INVESTIGATION INTO THE INCIDENCE AND DISTRIBUTION OF PIERCE'S DISEASE AND ITS VECTORS IN A PREVIOUSLY CONSIDERED "VERY LOW RISK" TEXAS WINEGRAPE GROWING REGION.

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Reporting Period: The results reported here are from work conducted May 2008 to September 2008.

ABSTRACT
An ecological model taking into account climactic, environmental and ecological factors was presented as a poster at the 2007 CDFA Pierce's Disease Symposium.(Lewis, 2007) The resulting risk map showed that conditions in the High Plains growing region of Texas were similar enough to other regions where Pierce's disease (PD) had been previously diagnosed to suggest this area could be vulnerable to the disease as well. In response, considerations for a new risk map for the state of Texas were proposed and sampling of suspect vineyards was carried out. Results of this sampling were used as a basis for the proposal of a program to examine the distribution of disease in this growing region. Additionally, possible agents of transmission were explored starting with an evaluation of the local insect population for putative vectors. The disease was found to be widely distributed in this growing region and a number of xylem specialists previously unrecognized to reside in this area were identified. Further analysis showed a percentage of these putative vectors to be positive for multiple strains of *Xylella fastidiosa*.

INTRODUCTION
There have been periodic observations and diagnostic results to indicate Pierce's disease (PD) has been an ongoing problem in the far west Texas grape growing region for decades. The vineyard originally diagnosed with PD in west Texas is situated at an elevation of 5410 ft. above sea level and experiences average winter low temperatures of 32˚ F with frequent drops well below freezing. When in 2007, testing for possible causes to explain symptoms observed in vineyards in far west Texas returned negative results, PD was considered as a possible cause. Sampling of all west Texas vineyards in commercial production indicated that PD had at that time become geographically widespread in this growing region. In the process of compiling statewide ecological and environmental information for addition to a GIS for vineyard information for the state of Texas, similarity of environmental and ecological conditions between these vineyards and those in what was then described as an "extremely low risk" wine grape growing region in the Texas High Plains became evident (Perry and Bowen 1974). Based on this realization, a new risk model for the state of Texas was initiated taking these factors into account.

During the compilation of information for this GIS, current and historical information regarding vineyard establishment and decline, disease symptoms, and cultural practices was obtained. During the course of this data collection, it was ascertained that for several years, growers in this area had reported symptoms of "unusual" leaf discoloration, decline in yield and fruit quality, higher than expected loss of vines to winter injury, and unexplained difficulty in successfully establishing previously successful varieties such as 'Chardonnay' (Lewis unpublished data, Burns personal communication September 2007). While this region has historically been considered to be in a zone of "very low risk" for PD, the combination of these factors lead researchers and extension personnel to consider the possibility that PD might be implicated in some of these previously unexplained grower reports.

While the premise for this study was realized well past the optimal season for the collection of plant samples for detection of *Xylella fastidiosa (Xf)*, the decision was made to begin a preliminary sampling of some of the vines in this area to be used both to potentially justify further investigation into PD in this growing region and to serve as a pilot study for a potential research program, should these results be positive. While certainly not conclusive, based in part on the deteriorated quality of the plant material at the time of sampling; this investigation returned positive results for *Xf* by QrtPCR from 31 of 39 samples in 12 of 12 vineyards with 3 negative and 5 inconclusive results.

Due to what was historically considered a very low potential for PD in this area, the High Plains was not considered a high priority area for the evaluation of potential vectors for *Xf*. Trapping in this area was limited to 12 traps in three vineyards, returning only 10 individual insects representing eight species of potential vectors over a three year period. The near absence of potential vectors in this area, presented as a contradiction in the understanding of how *Xf* infection may have become so apparently widespread across this region. Non-traditional possibilities that might account for disease spread in this area needed to be considered, however a more thorough investigation into potential vector populations needed first to be completed. It was in order to narrow the gap in knowledge and understanding of both the incidence of *Xf* and of the potential for the range of known vectors to extend into this region that this project was designed and initiated. Over time, a

comprehensive understanding of both the vector ecology and bacterial pathogenicity in this area may increase the understanding of the epidemiological effects of PD in temperate regions world wide.

