highly utilized plant species and increasing groundcover and naturalized non-crop areas with species less-utilized by vectors in ways that maintain landscape aesthetics, prevent erosion, favor beneficial insects, reduce vector refuges, and remove easy corridors into vineyards.

rable 1. Dacteria that cause grape rierce's disease and similar diseases.				
	Acro-	Typical		Geographical
Bacterium	nym	(occasional) hosts <sup>z</sup>	Diseases	distribution
Xylella fastidiosa	Xff	Grape (alfalfa,	Pierce's disease, alfalfa	North & Central
subsp. <i>fastidiosa</i>		almond)	dwarf, almond leaf scorch	America
X. fastidiosa	Xfm	Ornamentals,	Bacterial leaf scorch,	North, Central,
subsp. <i>multiplex</i>		weeds, trees, pecan,	phony peach, almond leaf	& South
		almond, plum	scorch, plum leaf scald	America

#### Table 1. Bacteria that cause grape Pierce's disease and similar diseases.

X. fastidiosa	Xfs	Oleander	Oleander leaf scorch	North America
subsp. <i>sandyi</i>				
X. fastidiosa	Xfp	Coffee, citrus,	Coffee leaf scorch, citrus	South America
subsp. pauca		weeds	variegated chlorosis	

<sup>z</sup>Host range varies depending on the isolate. Many native plants are host plants wherever *X*. *fastidiosa* subspecies occur, but most agricultural losses occur in introduced plants including European winegrape, almond, peach, oleander in North America, and on citrus and coffee in South America.

 Table 2. Insects that ingest primarily xylem sap are known or suspected vectors of Xylella fastidiosa bacteria.<sup>z</sup>

Order	Family	Common names
Hemiptera	Cicadellidae	Leafhoppers, sharpshooters
	Cercopidae	Spittlebugs
	Machaerotidae	Tube-building spittlebugs
	Cicadidae	Cicadas

<sup>z</sup>Almeida et al. 2005. Ann. Entomol. Soc. Am. 98(6): 775-786.

Table 3. Non-grape plants from which *Xylella fastidiosa* has been isolated in Texas. Plants in bold text are proposed indicator plants for Pierce's disease risk in central and southwest Texas. Bacterial load ranged from  $10^3$  to  $10^8$  cfu/g xylem-rich plant parts.

Common name(s)	Family	Species
Oleander	Asclepiadaceae	Nerium oleander
Western ragweed	Asteraceae	Ambrosia psilostachya
Giant ragweed	Asteraceae	Ambrosia trifida var. texana
Chocolate flower	Asteraceae	Berlandiera lyrata
Annual sunflower	Asteraceae	Helianthus annuus
Narrowleaf sumpweed	Asteraceae	Iva angustifolia
Seacoast sumpweed	Asteraceae	Iva annua
Mexican hat	Asteraceae	Ratibida columnifera
Goldenrod	Asteraceae	Solidago species
Slim aster	Asteraceae	Symphyotrichum divaricatum
Redbud	Fabaceae	Cercis canadensis
Texas red oak <sup>x</sup>	Fagaceae	Quercus nuttallii (Q. buckleyi)
Pecan	Juglandaceae	Carya illinoinensis
Red mulberry	Moraceae	Morus rubra
Sycamore	Platanaceae	Platanus occidentalis

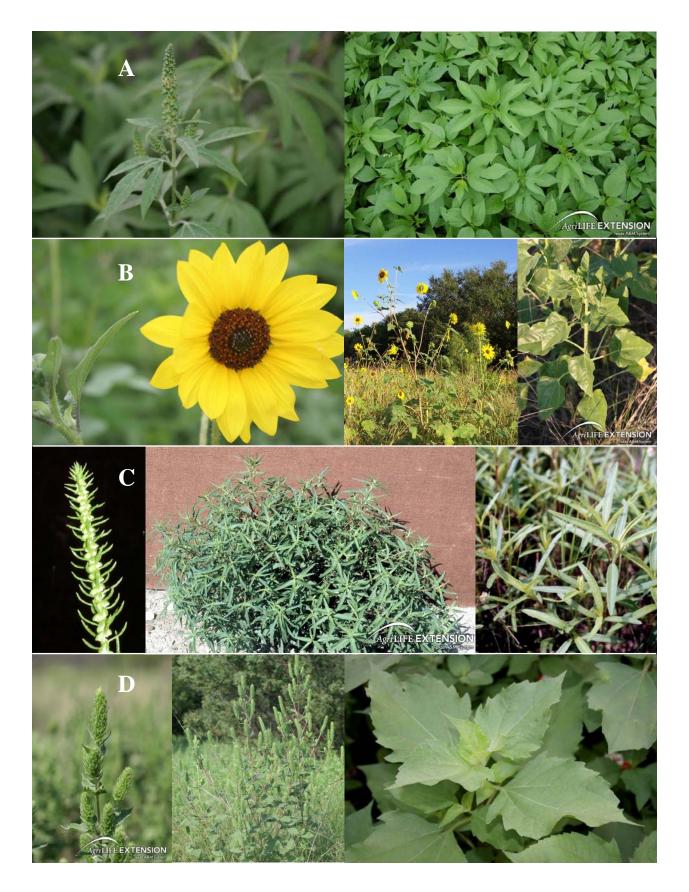
Western soapberry	Sapindaceae	Sapindus saponaria var. drummondii
Cedar elm	Ulmaceae	Ulmus crassifolia
Heart-leaf ampelopsis	Vitaceae	Ampelopsis cordata
Lavender	Lamiaceae	Lavandula sp.

