EPIDEMIOLOGY OF PIERCE'S DISEASE IN TEXAS VINEYARDS

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ABSTRACT
Multiple studies are being conducted to improve current recommendations for control of Pierce's disease (PD) of grapes in Texas. These studies focus on epidemiology of the pathogen, *Xylella fastidiosa (Xf)*, in several Texas vineyards. Sequential surveys in one of those vineyards for PD symptoms and vine mortality were conducted annually between 2005 – 2007. Very different disease progress and mortality rates were recorded for four varieties, including Chambourcin, Ruby Cabernet, Shiraz, Primitivo, and Blanc du Bois. Chambourcin exhibited the high mortality rate, while Blanc du Bois had the least mortality. Two different plantings of Shiraz responded very differently to the pathogen. Attempts were made to isolate the pathogen from throughout the vineyard and relate the success to the survey results. The pathogen was most easily isolated from vines in the advanced stages of disease development, but the pathogen could also be isolated from apparently healthy, symptomless vines. Vine recovery from infected plants was common in some varieties. The collection of isolates was analyzed using 5 Single Sequence Repeat (SSR) markers, confirming the population was exclusively in the *Xf* subsp. *fastidiosa* group. Three distinct strains were delineated within the pathogen population. Studies are ongoing to determine the significance of the strains with the population and how they may be influencing disease development. The results of these studies are particularly important to how we recommend roguing for control of within-vineyard spread of the pathogen.

INTRODUCTION
Xylella fastidiosa (Xf), the causal agent of Pierce's disease (PD) of grapes, is considered to be a native, endemic pathogen in Texas. PD is a limiting factor for growth of *Vinifera* varieties in many of the winegrape regions in the state. Current recommendations for PD control can be expensive and inconsistent. As a result, growers face a great deal of anxiety over sustained production in existing vineyards, as well as a lack of confidence in selecting varieties for replanting and establishing new vineyards. Information on disease progress in preferred grape varieties is needed to for growers in high risk PD areas.

Another problem for growers concerning PD relates to routine, timely and reliable diagnostic results. Each of the currently available methods for diagnosing PD has strengths and weaknesses when needed for locating diseased vines. This is particularly true when attempting to relate the appearance of visual symptoms to the results of diagnostic testing. The practice of roguing to reduce within-vineyard sources of inoculum is dependent on quickly identifying suspect vines. Failure to do so may lead to additional infections, but removal of falsely identified diseased vines will unnecessarily reduce productivity.

Vine to vine spread of *Xf* appears to be an important element in the development of a PD epidemic in a vineyard. Patterns of disease incidence in some vineyards suggest that there are clusters of diseased vines from which the pathogen is transmitted in distinct directions by sharpshooters (Tubajika et al. 2004). Roguing is intended to prevent this sort of spread. A better understanding of this process might be provided by analyzing the population structure of *Xf* within a vineyard. The existence of multiple subspecies of *Xf*, and the potential for the introduction of different subspecies into vines by sharpshooters, provides an additional source of unknown variation to the epidemiology of PD in Texas. An understanding of strain diversity within a subspecies might also be useful for identifying potential sources of inoculum and subsequent spread of the pathogen through a vineyard.

The goal of this project is to improve current recommendations for control of PD by learning more about the epidemiology of the pathogen. Different approaches are being used to analyze disease incidence and severity over time and space to reveal underlying influences on disease development.

OBJECTIVES
1. Compare rates of PD development among common grape varieties in a Texas vineyard.
2. Relate symptom development in diseased vines to the isolation of *Xf*.
3. Analyze population structure of *Xf* in a Texas vineyard.

RESULTS AND DISCUSSION

Disease progress rates. Sequential surveys of symptom development in individual vines were carried out annually in a vineyard near Brenham, TX. This vineyard, planted in 2000 and 2001, is located in the south eastern winegrape region of the state. Eight grape varieties were planted in 2000 and 2001 in blocks ranging from 512 – 1270 vines. The results from 7 of these vineyard blocks containing 5 varieties are reported here, including only those blocks containing greater than 1000 vines (**Table 1**). Vines were rated on a 1 – 5 scale, where 1 was a symptomless, healthy vine and 5 was dead. A vine rated 2 had a few leaves with typical foliar scorch, a 3 extensive scorching, and a 4 exhibited various combinations of matchsticks, green islands, and scorching combined with significant dieback.

Table 1. Attributes of 7 blocks containing 5 grape varieties in a vineyard near Brenham, TX.