OBJECTIVES

1. Assess the distribution of *Xf* infection and PD in the Texas High Plains Growing Region.
2. Asses the presence and distribution of putative vectors of *Xf* in the Texas High Plains Growing Region.
3. Evaluate putative vectors for association with *Xf*.

RESULTS

Objective 1

Assess the distribution of PD in the Texas High Plains Growing Region.

Twenty-six vineyards in the High Plains were selected for potential PD infections based on a number of criteria. Eleven vineyards had previously tested positive for *Xf* in a 2007 pilot survey with QRT-PCR. A representative sample was selected to include the range of growing conditions in the High Plains grape growing region. In addition to meeting ecological and geographic criteria, several of the selected vineyards had histories of unexplained symptoms and mortality. Each vineyard was sampled in May, July, and October. The sampling procedure consisted of collecting petioles with blades attached from vines exhibiting suspicious symptoms. A minimum of 3-4 petioles were removed, starting with the basal position and moving acropetally to the end of a cane. When no symptoms were present, a random sample of vines were included to represent the entire vineyard, bringing the total number of vines to 10/vineyard. The presence of foliar scorching, green islands, matchsticks and other appropriate observations were made for the sampled vines.

Samples were placed in an ice chest with blue ice and returned to the laboratory for processing with ELISA, QRT-PCR, and direct isolation of the pathogen on culture media. ELISA analyses were done according to manufacturer's instructions (Agdia, Elkhart, IN 46514). QRT_PCR procedures have been established and described in previous studies (Schaad et al. 2002). Culturing of tissue samples for pathogen isolations was done on PW media according to previously accepted practices.

Table 1. Summary of survey and diagnostic results for PD on vines testing positive in Texas High Plains vineyards in samples collected in June, 2008.

Vineyard No.	No. Samples Processed	No. ELISA Positive	No. QRT-PCR Positive	No. Isolations
1	20	3	2	0
2	19	1	1	0
3	13	1	1	0
4	19	1	2	0
5	18	1	0	0
6	22	3	1	0
7	21	2	2	0
8	16	0	1	0
9	22	4	4	0
10	19	1	0	0
11	16	0	2	0
12	16	0	1	0
13	16	0	1	0

Thirteen of the 26 surveyed vineyards tested positive for PD (**Table 1**). Seven of those 13 positive vineyards were among those testing positive in the original 2007 pilot survey. Although QRT-PCR proved to be more sensitive to detection of the pathogen in terms of numbers of vineyards, in a few cases ELISA positives were obtained with the sample testing negative by QRT-PCR. In no case was the pathogen isolated from the samples, regardless of the results of the indirect tests.
Due to the implications of expanding the recorded range of PD into a previously unaffected, major Texas grape growing region every precaution has been taken to insure the reliability of the diagnostic results. The results of the ELISA and QRT-PCR are considered to be sufficiently reliable to conclude that *Xf* infections of grape are widespread on the Texas High Plains. However, the diagnostic step of isolating the pathogen and completing strain analyses will continue to be pursued in order to further define the status of PD on the High Plains.

The results from the 2008 sampling extend the range of PD in Texas well into the High Plains growing region. The apparent widespread distribution of the disease within this region would suggest that it has been present in this area for several years and that a competent vector of the bacteria is active in the environment. The High Plains of Texas is the most productive wine-grape region in the state, with approximately 1500 acres in commercial production with production expanding yearly.

Environmental conditions are very different from most other grape growing regions of the state where PD has become a limiting factor. It is possible that PD has been responsible for many of the chronic problems for vine production in the past on the High Plains. The environmental conditions on the High Plains may be less conducive to epidemic development of PD, such as that seen in the more southern Texas growing regions. If so, the economic impact and prospects for control may be vastly better than in those areas where the disease has been historically devastating.

Objective 2

Assess the presence and distribution of putative vectors of Xf in the Texas High Plains Growing Region.

A number of trapping sites within the High Plains growing region were selected in an effort to identify the presence and distribution of putative vectors for *Xf* in grapevines. In order to identify occupant vector species in the High Plains, a trapping program which included both active and passive trapping methodologies was designed and implemented during the 2008 growing season. Results available for this report come from the passive portion of this program.