<sup>z</sup>D.N. Appel laboratory and F. Mitchell/Jeff Brady laboratory.

Table 4. Cool season wildflower and forage plants considered safe or unsafe near vineyards in central and southwest Texas where Pierce's disease risk is high. Plants were mechanically inoculated with *Xylella fastidiosa* isolates representing three subspecies were later tested with serology.

Common name(s)	Family	Species	Flowering <sup>z</sup>
	PD saf	fe species	
Cilantro	Apiaceae	Coriandrum sativum	April to July
Plains coreopsis, wildtype & 'Dwarf red'	Asteraceae	Coreopsis tinctoria	Spring to early summer
Catchfly	Caryophyllaceae	Silene armeria	Spring
Annual ryegrass	Poaceae	Lolium multiflorum	Spring, forage
Buckwheat	Polygonaceae	Fagopyrum esculentum	Late spring, drought sensitive
Tuber vervain	Verbenaceae	Verbena rigida	April to October
	PD unse	ife species	
Yarrow	Asteraceae	Achillea millefolium	Spring to summer
Blanketflower	Asteraceae	Gaillardia aristata	Spring
Indian blanket	Asteraceae	G. pulchella var. pulchella	Late spring to early summer
Tahoka daisy	Asteraceae	Machaeranthera tanacetifolia	Late spring to summer
Texas bluebonnet	Fabaceae	Lupinus texensis	Spring
Burr medic 'Armadillo'	Fabaceae	Medicago polymorpha	Spring
Small burr medic 'Devine'	Fabaceae	M. polymorpha	Spring
Crimson clover	Fabaceae	Trifolium incarnatum	Spring
White clover 'Durana'	Fabaceae	T. repens	Spring and fall
Scarlet flax	Linaceae	Linum rubrum	April to September
Showy primrose	Onagraceae	Oenothera speciosa	April to July
Drummond phlox	Polemoniaceae	Phlox drummondii	Spring
Petunia 'Laura Bush'	Solanaceae	Petunia x violaceae	Spring to frost

<sup>z</sup>Plant height and flowering duration are influenced by rain and irrigation.



**Fig. 1** (previous page). Pierce's disease risk indicator species in central and southwest Texas. **A**, Giant ragweed. **B**, Annual sunflower. **C**, Narrow leaf sumpweed. **D**, Seacoast sumpweed. Some photographs are from <u>http://essmextension.tamu.edu/plantsdev/</u> and <u>http://www.sbs.utexas.edu/bio406d</u>.



Fig. 2. Goat browsing weeds. Photo by Rick Machen, Texas AgriLife Extension Service.



Fig. 3. Irrigated wildflower small plots between vineyard blocks in May 2009.

### **Disease Control Through Roguing of Infected Grapevines**

### - David N. Appel

To rogue is to remove or destroy diseased plants or their parts with the intent of reducing, or eliminating, the pathogen from doing further harm to the crop. In the case of Pierce's Disease, roguing would consist of pulling diseased vines immediately when the diagnosis is confirmed so that sharpshooters would have no opportunity to further transmit X. fastidiosa and induce new infections. Under some circumstances, pruning diseased canes may be appropriate in an attempt to extend the productive life of a vine as well as reduce sources of infection, particularly in tolerant grape varieties.

