Unraveling Pierce's Disease in Its Ancient Environment

With hopes of decreasing the widespread death of wine grapes, Texas researchers seek answers in the vectors and bacterial pathogens that fuel Pierce's disease in their region.

By the Texas Pierce's Disease Task Force

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For most of Texas, Pierce's disease (PD) is the greatest limiting factor in cultivating *Vitis vinifera* and most hybrid wine grapes.Widespread death of wine grapes has been a common occurrence in Texas since the first introduction of old-world varieties brought by European settlers over four hundred years ago. Although the scientific knowledge of Pierce's disease has grown significantly in recent years, little was known about the variety of vectors or how the bacterial pathogen (*Xylella fastidiosa*) colonizes the plants and xylem-feeding insects in Texas ecosystems where the disease has long been endemic.

In the 1970s and early 1980s, PD risk was thought to be strongly correlated with proximity to the Gulf of Mexico. Mild winters and a diversity of plants suitable for sharpshooter feeding and reproduction made the coastal areas of Texas ideal for both vector and pathogen.

There are numerous scientific advantages to studying a disease where it is highly endemic, including: survey of plant reservoirs, evaluation of resistance in native grape species and hybrid varieties, and genetic diversity of the pathogen. Of equal importance, entomological studies can evaluate vector diversity and population levels in a natural environment (as opposed to California where the Glassy-winged sharpshooter is still in outbreak mode), and discover potential vector parasitoids and pathogens.

In the mid 1990s, the incidence and severity of Pierce's disease escalated dramatically in the Texas Hill Country (west of Austin and north of San Antonio). While this area of Texas was once thought to be a transition zone between high- and low-disease probabilities, many established Hill Country vineyards have seen increased vine mortality due to PD .

It is speculated that a series of warm winters allowed the pathogen to become more widely distributed throughout the native plant community, providing the initial inoculum for vineyard infection. While the disease is not known to occur in the northern Panhandle of the state, recent outbreaks in areas thought to be at relatively low risk in far-west Texas have changed the thinking on where the pathogen and vectors can ultimately survive and move into commercial grape plantings.

Project Background

From its modest beginnings in 1998, members of the Texas Pierce's Disease Task Force started to investigate how the disease and its vectors interact in their native habitat. Initially, limited industry and university funding provided the first insight into the diversity of sharpshooters active in and around Texas vineyards.

In 2003, Texas A&M University entered into a cooperative agreement with USDA and APHIS (Animal and Plant Health Inspection Service) that resulted in the formation of a multidisciplinary and multi-institutional research team. The team broadened the scope of previous work to include identification of supplemental *Xylella* plant hosts, spread of PD within commercial vineyards, year-round insect surveys across the state to identify potential vectors and gain an understanding of their seasonality, and utilization of GPS/ GIS (global positioning system/geographic information system) to gain an understanding of where the disease occurs and why. Program budgets grew in 2004, and a targeted annual budget of $2 million is anticipated by 2006.

Xyllella fastidiosa In Its Center of Origin

The native range of *Xylella fastidiosa*, the bacterial pathogen that causes Pierce's disease, includes the Gulf Coast of the US which may also be the center of origin for this bacterium. The Texas Gulf Coast region has warmer temperatures and much higher humidity than other grape-growing regions and subsequently has enormous disease pressure for Pierce's disease. Evaluation of specific questions in this PD hot zone allows for a better understanding of the ecology and epidemiology of the disease.

With respect to plant reservoirs for PD, we are establishing which plants routinely test positive for *X. fastidiosa*. The advantage of testing plants in an area with intense disease pressure is that it allows for evaluation of plants serving as persistent reservoirs rather than sporadic positives during an outbreak, as those positives may not survive. By testing plants from more than 45 different plant families in the area, we also will be determining trends in structure or evolutionary relatedness among tolerant plant reservoirs.