Nineteen sites were selected for the placement of yellow sticky traps. These traps were scheduled to be collected on a 14 day rotation, which was at times amended due to adverse weather or vineyard conditions which made trap collection on the planned day impossible. Other factors forced researchers to change the collection schedule for three trapping areas to once monthly.

Traps placed within vineyards were placed at a density of 10 traps per acre. A trapping protocol developed to both address the unique environmental conditions and lack of knowledge of target species was utilized (unpublished). Trap height varied with trellis height but was standardized primarily to be wholly or partially within the canopy level for that location. Additionally, in some locations traps were standardized to heights both "short" approximately two feet from the ground and "high" approximately four feet from the ground. Finally, in some "non-vineyard" locations, traps were set using local vegetation as the indicator for trap height. This technique helped to increase trapping efficiency for a variety of insect species, giving a more comprehensive representation of the resident population of putative vectors.

Trapping locations within vineyards were selected based on the following criteria:

1. The 11 vineyards that tested positive for presence of *Xf* in the 2007 pilot study were given priority.
2. Vineyards not included in any pre-existing trapping programs were prioritized over those that were.
3. Vineyards were grouped into five geographic areas in order to properly assess the entire growing region. A minimum of one vineyard per geographic region was selected.
4. Vineyards were further selected based on surrounding vegetation in order to include as many ecological/environmental types as possible.

Trapping sites outside vineyards were selected in order to adequately represent as many of the environmental and ecologically distinct areas within the region as possible. In addition, both areas directly adjacent to and several miles from the nearest vineyard were selected.

This report contains the first nine trapping periods in the 2008 cycle beginning in May and ending in September. As previously stated, some trapping periods were either shortened or lengthened as a result of weather conditions, or other vineyard considerations including pesticide applications and harvesting (**Tables 2 and 3**).

This program resulted in the capture of approximately seven distinct species of xylem feeders, six which are known vectors of *Xf* in grape. Of these, the range of at least two were not known to extend into this area and at least one species is currently classified as "undescribed." Previous to this study, 10 individual insects representing seven species of xylem feeders had been observed by vineyard trapping efforts in this area over a three year period (Lauzière et al. unpublished).

Table 2. Putative vector's captured during the months of May through August of 2008 on traps set inside vineyards. In addition to total # of xylem specialists recovered, data is reported for the three most commonly occurring species in this study.

Trapping Site	Total # Xylem Specialists	*H. vitripennis*	*G. hieroglyphica*	*Cuerna sp.*
1	15	0	8	3
2	8	0	5	2
3	2	2	0	0
4	2	0	2	0
5	0	0	0	0
6	7	0	0	7
7	7	0	0	7
8	17	0	0	17
9	8	0	1	7
10	1	0	1	0
11	6	0	0	4
12	4	0	0	4
13	10	0	0	6
14	13	0	0	12

Table 3. Putative vector's captured during the months of May through August of 2008 on traps set outside vineyards. In addition to total # of xylem specialists recovered, data is reported for the three most commonly occurring species in this study.

Trapping Site	Total # Xylem Specialists	*H. vitripennis*	*G. hieroglyphica*	*Cuerna sp.*
15	59	0	53	1
16	2	0	0	2
17	1	0	1	0
18	199	0	198	0
19	1	0	1	0
20	32	1	2	26
21	2	1	0	1

Objective 3

Evaluate putative vectors for the presence of Xf.

A number of insects from this region representing eight species were examined by PCR using an established protocol (Schaad et al. 2002), for the presence of *Xf*. Of those tested, approximately 30% tested positive *for Xf*. The insects that were selected for testing were chosen based on overall condition and preservation of the available specimens and represent a random sample of insects with regard to where within the research area they were captured. Specimens from this area are in the process of being analyzed in order to determine which specific strain of the bacteria is present in these insects. As of the time of this report, this data is still pending. The presence of *Xf*, in xylem specialists captured on the High Plains would suggest that it is likely that vectors are active in this region. It appears likely that at least some portion of the widespread presence of *Xf* and PD in this growing region is the result of vine to vine transmission by insect vectors.