Variety	No. of Vines	Year Planted	Rootstock
Chambourcin	1071	2001	own
Shiraz (4)	1270	2001	101-14
Primitivo (4)	1270	2001	SO4
Primitivo (3)	1280	2000	101-14
Shiraz (3)	1280	2000	101-14
Ruby Cab	1152	2000	101-14
Blanc du Bois	1071	2001	own

The surveys revealed a variety of responses to PD. In **Figure 1**, the decline in vines rated "healthy", with no scorching (Rating 2) is depicted for each of the varieties. A few of the varieties, such as Chambourcin, Shiraz block 4, and the Ruby Cab block 5 had little decline in the numbers of healthy vines. In contrast, the decline in symptomless vines dropped steeply for the Chambourcin, Blanc du Bois, Ruby Cab block 5, and Shiraz block 4. There was a noted trend of recovery of Chambourcin, Blanc du Bois, Shiraz and Ruby Cab Block 5 in 2006, but the proportions of symptomless vines declined again the following year.

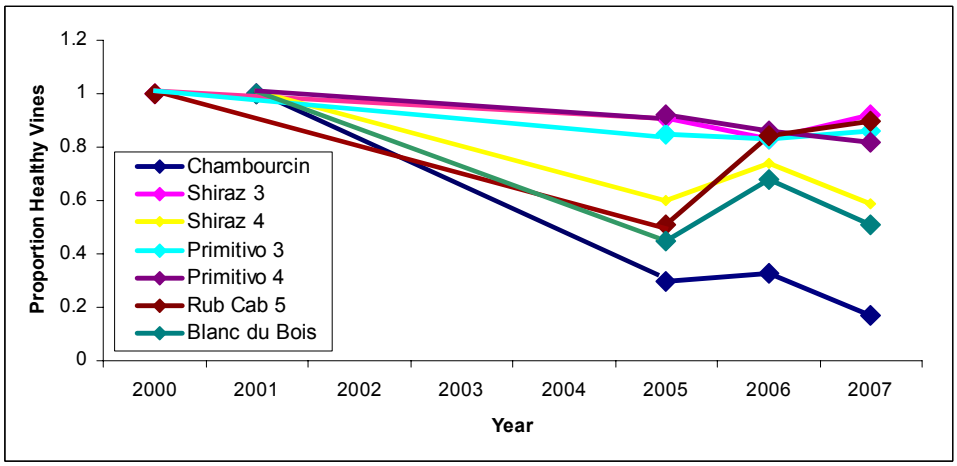

Figure 1. Proportions of healthy, or symptomless vines, in 7 vineyard blocks containing 5 grape varieties in a Texas vineyard.

Figure 2 depicts the mortality curves for the same varieties. The two steepest rates of mortality are exhibited by the varieties Cahmbourcin and Shiraz Block 4. As would be expected, Blanc du Bois, a muscadine hybrid x French varietal cross, had the lowest mortality rate during the 2 years of the survey. Shiraz Block 3 also had one of the lowest mortality rates, in contrast to Block 3 of the same variety that was planted a year earlier and was located just a few yards away.

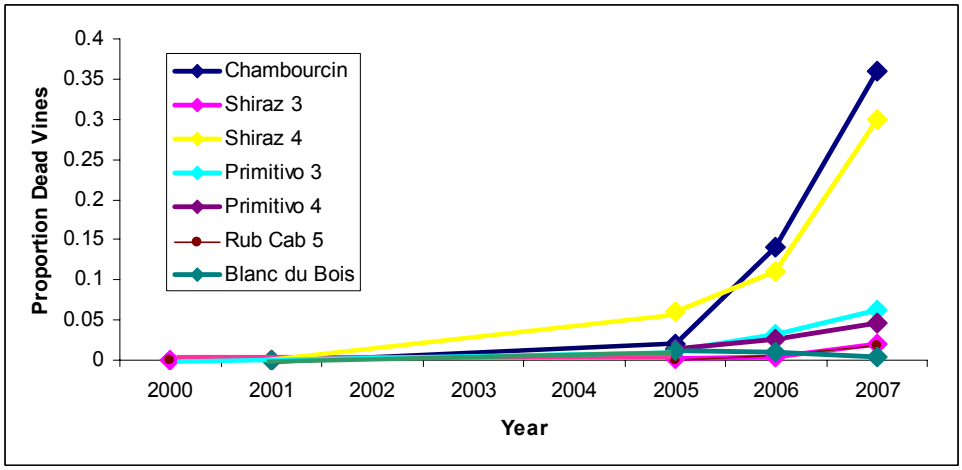

Figure 2. Disease progress in seven grape varieties as measured by vine mortality.

Chambourcin is a French-American hybrid producing red grapes with an uncertain genealogy. There is some evidence that this variety is prone to overcropping, and without compensating for this tendency can undergo significant vine stress and decline. Blanc du Bois is a wine grape developed in Florida as a result of a cross between another American hybrid and the Cardinal table grape. It is known to be resistant to PD. Although these mortality rates are largely consistent with expectations concerning susceptibility to *Xf*, the differences in mortality between the two blocks of Shiraz were unexpected. The reasons for the dramatic differences in response are unknown. The Shiraz block 3 was planted in 2000, and block 4 in 2001. Although they both came from the same nursery, the different disease responses suggest there are some differences in the genetic backgrounds of the two blocks. They are on the same rootstocks and located adjacent to one another, making site differences unlikely.