Initial evaluation of the genetic diversity of *Xylella fastidiosa* strains across Texas is underway. Partial sequencing of several strains suggests homology to other published strains of *X. fastidiosa*, but preliminary work on strain diversity suggests that there are multiple strains within Texas vineyards and within the wild plant reservoirs. *X. fastidiosa* isolates from grape, other crops, ornamentals and weeds have some visible differences in the laboratory on culture media (fast- vs. slowgrowing isolates; large vs. small colonies).

Big unknowns include the stability of *X.f.* strains and potential for strains to exchange genes. Does genetic recombination occur when two or more *X.f.* strains colonize an insect or plant? Prior to the establishment of the current Pierce's disease project, the only thing really known about the pathogenin Texas was that vineyards were sustaining alarming losses of vines.

Valuable research in California has illustrated that many factors are responsible for influencing Pierce's disease epidemiology; however, conditions in Texas are different, requiring further research to understand how the pathogen behaves in this environment.

Growers who have successful vineyards along the Gulf Coast have done so by planting American hybrid varieties, including Black Spanish (Lenoir), Blanc du Bois, and Cynthiana (Norton). We have done an extensive evaluation of the bacterial levels of these hybrids in this intense disease area. Specifically, we have evaluated bacterial levels across the growing season and over several years and are analyzing the effect of bacterial load on yield in these varieties. It appears that hybrids vary in the level of resistance to PD and potentially in the mechanism as well.

One such variety, Cynthiana (*Vitis aestivalis*), has been reported to be tolerant and has been planted in some parts of the state. Current research however shows that Cynthiana is not as resistant as might be expected. Cynthiana vines growing in greenhouses were artificially inoculated with *Xylella fasitidiosa*, only to find that the bacterium could grow and thrive throughout the entire vine. Levels of colonization resembled those of the known susceptible varieties, Cabernet Sauvignon and Chardonnay (*Vitis vinifera*). The difference was that Cynthiana showed no signs of infection, unlike the known susceptible varieties.

In the following year, Cynthiana did decline in vigor, a sign that *Xylella* was indeed affecting the vines. Field observations confirm this finding in that Cynthiana plantings have reduced yields and vigor over time in spite of not showing classical PD symptoms. In fact, these results illustrate that growers need to be aware that such varieties could indeed produce while enduring high levels of infection in the vineyard, but they could also serve as sources of inoculum that could destroy more vulnerable varieties nearby.

Diagnostics

One troubling part of the Pierce's disease scenario has been the uncertainty associated with decisive, reliable and rapid diagnosis of the disease. Each method seems to have advantages and disadvantages in such areas as cost, speed and consistency.

One of those methods, ELISA (Enzyme Linked Immunosorbent Assay), has been regularly used for many years, but criticized for consistency and sensitivity. On the other hand, a new technique named RT PCR (Real Time Polymerase Chain Reaction) holds great promise to overcome the ELISA limitations. In side-by-side tests on the same plant tissues, there was fairly consistent agreement between the two techniques, with a similarity rate of 75 percent on samples assumed infected with the pathogen. Yet the RT PCR did perform more reliably on the tissues, and had a lower rate of false negatives and false positives on uninfected tissues.

With repeated use, the RT PCR may eventually come to be the diagnostic tool of choice, but cost of the machine and the per-sample reagent costs are very high when compared to other methods. Two conclusions were clear from these studies: no diagnostic technique is completely reliable, and the consistency of the results depends on sampling routines as much as on the technique being implemented.

The diversity of phyto-chemicals in some plants is commonly blamed for false positive ELISA results or inhibition of PCR enzymes creating false negatives. Although steps can be taken to reduce these risks, the novel use of indirect immuno-fluorescence has been employed in Texas.

The technique indirectly attaches fluorescent antibodies to *X. fastidiosa* cells within the plant sap. A small amount of xylem fluid from wild plants (or grapevines) can be evaluated under a fluorescent microscope to confirm either a positive ELISA or PCR result. This immuno-fluorescence technique allows for the visualization of cells from a variety of plants, and its application to multiple *X. fastidiosa* strains suggests differential expression of antigen (brighter signal) and different cell morphologies among strains.