Roguing may be most effective in vineyards that are free of any appreciable level of Pierce's Disease rather than those where the disease is present in high levels. This would include new vineyards or those where the disease is being successfully suppressed by other management practices such as



applications of insecticides. Where disease levels are already high and the vineyard is being managed to maximize production on declining numbers of vines, pruning may be more inappropriate. Removal of only the symptomatic vines may not be sufficient to reduce the infection rate because adjacent vines may also be infected but not yet showing symptoms. For this reason, symptomless vines next to those in the advanced stages of infection should also be removed.

Early diagnosis and prompt removal are essential for roguing to be effective. The longer the diseased vine is allowed to remain in the vineyard, the greater the chances that sharpshooters will sustain disease development. Therefore, vineyards should be continually monitored for symptoms of infection. In addition, the need to supplement rouging with other recommended disease management practices is emphasized.

### Maintaining Vine Health - Jim Kamas

Although the goal is to avoid "hot sharpshooters" feeding in the vineyard, it is important to realize that a single inoculation event does not necessarily mean disease development. There is a tremendous difference in a 'Chardonnay' plant in a West Texas vineyard experiencing a single feeding event from a hot sharpshooter and the same vine in Southeast Texas that is being fed upon twenty times per day by hot sharpshooters. The West Texas vine has a chance of not developing disease while the vine in Southeast Texas is probably in serious trouble. When a plant's defense mechanisms are employed, and the plant is not under other stresses, it is possible for a plant to resist the invasion of a pathogen. *Xylella* is a variable and somewhat delicate pathogen. There have been occasions when a vineyard block has shown wide-spread infection, has gone through a mild, wet growing season, and has become nearly asymptomatic. Our goals as grape growers is to create an environment in and around our vineyards that are very inhospitable to sharpshooters and to maintain our vines in the best health possible.

#### Vineyard Floor Management



A Weed-free Strip Under the Trellis and Tightly Mowed Row Centers Are Ideal for Maintaining Vine Health and Deterring Sharpshooters Immaculate weed control is an integral part of reducing the risk of Pierce's disease. Because of the wide diversity of insects that can transmit PD, it is not possible to selectively remove only plant species that serve as supplemental feeding sources for a select few species. With over 30 species of competent vectors, this group represents a wide selection of both grass and broadleaf weed feeders. So, whether it is annual or perennial grasses or

broadleaves, when weed stature exceeds three or four inches, it can serve as a feeding source for some kind of sharpshooter.

While cultivation certainly eliminates unwanted plants, there are a number of reasons that row center

cultivation is not recommended. Most vine roots are in the top few inches of soil where there is the greatest oxygen content in soil free space. Cultivation destroys valuable roots needed for water and nutrient uptake leading to a dwarfing effect in the vineyard. Cultivation also decreases soil structure and leaves vineyards subject to soil erosion from high rainfall or high wind events. In all areas of Texas, successful grape production relies on the timely application of fungicides. When a vineyard floor is cultivated, and rain falls triggering the need for spraying, vineyards are commonly impassible with spray equipment, or results in serious rutting and compaction if sprayers are forced to operate on wet cultivated ground. It is impractical to try to cultivate a specific row center cover crop during the growing season. Most growers simply manage existing native vegetation with a shredder or flail mower. Close, frequent mowing is perhaps the best method for both ensuring equipment has proper footing for passage in wet weather and still creating an environment inhospitable for sharpshooters. During the dormant season, cool season cover crops such as annual rye grass or oats are recommended to improve soil structure and once again support equipment movement in the vineyard during spring activities. Grass feeding sharpshooters do not pose a hazard to dormant vines, so there is no threat of additional PD risk. Winter covers can be killed with herbicides at bud-break or simply kept mowed until they die from early summer heat.

Herbicides are recommended for managing competitive vegetation under the trellis. Preemergence herbicides act by inhibiting the germination of weed seeds, but generally have little effect on existing perennial vegetation that may arise from roots or stolons year after year. They also typically depend on varying amounts of rainfall to incorporate them into the soil where they become active. The application of a pre-emergence material after weed seed germination typically has no effect on controlling those weeds, so applications must be made on a timely basis and the weather needs to cooperate to have success with this approach.

Contact herbicides do not depend on rainfall for activity and are more commonly used by a majority of growers to manage weeds in the vine row. Contact materials vary in their systemic activity, so caution must be made in choosing the correct material at a specific stage of vine growth to minimize the chance of injury. Windy spring conditions often complicate and greatly limit spraying opportunities, so relying on contact herbicides can also present its own set of challenges.