CONCLUSIONS

This study was designed as a first step to examining the occurrence of *Xf* infection in the High Plains growing region as well as the potential for increased incidence of infection over time.

Findings from Objective 1.would suggest that *Xf* infection occurs widely in this area and that PD should be considered in previously unexplained grower reports of vine decline and reductions in fruit quality and yields as well as other symptoms such as higher than expected percentages of vine loss due to winter injury. During this study, symptoms indicative of PD

including leaf blade scorching, petiole retention and uneven periderm formation were observed in vineyards across the region.

The presence of *Xf* in samples taken in early June, supports the hypothesis that the bacteria is able to over-winter in vines in this area both serving as a source for inoculum for subsequent spread within and among vineyards, and potentially impacting vine health from season to season. The widespread distribution of infection appears to imply that infection may have been actively spreading in this area for some time. Coupling the current distribution of the disease with the historical accounts reported by growers further supports the conclusion that *Xf* has been present and active in this growing region for a number of years. Lack of suitable historical records and the relatively new nature of large scale grape production in this area will make it extremely difficult to ever make an accurate estimate of how long PD may have been impacting vineyards in this region.

The data collected in order to satisfy Objectives 2. and 3. support the conclusion that the potential exists for continued spread of *Xf* in the High Plains region of Texas. While the first season of trapping recovered low numbers of potential and known vectors, the diversity of these putative vectors was moderately high and species known to be very competent vectors of the bacteria were among those recovered. Of the species captured, 30% of these species were found to be carrying *Xf*. Because the 2008 trapping program results contained in this report represents only a single "short" (May-Aug) season of collection and the trapping protocol was in the process of being refined, this data is far from comprehensive. Only with several seasons of collection and an increase in trapping intensity can an in depth understanding of the vector ecology of this area begin to become clear. A solid understanding of how vectors behave in the environment over time is necessary in order to develop targeted strategies for management of this disease.

The newly recognized range of PD in this growing region brings attention to the danger of making assumptions about the susceptibility of vineyards to disease based on geographical location or historic climactic patterns. While it is impossible to be certain, it is logical to conclude that a lack of recognition of disease and the subsequent complacency with regard to preventive strategies to control the spread of the bacteria may be at least partially responsible for the widespread presence of the bacteria in this region today. Based on the preliminary findings of this study, it seems prudent to re-evaluate the potential for vineyards across much of temperate North America to be affected by PD, and to reconsider the potential for further disease spread into regions currently considered to be at very low risk.

Continuation of this study will further define the vector population in this area as well as serve to provide an initial understanding of the local ecology of the guild of xylem specialists in this and other temperate regions. More in depth knowledge of possible migratory, reproductive, and over-wintering behaviors as well as feeding strategies of the individual species in this group will aid in both an understanding of the current and historical epidemiology of the disease as well as the development of control strategies specifically targeted at this region. Preliminary data suggests that this guild may have a very different species composition and exhibit behaviors that differ from their functional equivalents in warmer and/or higher precipitation areas. Further, continued investigation into the long term health and productivity of individual vines and vineyards will aid in the understanding of the epidemiology both at the vineyard and the landscape level of this disease in more temperate regions. This is an area of knowledge for which there is currently very little data and could prove invaluable in the future protection of vineyards in these lower temperature regions.

REFERENCES CITED

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FUNDING AGENCIES

Funding for this project was provided by the Texas Department of Agriculture.

ACKNOWLEDGMENTS

Sincere thanks to the following individuals for their contributions to both the collection and continued analysis of data and overall individual and project support: James S. Kamas, Dr. James Supak, Cruz Torres, Julia Cope, Megan Morley, Danny McDonald, Teresa Burns, and Dr. Steven Presley

Additionally we would like to thank the Texas Pierce's Disease Research and Education Program and the USDA for programmatic support and use of equipment.

Finally we would like to recognize the wine-grape growers of the High Plains region for providing open access to all vineyard locations and uncommon cooperation in both research efforts and dissemination of information to researchers and the growing community.