

Overview of the High Plains Pierce's Disease Vector Assessment Program— Jacy Lewis & Isabelle Lauzière

Funded by a generous grant from the Texas Department of Agriculture and supported by AgriLife Research, AgriLife Extension and the Pierce's Disease Research and Education Program, the High Plains Pierce's Disease Vector Trapping/Evaluation Program was initiated in April 2008 in response to the newly discovered threat of Pierce's disease in the Texas High Plains grape growing region. After initial diagnostic positives of *Xylella fastidiosa* from grape vines in the High Plains region in late 2007, and the subsequent confirmation of those findings in the early summer of 2008, the threat of Pierce's disease on the High Plains appears to be substantiated. This program's goals are to identify potential insect vectors for *X. fastidiosa* on the High Plains as well as to evaluate the extent of *X. fastidiosa* infection and to understand the dynamics and consequences of Pierce's disease in this grape growing region.

The entomological goals of this project include an assessment of the potential vector populations in this region, a refinement of trapping protocols for those specific vector species, development of trapping techniques that withstand environmental conditions of this area. Unlike other areas in the state, the High Plains area has many specific challenges to the assessment of vector populations and a goal of this program is to address this issue.

While the first quarter of this program has been dedicated to the selection of participating vineyards and development of trapping protocols and strategies, a number of interesting findings have already been made. So far, insects that have been confirmed from High Plains vineyards include *Cuerna costalis*, *Cuerna striata*, *Cuerna obesa*, and one species of *Cuerna* that is yet undescribed in the lit-

erature. These trapping results have documented the first findings of some of these species in Texas vineyards or from this area of Texas. *Cuerna costalis* is known to be a competent vector of *X. fastidiosa* and by extrapolation one can assume that the other *Cuerna* species, which are anatomically very similar, are capable of vectoring the pathogen as well. Some of these species are in fact so similar to one another that they can only be discerned by time-consuming and delicate dissection of the genitalia in an insect of total length of about 0.25 inch. This large number of specimens came as a bit of a surprise, as in the three years prior, there have only been three individual specimens of *Cuerna* identified on the High Plains. As of the most current trap scoring cycle, approximately 50 *Cuerna* have been recovered from High Plains traps and approximately 100 xylem specialists. This would most likely represent an increase in trapping efficacy and not a change in the population numbers of *Cuerna* or other xylem feeders in this area though



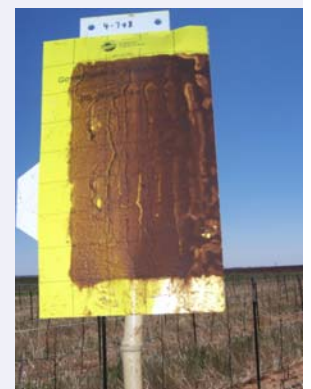
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Additional Articles Contributed by Members of the Texas Pierce's Disease Research and Education

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**Insect Trapping in the High Plains Presents Its Own Set of Challenges
Dust Storms Can Render Traps Ineffective**



High Plains Vector Survey, continued

of course this can not be certain. Additionally, a single specimen of *Homodisca vitripennis* (glassy-winged sharpshooter), one *Xyphon flaviceps*, 16 *Graphocephala hieroglyphica*, all identified by Dr. Isabelle Lauzière and Research Assistant Megan Morley have been found on traps in the High Plains area. The *H. vitripennis* was trapped in Terry County, while *Cuerna* have been captured in Terry, Lubbock, Hockley, Yoakum, Dawson and Hale counties, *Graphocephala hieroglyphica* has been found in the above counties as well with the exception of Lubbock. These potential vectors are soon to be tested to determine whether or not they are carrying the bacterium that causes Pierce's disease, the insects are now in Stephenville where Dr. Forrest

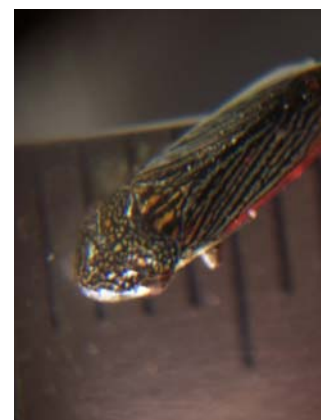
Mitchell's team will carry out DNA extraction and QRT-PCR analyses.

The extent and distribution of *X. fastidiosa* infection in High Plains vineyards is another goal of this project. All vineyards in the High Plains Trapping Program are also being assessed for symptoms of Pierce's disease and tested for the presence of *X. fastidiosa*. The testing and sample collection are being done by the laboratory of Dr. David Appel assisted in the field by his Research Assistant Cruz Torres and Technician Julia Cope. The first round of sampling attempted to sample previously tested plants, when possible, and a selection of vines both random and apparently symptomatic.