In addition to keeping sharpshooters out of the vineyard, solid weed control is one of the foundations of successful vineyard management. Weeds compete for nutrients, but the greatest threat of undesirable vegetation is competition for water. Especially during drought conditions, unmanaged weed growth can greatly inhibit the establishment and maintenance of a healthy canopy and crop. While we can apply supplemental water with drip irrigation, when available water is removed from areas of the vineyard floor not supplied with irrigation, the roots in those areas become quiescent and no longer function. This results in stressed vines less capable of ripening a crop or fending off infection from a multitude of pathogens. With or without Pierce's disease, the one thing that successful vineyard operations have in common is good vineyard floor management.

#### Crop Load Management

The greatest way to guard against overly stressed vines is to not exceed vine bearing capacity. Remember, you need a vigorous vine to bear a full crop. A vine's vegetative growth is the result of being supplied with ample water, adequate nutrient levels, good weed control and an appropriate crop load. A vine's bearing capacity is expressed through annual dormant pruning weights. If for whatever reason, a vines pruning weights are less than the previous year, its ability to bear and ripen a crop is proportionally reduced. Failing to observe and follow this phenomenon will result in a plant with weakened defense mechanisms that is not only more susceptible to infection from Pierce's disease, but other bacterial and fungal pathogens as well. Maintaining this vine balance of vegetative growth and fruit production is the first rule of maintaining vine health.

There is no number of tons per acre that can be identified as a universal target for appropriate yield. This is a variety/vine

health/site/season/management interaction. For example, in some locations in Texas 3.5 tons per



Monitor and Adjust Crop Loads to Ensure High Quality Fruit and Optimal Vine Health

acre for 'Merlot' may be an appropriate cropping level, while a 10 ton crop 'Chenin Blanc' may be entirely appropriate. The goal is to ripen a sustainable crop level of very high quality fruit without negatively impacting overall vine health. Whether from winter injury, crown gall or fungal pathogen infection or Pierce's disease, over cropping vines is the quickest way to predispose grapevines to injury.

### Maintaining a Healthy Canopy



Healthy Canopies Depend on Proper Vineyard Fertilization Grapevines need to be supplied with appropriate amounts of nutrients on an annual basis. It is important for growers to understand nutrient availability, uptake, and the potential competition between nutrients in order to design a fertilization program capable of supporting a healthy canopy. Nitrogen is typically applied every year, but in most cases vineyards can function well on small amounts of soluble nitrogen applied through the drip system. Since nitrogen is subject to leaching, in wet years, foliar applications of nitrogen may be needed simply to keep an existing canopy healthy well into the fall. Other elemental deficiencies such as potassium, magnesium and zinc may also have a negative impact on canopy health, but petiole samples can help growers accurately determine fertilization needs in order to promote good vine health.

Fungal pathogens, especially powdery mildew and

downy mildew, can cause rapid deterioration of grape foliage. While most growers rightly focus on disease control from bud-break to harvest, it is a common mistake to ignore fungal pathogens

in late summer and fall. In addition to building up inoculum for the coming growing season, these diseases can cause premature defoliation leaving vines in a weakened condition. The use of strobiluran and ergesterol-inhibiting fungicides is probably best left to critical times of fruit protection, but inexpensive fungicides like sulfur and copper can play an important part managing foliage diseases after harvest. Canopy management also impacts overall vine health. Ensuring good air movement and ample light interception helps reduce the potential for loss of canopy from fungal pathogens and simple shading. Growing a healthy canopy is a labor intensive enterprise, but necessary for optimal crop maturity and overall vine health.

### Supplemental Irrigation

All Texas grape growing regions are subject to drought. A vine with inadequate water is incapable of growing and maintaining a healthy canopy and ripening a crop. As vines start to lack water, they begin dropping foliage in order to reduce the transpirational stream. As water deficit accelerates, basal leaves begin to senesce and the drop. The net result is a vine that becomes less photosynthetically efficient, less capable of ripening a crop, less able to mature canes and put into a weakened state going into winter. These vines are also far less capable of fending off an inoculation of *Xylella* than a plant with a healthy canopy. While we do not have control of the weather, supplemental irrigation systems need to be designed to be able to supply sufficient water to as much as the vines over the course of the worst imaginable drought situation.

### Using Trap Hedges to Manage Vectors - M.C. Black

"Trap crop" is a pest control strategy designed to engage and reduce or eliminate a pest before it gets established on agricultural plants. The goal for Texas vineyards is attraction of glassywinged sharpshooter (GWSS) and other vectors of the Pierce's disease (PD) bacterium, *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*), to non-grape plants outside a vineyard where the insects can be killed before they reach the nearby PD susceptible vines. This will probably be deployed as a hedge around vineyard blocks rather than large block adjacent to vineyards, so we are referring to this strategy as "trap hedge."

