

PD Research Center Construction is Underway

The long awaited groundbreaking of the new Pierce's Disease Research Center began last month at the Gillespie County Business Park on the outskirts of Fredericksburg.

Local businessman Sam Golden designed and financed the building of these office, laboratory and greenhouse facilities which will be leased back to the program

funded by a USDA/ APHIS cooperative agreement with the Texas Agricultural Experiment Station. Home to much of the entomological research, this facility will also provide the opportunity for interdisciplinary work among all cooperating scientists.

In addition to the lab, a research vineyard is also being established. The

one acre planting of 'Viognier', 'Semillion', 'Sangiovese', 'Tempranillo', 'Syrah' and 'Malbec' will serve as critical infrastructure to support field research on the movement of *Xylella fastidiosa* into and within vineyards. Both vineyard and facilities are expected to be completed by late spring.

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Work Begins on New Lab and Greenhouse Facilities



Trellis Installed, Irrigation System and Planting to be Completed this Spring

PD Program Funding Apparently Secure, for Now

In the wake of the elimination of other viticultural research support, the latest word is that level funding appears to be in place for the Texas PD Cooperative Agreement for the upcoming fiscal year.

The loss of "earmarked appropriations" in late January resulted in a loss of approximately \$5 million of CSREES support that was directed at PD research administered through the California

IPM program. It also meant a loss of all funding administered through both the eastern and western Viticultural Consortium. While efforts will be made to reinstate these important sources of support, the one year loss of funding will delay other prioritized areas of research.

The current budget for the APHIS Glassy-winged sharpshooter project has been reduced to approximately \$24.25

million. The majority of these funds go to monitoring and limiting the spread of glassy-winged sharpshooter in California but also includes \$1.6 million in what the agency considers critical research. The Texas program currently receives \$1.2 million of this research funding. Nothing is certain until congress passes the upcoming budget, but at this point, our position appears stable.

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Feature Article– Using Real Time PCR to Distinguish Grape and Non-grape Strains of *Xylella fastidiosa*

By Lisa Morano and Blake Bextine

The genetic diversity of *Xylella fastidiosa* strains is a critical component of the natural history of Pierce's disease within Texas. In addition, genetic fingerprints for Texas strains can answer important epidemiology questions such as the rate and direction of pathogen spread. A critical first step in the genetic analysis is the characterization of a *Xylella fastidiosa* isolate as either a grape or non-grape strain. One way to do this is to copy and sequence a gene which is known to differ between strains. Another more efficient method (in terms of time and money) is to compare melting temperature data collected by a Quantitative Real Time Polymerase Chain Reaction (QRT PCR) machine.

Polymerase Chain Reaction (PCR) is a method where total DNA of a sample is copied many, many times. Special DNA primers are designed that stick to either side of a gene of interest and enzymes are added to build DNA off of the

original DNA template. Within one to two hours, millions of copies of a gene from the original DNA template are made. Real Time PCR is a more precise methodology where fluorescent molecules are added into DNA molecules as they are built. This allows for a fluorescent signal to be produced which is directly proportional to the starting amount of DNA. This allows scientists to quantify exactly the concentration of *X. fastidiosa* and therefore the amount of bacteria in a

sample. Additionally, small genetic differences in sequences can be detected by QRT PCR using a melting curve. By slowly warming a sample and monitoring a change in fluorescence the machine can detect the smallest differences between grape and non-grape strains (Figure 1). After this important first step of identifying cultures as grape or non-grape strains, additional molecular tests can be used to describe differences that may exist within strains.