Vector Diversity and Behavior

Glassy-winged sharpshooter (GWSS) and *X. fastidiosa* are almost certain to have had a long history of coexistence in much of Texas.While incidence and severity of Pierce’s disease in Texas appears to be strongly correlated with GWSS numbers, by no means is that the only vector responsible for the spread of the disease.

Of about 110 Homoptera species captured over the past two seasons, a total of 20 species were identified as xylem feeders, all with the potential to carry and transmit *X. fastidiosa*. In 2003, insect surveys were conducted in 20 vineyards across the state, and in 2004, sampling was increased to 40 vineyards with an emphasis on the Texas Hill Country. Work continues to assay insects for their ability to acquire *Xylella* and to infect grapevines.

Initially, GWSS was thought to have been introduced into California from Florida. Two recent, independent studies have shown that the genetic footprint of the California introduction is most closely related to that of populations found in Texas. Over the past two seasons, Texas insect surveys have pointed out irregularities in GWSS behavior and population densities. One striking difference is that in California, GWSS can be readily found feeding on arm winter days while in central Texas, the insect is almost entirely absent from late fall through late spring.

Throughout the fall and winter, surveys will be initiated in an attempt to unravel the behavior of these insects during overwintering (reproductive diapause). Understanding this migratory or diapausal behavior may provide insight as to what degree the insect can ultimately establish itself in different climates.

In early 2005, additional surveys will take place to identify natural enemies of GWSS and other large sharpshooter species. Very low population numbers in areas where winter temperatures regularly drop into the single digits could indicate that biological control agents may be playing a role in limiting GWSS populations. If confirmed, there may well be application for biological control in northern California should GWSS become established.

Preliminary observations of GWSS egg-masses show a high degree of parasitism in many areas of the state suggesting there may be parasitoids that would enhance the current proposed long-term sustainable strategy in California.

The Anomalies

The Texas Hill Country is the state’s fastest growing wine region. Over the past eight years, many established vineyards have been hit hard by Pierce’s disease, but interest in new vineyards and wineries continues. In the northern part of the Hill Country, there are numerous vineyards where vectors, including Glassy-winged sharpshooter, are routinely trapped, but where PD is not known to occur.

While most of these vineyards have been established in the last 10 years, one vineyard that is over 25 years old has never had a confirmed case of PD. One aspect of this research program focuses on how this area remains disease free in the presence of abundant numbers of vectors. In this northern part of the Hill Country, we are intrigued by absence or rarity of *Xylella fastidiosa* in weeds and brush around some vineyard sites within this anomalous zone, and by the high frequency in many of the same plant species around other vineyards to the south and east. With an endemic disease and abundant vectors, investigations on what is breaking the disease triangle at these locations may have nation-wide implications.

Geographical Information System

A geographic information system (GIS) is under development to improve our understanding of the distribution of Pierce's disease and sharpshooters in Texas, in relation to geographical, environmental and viticultural factors. The GIS will combine layers of broaderscale geographical data on state maps with site-specific data collected at all vineyard locations within the state. Geographical data will include climatic factors, topography, and vegetation. The vineyard surveys will obtain data on cultivars, acreage, weed management, surrounding vegetation, presence of supplemental hosts of *Xylella*, proximity to water, imidacloprid use, and presence/absence of PD and sharpshooters.

Completion of the GIS and spatial analysis of the layers of data may reveal patterns that provide insight into factors that favor development of Pierce's disease. Greater knowledge of these risk factors will lead to improved recommendations to producers on management practices and site selection to reduce losses from PD.

Much More Than Just a Regional Program

While this research has already paid dividends on how to manage the disease for Texas growers, several aspects of this work have nationwide implications. Identification of robust parasitoids or vector pathogens, the understanding of the nature of disease tolerance in hybrids and native species, and a greater understanding of *Xylella fastidiosa* populations in its native range may provide important knowledge that could ultimately provide a breakthrough in Pierce's disease management.

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