### Regions

This strategy mostly applies to a wide swath of the state of Texas from south and southwest Texas to north and northeast Texas. The Panhandle has low PD risk due to colder winters, and we have no evidence to date that the obligatory PD tolerant varieties grown in southeast Texas would benefit from reduced PD challenges from insect feeding. Short statured annuals are typically trap crop species of choice for most other agricultural crops, but we are pursuing a mixture of annuals, perennials, and woody plants in a range of heights and phenologies (timing of vegetative growth, flowering, seed set, senescence or winter dormancy) for reducing PD risk in winegrape in Texas.

### The Trap

Knowledge of *Xff* insect vector behaviors informs our plan for a hedge highly enticing to GWSS and other vector species. At peak flight activity, 97% of incoming GWSS trapped between 1 and 7 m were trapped at 5 m or lower (M.J. Blua and D.J.W. Morgan. 2003. J. Econ. Ento. 96:1369-1374). A trap hedge near tall riparian trees should have foliage from near ground level to at least 16 or 17 ft.

GWSS prefers to make short flights to adjacent plants, and locates plants by sight, smell, feel, and taste, in that order of importance. Provide mowed perimeters 1) between the trap hedge and riparian habit (uncontrolled vegetation, especially near seasonal and permanent bodies of water); and 2) between the trap hedge and the vineyard. We have not yet worked out minimum widths or width ratios for these mowed strips, but suggest a range of 50 to 100 ft mowed on each side of the trap hedge. Greater widths are suggested where the trap hedge is near tall riparian trees or an irrigated orchard. The gap between vineyard and hedge should probably be greater than the gap between hedge and suspect vegetation. Without a trap hedge, studies suggest 328 ft (100 m) of mowed area to minimize GWSS movements from mixed vegetation to the vineyard (R. Mizell). Therefore, the trap hedge may reduce the amount of non-crop land around vineyards.

Individual GWSS adults may feed on several plant species in one day during the warm season in order to meet nutritional needs for reproduction and dispersal (A. Purcell, *personal communication*, R. Mizell). Plant preferences change throughout both warm and cool seasons of

the year. All species in a trap hedge should have a history of use by GWSS or other vectors during nymph (immature) and/or adult feeding. A plant used only for egg lay (oviposition) should perhaps be avoided. A trap hedge should be diverse and include one or more grasses to attract Johnson grass sharp shooter (JGSS, *Homalodisca insolita*) and other grass feeding vectors.

GWSS prefers to feed on tender new growth. Some woody species in a trap hedge should be pruned periodically and/or irrigated in the warm season to stimulate additional terminal growth.

GWSS is attracted to the fragrance from certain flowers. A trap hedge should include some odiferous (hopefully fragrant to humans) flowering plants with staggered bloom dates. A bonus is that owners, workers, and tourists enjoy flowers too!

### The Kill

The insecticide of choice is thiamethoxam (Platinum®, Flagship®) drenched on roots at approximately 8 week intervals. This neonicotinoid insecticide is less repellant to GWSS than imidicloprid (Admire®, Merit®) yet is toxic to this insect species (N. Toscano). Growers could inject thiamethoxam through drip irrigation. Platinum has 4X the solubility of and shorter residual than Admire, and should last 2 months. Leaching of the more soluble soil applied neonicotinoid insecticides may be a possibility for a trap hedge section near a shallow water table, on permeable soils, or in a drainage area. A carefully applied foliar spray of acetamiprid (Assail®) may be more appropriate if leaching is a concern, controlling GWSS for about 2 weeks and sometimes 1 month (N. Toscano, personal communication).

The trap hedge experiment underway in Uvalde County already has clear differences in establishment success among plant species after the severe drought year of 2011 (Table 1), but GWSS did not colonize the site in 2011. We will continue to evaluate feasibility, estimate costs, and monitor GWSS interactions with plant species phenologies.

### Poor soil sites

The route of a trap hedge may transect very poor soil sites, such as caliche or rocky outcrops. Substitute natives at these sites rather than planting something clearly not adapted. For example, Texas mountain laurel and evergreen sumac may survive in some difficult sites where crape myrtle or bigtoothed maple would have little chance of establishing even with drip irrigation. Xeric species such as yucca, stool, and cacti may also be options in very well drained situations.