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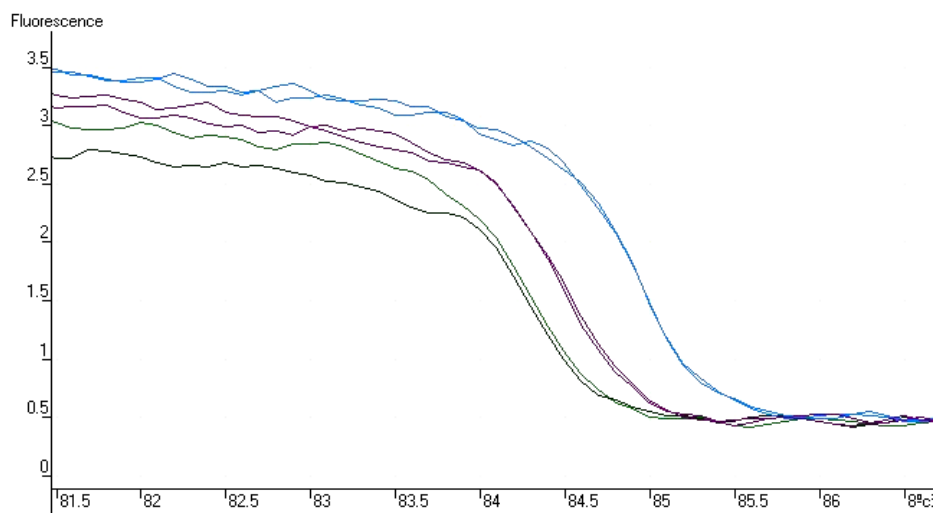


Figure 1. Fluorescence of double-stranded DNA as a function of melting temperatures. Small changes in DNA sequences result in different melting temperatures for DNA from different strains. The three curves above represent the melting curves of three different strains.

Update on The Texas Vineyard Survey – Jacy Lewis

The Texas Vineyard Survey is virtually complete with the exception of a handful of vineyards in the extreme southern and coastal regions of Texas. We are now onto the task of updating information about variety and acreage as well as insecticide treatments that may have changed since the vineyards were originally surveyed. Many growers can expect to be contacted by their new area Viticulture Extension Advisors or myself in order to gather this updated information.

I currently have identified over 25 counties in the state where commercial vineyards are currently confirmed or suspected of having Pierce's disease. I am moving forward with analyses that may draw connections between PD and several suspected environmental and ecological conditions.

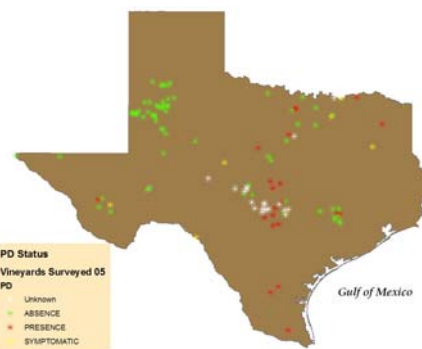
Many may ask "why?" We need a solution to PD, what difference does it make what environmental or eco-

logical conditions may be indicators of PD outbreaks? This information could potentially lead to the development of new management techniques for Pierce's disease. Of equal importance, this information may be used to develop a risk assessment model for potential new vineyard sites in the state. In recent years we have seen an expansion of Pierce's disease into areas previously considered too cold for disease development. This information may additionally indicate areas that are currently considered "PD safe", where early management intervention may limit or eliminate a future outbreak, as well as saving treatment costs for areas where the risk for outbreak is very low based on solid decision making criteria.

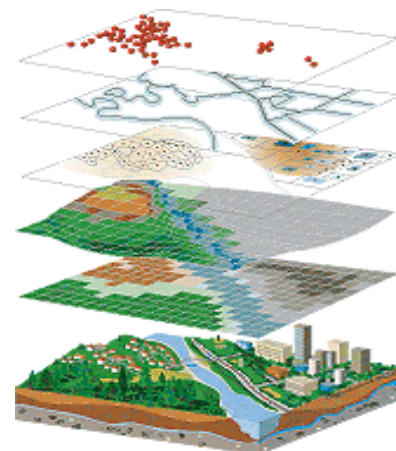
In order for disease to develop, conditions must be right in order to sustain both the agent of disease (bacteria) and the vector. At this point, very little is understood about the range

and potential ranges of either within the state of Texas. An understanding of these two factors is necessary in order to understand both how to predict the future spread of the disease as well as how best to control the disease in a given situation. An understanding of all facets of the disease may lead to a way to target control of the disease by taking advantage of the environmental vulnerability of either the pathogen or the vector. For the 2007 season, I plan to continue to update the database with new vineyard information about previously surveyed vineyards, the inclusion of newly planted vineyards and any that may not have been surveyed to date. I plan to concentrate the bulk of my time on landscape level analyses as well as analyses specific to the host and bacteria in order to shed further light on the epidemiology of the disease.

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Work Continues to Update the Preliminary Data Collected During the 2004-2005 Growing Seasons



Overlaying Layers of Data Helps Reveal Factors That May Be Critical to PD Disease Development

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