Sampling random non-symptomatic vines can give some indication of how strongly visual symptoms of Pierce's disease are correlated with *X. fastidiosa* infection. Subsequent sampling will attempt to sample primarily symptomatic vines and those vines that tested positive in the first sampling cycle. Vineyards will be sampled a total of three times this year. Thus far several vineyards on the High Plains have been confirmed as positive for a more in-depth analysis of these findings, see Dr. David Appel's article on the results and sampling portion of this program.



Trap Design, Height and Location Has Been Manipulated to Meet High Plains Conditions



High Plains Grapevine Sampling– David Appel

In the fall of 2007, samples from grape vines growing in vineyards on the Southern High Plains and far West Texas were processed for Pierce's disease by lab technicians working with Dave Appel at Texas A&M University. The vines were selected because they were suffering from undiagnosed disorders, and, in some cases had some signature symptoms of PD such as leaf scorch, green islands on canes, and/or match-sticking. The field symptoms were not sufficiently clear to provide a confident diagnosis. Therefore, quantitative real time polymerase chain reaction was

used to process the samples to aid in diagnosing the problem. Direct culture for *Xylella fastidiosa* was used on some of the West Texas samples. Samples consisted of petioles from the suspect vines.

The laboratory tests yielded a high number of positive results. Obviously, the implication of this find for grape production in those areas is highly significant, particularly for the Southern High Plains. Previous testing of West Texas vineyards more than a decade ago

resulted in positive finds, so the confirmation that *X. fastidiosa* occurs in that region was less surprising. These are highly definitive results. We did find that the organism was far more widely distributed than previously suspected. The samples from the Southern High Plains were only tested by qrt-pcr. Although they were consistently positive, this test does require some interpretation. As we have explained in several meetings held during the ensuing months, caution must be used when relying on one diagnostic protocol for Pierce's Disease.

Intensive Sampling Efforts Have Given Us the First Idea of Insect Species Possibly Involved in Disease Movement in the High Plains



High Plains Grapevine Testing, continued

Qrt-pcr tests are highly sophisticated methods for detecting the presence of bacterial DNA in the sample. The potential for false positives, and false negatives, exists with qrt-pcr and must be considered when interpreting results. Results can be particularly variable when obtained from samples collected late in the growing season, for example. This variability is due to the accumulation of compounds in the grape petioles that interfere with the ability to detect the DNA. Every contingency was examined in the testing process, so our confidence is very high that the results were accurate. Nonetheless, the need for further testing in the 2008 growing season was considered to be a very high priority. As part of the 2008-2009 PD Research Program, a comprehensive survey was designed to sequentially sample 26 vineyards on the Southern High Plains on three dates for PD. The results from the first sample date in May are complete. Samples were processed for testing with all three protocols, including qrt-pcr, ELISA (Enzyme Linked Immunosorbent Assay), and direct isolation of the pathogen. The qrt-pcr and ELISA results

were very consistent with those achieved in the fall of 07. Many of the vineyards testing positive in the initial sample again tested positive in May of 08. However, all attempts to isolate the pathogen in May failed, although this would be expected. Previous research has clearly shown that populations of *X. fastidiosa* in the spring and early summer are very low, making isolation difficult. Further attempts will be made to obtain cultures of *X. fastidiosa* from samples collected later in the growing season in Southern High Plains vineyards, because isolation is the most definitive of the three methods for PD diagnostics. It becomes increasingly likely that PD is present on the Southern High Plains. Although this information will be extremely useful to grape producers as they manage their crop, it also raises questions. When was the pathogen first introduced into the region? How did it get so widely distributed? These and other questions will be the subject of research and speculation for years to come. In the mean time, what must grape growers do to minimize the potential damage caused by *X. fastidiosa*? The answer to this question relies, in part, on the results of insect surveys currently being

conducted and described in this Newsletter. Vector management is a critical part of managing vineyards at high risk to infection by PD, as is weed control, irrigation management, and any other measure that could influence stress in the vines. PD control will be outlined in greater details in future newsletters, and can be found at the Texas Pierce's Disease Research & Education Program's website (<http://pd.tamu.edu>). Certainly the unique growing conditions found in the Southern High Plains will influence the development of Pierce's Disease and subsequent management. The cold temperatures occurring the region will be a beneficial factor, and when combined with pruning may be an effective factor in delaying disease development and any loss in productivity. This very factor may be why grapes have been grown successfully for many decades on the Southern High Plains under disease pressure from PD for many years. As previously stated, further research is going to be needed to address these questions, just as further surveys are still needed to fully understand the extent and impact of PD in the region.



ELISA Plates Used For Disease Diagnostics
Colored Wells Indicate Positive Reactions



While More Subtle, High Plains Vineyards Do Exhibit Classical PD Symptoms Such as Leaf Scorch (above) and Match-sticking (below)



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