### Maintenance

Weeds will be an issue at least initially in a hedge planting. Our year-1 control efforts included 1) spreading mulch (coarse shredded yard waste) in the hedge area to cover all soil that would receive irrigation, 2) hoeing and hand weeding, and 3) spot treatments with glufosinate-ammonium (Rely®) herbicide in a hooded backpack sprayer, or grass selective herbicides for

bermudagrass, johnsongrass, etc. Winter trimmings from cutting-back annuals and perennials, and pruning woody plants, could be used directly or shredded on site for replenishing the mulch. This will help with reseeding at least some desired plants in the hedge.

Grasses and perennials must be cut back to near-ground level in winter for reasons that include fire hazard. Annuals must be re-seeded or transplanted in fall or spring unless there are volunteers from a seed bank in soil. Shrubs should be pruned to remove dead or broken branches, and to force vigorous new growth in the spring. Trees that exceed 16 to 20 feet tall should be cut back in late winter dormancy to avoid excess shade on nearby smaller plants. After spring growth stops on woody plants, some should be pruned lightly in summer on staggered dates to encourage new terminal growth preferred by GWSS and other vectors.

Birds droppings contain viable seeds of several vines and brush, and there will be volunteer plants ranging from poisonous ivy (*Toxicodendron radicans*) to hackberry (*Celtis laevigata*). These can be spot treated with a labeled herbicide in the seedling stage in the warm season or as cut stumps (dormancy may be safer for nearby grapevines).

### Other diseases

Some sites may see some plant mortality from cotton root rot disease, caused by the endemic soil fungus *Phymatotrichopsis ominvora* (syn. *Phymatotrichum omnivorum*). This fungus flares up in alkaline soils (usually  $7.0 < pH \le 8.3$ ) in the warm season primarily on introduced ornamentals, but also on native plants where soil is periodically saturated with rain or irrigation. Diversity in the hedge (including resistant grasses) should limit plant losses, and any replanting should use more resistant species (usually natives will survive).

Some species are susceptible to one or more water molds in southwest Texas soils, (*Phytophthora* spp.) including some cultivars of Madagascar periwinkle that are highly susceptible to *Phytophthora parasitica*.

Table 1. Thirty-five species<sup>z</sup> from 20 plant families are being evaluated near Cook's Slough Nature Park, Uvalde, TX for attraction of migrating glassy-winged sharpshooter (*Homalodisca vitripennis*) and other xylem-feeding insects to a hedge treated with insecticide. The trap hedge strategy (with fewer species) would be deployed around Texas vineyards at risk for Pierce's disease to reduce insect vectors of *Xylella fastidiosa* entering vineyards. Short lived annuals alternate between warm and cool seasons. Grape is included as a control treatment.

Common name	Family Short-	Plant species	Established, % 5Oct11
Fennel	Apiaceae	Foeniculum vulgare	100
Plains coreopsis	Asteraceae	Coreopsis tinctoria	100
Engelmann's daisy	Asteraceae	Engelmannia peristenia	64
Maximillian sunflower	Asteraceae	Helianthus maximiliani	100

Western ironweed	Asteraceae	Vernonia baldwinii	100
Esperanza (yellow bells)	Bignoniaceae	Tecoma stans	100
Catchfly	Caryophyllaceae	Silene armeria	100
Rough-leaf dogwood	Cornaceae	Cornus drummondii	100
Black dalea	Fabaceae	Dalea frutescens	72
Texas bluebonnet / Madagas-	Fabaceae /	Lupinus texensis /	100 / 96
car periwinkle (vinca) 'Cora' (burgundy flowers)	Apocynaceae	Catharanthus roseus	
Vasey shin oak	Fagaceae	Quercus pungens var. vaseyana (Q. vaseyana)	100
Autumn sage	Lamiaceae	Salvia greggii	100
Big bluestem	Poaceae	Andropogon gerardii	92
Sideoats grama	Poaceae	Bouteloua curtipendula	100
Switchgrass	Poaceae	Panicum virgatum	100
Yellow indiangrass	Poaceae	Sorghastrum nutans	100
Annual ryegrass / Orange	Poaceae /	Lolium multiflorum /	100 / 100
cosmos	Asteraceae	Cosmos sulphureus	
Common lantana	Verbenaceae	Lantana urticoides (L. horrida)	100
Tuber vervain	Verbenaceae	Verbena rigida	100
Grape 'Blanc du Bois' on '5BB' rootstock	Vitaceae	<i>Vitis</i> hybrid 'Blanc du Bois' on <i>V. berlandieri</i> x <i>riparia</i> '5BB' rootstock	96