Attempted culturing for isolation of Xf. Six blocks in the Brenham vineyard were extensively sampled for laboratory culturing and isolation of *Xf* (**Table 2**). Vines were randomly distributed from throughout each of 6 blocks representing 4 different varieties in order to obtain a representative sample from throughout the block and from vines in various states of health. Samples were collected during the first two weeks of June and rated two months later in August. Samples were surface sterilized and cultured on PW media. From a total of 103 culture attempts in these blocks, the pathogen was successfully isolated from 66 vines after one or two attempts. The health status of the sampled vines is included in **Table 2**. The 66 vines testing positive for isolation of *Xf* in June of 2007 were largely symptomless in 2005, two years before they were sampled. Twelve of the vines testing positive were ranked symptomless at the end of the growing season, illustrating the ability of some vines to recover from infection and survive. The majority of vines testing negative were rated symptomless or in the earliest stages of disease development in 2007 as well as the two years previous.

Table 2. Numbers of vines testing positive or negative for isolation of *Xf* ranked according to health where: 1 = healthy, no symptoms, 2 = incipient symptoms with 1 or a few scorched leaves, 3 = majority of foliage symptomatic, 4 - 6 = scorching, dieback, matchsticks, green islands, dead or removed. Vines were rated at the end of the season after samples were taken.

	Numbers of Positive Vines				Numbers of Negative Vines			
Year	Rating = 1	Rating = 2	Rating = 3	Rating = 4-6	Rating = 1	Rating = 2	Rating = 3	Rating = 4-6
2005	30	33	2	1	24	10	0	1
2006	13	24	21	8	26	5	3	1
2007	12	6	20	28	19	7	3	6

Strain differentiation of isolates. Repeated sampling from several vines and sampling of vines from some smaller blocks in the Brenham vineyard resulted in collection of 97 *Xf* isolates. Simple sequence repeat (SSR) markers (Lin et al. 2005) are being utilized to analyze the population structure of the isolates. When 5 SSR markers were used with conventional PCR (polymerase chain reaction), hierarchical clustering analysis resulted in delineation of 3 strain groups containing 14 – 44

isolates per group. There appears to be no selection of strains for different cultivars. Spatial relationships and the association between the occurrence of the strains and variation in disease progress will be analyzed.

CONCLUSIONS

Differences in susceptibility, tolerance, and resistance to *Xf* are being observed in popular grape varieties in Texas vineyards. However, these differences are not entirely consistent with expectations based on previous observations (Fry and Millholland 1990). Blanc du Bois proved to be extremely resilient, as expected, even though large proportions of the vines exhibit low levels of scorching. Even though we were unable to isolate the pathogen from the Blanc du Bois, vines in this vineyard have tested positive with ELISA (enzyme linked immunosorbent assay) in other studies. Another hybrid, Ruby Cab was also extremely tolerant in the Brenham vineyard. The two blocks of Shiraz responded very differently and illustrate the difficulties that still remain in predicting the course of Pierce's isease. One planting is sustaining heavy losses, while a nearby planting is proving very tolerant. The reasons for these differences are unclear. Insect control, irrigation, and other management practices for the two Shiraz blocks were the same. Since the two blocks were planted in different years, the relative susceptibilities may result from different genetic backgrounds of the grapes.

There are always questions concerning the use of symptoms, particularly scorching, as basis for diagnosing PD. Minor scorching early in the season was prevalent on all varieties throughout the vineyard annually, and was used to select potential vines for sampling and laboratory isolations. These isolations illustrated that the pathogen was widely distributed throughout the six vineyard blocks. Consistent isolation of the pathogen does not occur until the vines are in advanced stages of disease severity. Yet the pathogen was also isolated at lesser frequencies from vines that were entirely symptomless by the end of the growing season. These results show why there are uncertainties in knowing when to rogue for disease control. Although scorching may reflect infection, it is not a good symptom for determining the ultimate fate of the vine. Also, infected vines may be symptomless and continue to yield. Even though they may be colonized at levels too low to serve as inoculum sources, they may unexpectedly collapse and thus become infective. This unpredictability will continue to confound efforts to successfully rogue.

All of the isolates obtained from this vineyard were grape strains in the subsp. *fastidiosa*. There has been some concern in Texas that ELISA results may be identifying other subspecies in grapevines, but in the Brenham vineyard this does not appear to be the case. The significance of the three strains delineated with the 5 SSR markers has yet to be determined. The strains do not, however, appear to be associated with differences in disease development observed in the grape varieties.

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