Intermediate					
Prairie flameleaf sumac	Anacardiaceae	Rhus lanceolata	100		
Evergreen sumac	Anacardiaceae	Rhus virens	100		
Possum-haw, female	Aquifoliaceae	Ilex decidua	100		
Goldenball leadtree	Fabaceae	Leucaena retusa	100		
Texas mountain laurel	Fabaceae	Sophora secundiflora	100		
Crape myrtle 'Zuni' (lavender flowers)	Lythraceae	Lagerstroemia indica x fauriei 'Zuni'	100		
Satsuma citrus 'Seto' on 'Sour orange'	Rutaceae	<i>Citrus unshiu</i> 'Seto' on <i>C.</i> <i>aurantiurm</i> 'Sour orange' rootstock	96		
Mexican-buckeye	Sapindaceae	Ungnadia speciosa	100		
Tree tobacco	Solanaceae	<i>Nicotiana glauca</i> (tree tobacco)	92		
Vitex (purple flowers)	Verbenaceae	Vitex agnus-castus	100		

		<i>Tall</i>	
Big toothed maple	Aceraceae	Acer grandidentatum var. sinuosum	62
Texas red oak, Spanish oak	Fagaceae	Quercus buckleyi	92
Lacey's oak	Fagaceae	Quercus laceyi, syn. Q. glaucoides	92
Texas ash	Oleaceae	Fraxinus texensis	100
Paper-shell pinyon	Pinaceae	Pinus remota	92

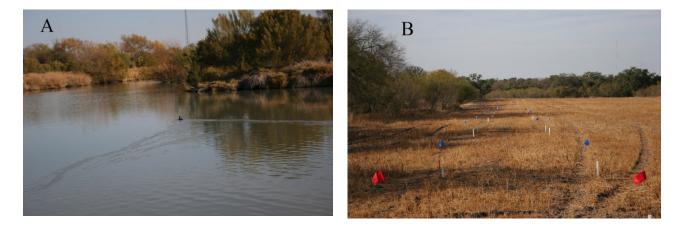
<sup>z</sup>Species list would vary for different sites due to soil, winter temperatures, irrigation, personal preference, new data, etc. One objective of this study is to prioritize plants for usage by GWSS and other vectors so growers can optimize attraction in a trap hedge.







**Fig. 1.** Experimental trap hedge near Cook's Slough Nature Park, Uvalde, TX. **A &B**, **6**May2011, linear layout <10 ft wide with layflat irrigation main line for five headers for the five replications, drip irrigation lines being covered with mulch (shredded yard waste) and electric fencing. Tall species are in center, intermediate sized species are mid-way to both edges, and small species are on both edges. **C & D**, 21July2011, significant growth and some flowering have occurred. **C**, 'Blanc du Bois' winegrape control plant supported by a T-post, with inevitable pigweeds (front left). **D**, Western ironweed (*Vernonia baldwinii*) in flower (center front). **E & F**, 4October2011, some plant canopies have overlapped, leaf scorch from drought and heat have affected some woody species. **F**, Tree tobacco (back left) and switchgrass (center) had high growth rates compared to Mexican buckeye (right front).



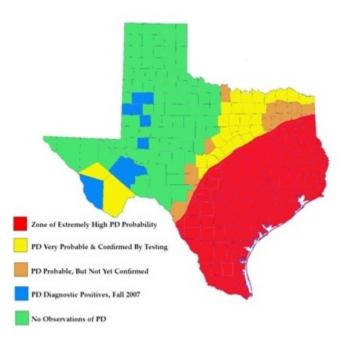
**Figure 2. A**, Artificial wetland with riparian vegetation at Cook's Slough Nature park, Uvalde, TX, 75 m south of the trap hedge experimental site. **B**, Linear layout of trap hedge plots during drought, 17Dec2011. Cook's Slough flows through live oaks in the background (west). Mesquite brush on the left was cleared back to a fence line with mostly hackberry.

## Synopsis of Recommended Practices for Successful Management of Pierce's Disease - *Jim Kamas*

**1. Determine Relative Risk-** For Texas, historical maps can give you a good idea of the relative disease pressure where you intend to locate your vineyard. Don't deceive yourself. Just because you own or are very attached to a field or an area, the risk of Pierce's disease will not go away with will power. For growers outside of Texas where Pierce's disease exists, areas that receive 700 hours of winter chilling or less should be considered extremely high risk areas. 700-850 hour chilling zones are moderate to high risk and 850 and greater chilling zones are low to moderate risk.

<u>Extremely High Pressure Areas</u>- If you are in a very high PD risk area, it is strongly recommended that tolerant or resistant varieties be planted. As with any new vineyard venture, talk to a winery about specific needs and make variety choices accordingly. If susceptible varieties are planted, be sure to isolate them from other blocks of tolerant varieties.

<u>High to Moderate Risk Areas</u>- Pierce's disease will definitely be a limiting factor to the production of susceptible grape varieties. Even with superior management, high levels of vine loss may be encountered. Site selection plays an extremely important role in mitigating this risk. <u>Moderate to Low Risk Areas</u>-



Moderate risk zones may still have relatively high disease pressure, and again, site selection can help mitigate relative risk. In some northern areas where disease presence has been confirmed, Pierce's disease may be more of a chronic problem rather than an acute one, and vine losses are still possible. Rouging strategies may be less aggressive in low risk zones as compared to areas of relatively higher risk.

**2.** Select Site With Risk Management in Mind- Avoid planting vineyards near native perennial vegetation. Because xylem feeding insects prefer a diversity of vegetation well supplied with water, creek and river bottoms pose an inherently higher risk. There is no set distance from a bottom-land site that is considered safe. The farther the better.

**3. Create Buffer Area-** When selecting an area for a vineyard, be sure to have control of the vegetation several hundred feet in any direction from the prospective site. It is important to have the ability to manage vegetation adjacent around the vineyard. Removal of perennial trees and shrubs and mowing of fields will keep xylem feeding insects from colonizing areas in close proximity to susceptible vines. Like site selection, the greater the distance to perennial or unmowed areas, the better

**4. Remove Suspected Supplemental Hosts-** Wild grapevines are capable of being colonized by *Xylella fastidiosa* and seldom show typical symptoms of Pierce's disease. Removal of wild vines is recommended to whatever distance that is practical. Become familiar with other plants capable of hosting *Xylella* and take steps to remove these plants.

**5. Use Neonicotenoid Insecticides-** After planting, apply imidacloprid or other nicotenoid through the drip system. First and second leaf vines can be treated with half the full labeled rate. Treat third leaf and older vines with the full labeled rate. Become familiar with logistical practices and timing of effective insecticide application.

6. Learn to Identify Insect Vectors of Pierce's Disease and Monitor Vector Presence and Seasonality. There are many vectors of Pierce's disease across Texas and the Southeastern



United States. Become familiar with what they look like and use yellow sticky traps to monitor vectors in and around the vineyard. This practice can be reinforced when vectors are present and may help identify which direction vectors are entering the vineyard from. This practice may indicate that removal of problematic supplemental feeding and reproduction hosts may be necessary.

**7. Maintain superior vineyard floor management**-Grapevines are not necessarily the favorite dining spot for xylem feeding insects. Sharpshooters need to change feeding hosts frequently to meet their dietary needs and having a vineyard with weeds favors infestation by sharpshooters. The recommendation is to maintain a 3 to 4 foot weed-free area under the vines and maintain vineyard row centers with close, frequent mowing.

**8. Keep Vegetation Surrounding the Vineyard Well Managed-** For the same reasons outlined for superior vineyard floor management, vegetation should be mowed frequently around the vineyard to keep sharpshooter populations low. Allowing adjacent fields to grow for any length of time will attract a diversity of insect species. Mowing infrequently chases these populations into the vineyard looking to find new food sources. Timely mowing beginning in late winter can prevent these near-by populations of vectors from becoming established.

**9. Become Familiar With the Symptoms of Pierce's Disease**- Disease symptoms can change subtly from variety to variety. Become familiar with what the disease looks like and be prepared to take action. Hoping for a cold, curative winter is not advisable.

# **10. Submit Grapevine Tissue of Suspected Infected Vines for Laboratory Analysis-**

Contact your local extension agent or extension specialist to identify a laboratory that can conduct appropriate diagnostic tests for PD. Other pathogens or environmental stresses may produce symptoms similar to PD, so it is important to confirm or deny vine infection status. It is economically impractical to submit every suspected vine for laboratory diagnosis, but it is very important to use a diagnostic lab to confirm suspected visual symptoms until you become confident of your ability to diagnose the disease in your vineyard.



**11. Follow Vine Roguing Strategies Appropriate for Your Production Area**- Relative risk of disease spread varies across growing regions. Learn which roguing protocol is appropriate for your area and act immediately upon confirmation of vines being infected. East of the Rocky Mountains, sharpshooter species can rapidly spread the disease within a vineyard. Removal of disease sources is essential to managing the epidemic.

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