

EPIDEMIOLOGICAL ASSESSMENTS OF PIERCE'S DISEASE, AND MONITORING AND CONTROL MEASURES FOR PIERCE'S DISEASE IN KERN COUNTY

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ABSTRACT

Vineyards in the 7 grape production areas of Kern County's area wide management project were surveyed for PD again in 2004. Incidence of PD in the highly affected areas (General Beale and North) peaked in 2002, and declined dramatically in both 2003 and 2004. Treatments to reduce GWSS and to identify and remove PD infected vines each year were associated with these dramatic reductions. Survey and epidemiological data is being processed at CAMFER, a GIS-based research institute at U.C. Berkeley. More than 98% of the vines infected with *Xylella fastidiosa* in the recent epidemic in the General Beale area of Kern County were of the two most susceptible varieties: 6 Red Globe and 2 Crimson vineyards. Thirty-two other nearby or contiguous vineyards of four less susceptible varieties were almost unaffected. A hypothetical mechanism for this varietal difference is proposed.

INTRODUCTION

These two projects have complimentary objectives and methods, and were thus pursued and are being reported here cooperatively. This combination of people and resources has resulted in synergistic efficiency and maximum utilization of resources.

The cooperative area-wide pest management project for the control of GWSS has defined 7 distinct grape growing areas in Kern County. The PD epidemic that peaked in 2002 only affected two of these, the General Beale and the adjacent Northern area. These were also the only areas where the populations of GWSS exploded in 2000 and 2001 to extremely high populations not seen elsewhere in the county. Insect control measures begun in winter 2001-2002 brought the GWSS populations down dramatically. During this time the population dynamics and control methods for controlling GWSS were studied extensively with effective results. However our understanding of how to control the disease (goal of project 1) and the epidemiology of PD when the causal bacterium is transmitted by GWSS (goal of project 2) had been based on limited actual field data. These two projects began in 2002 as 5 year projects to obtain extensive data about the incidence and control of the disease. This disease information would compliment the insect information to enable understanding of the dynamics of the epidemic and methods to control other potential outbreaks. A total of 216 vineyards with 4060 acres and 2,015,698 vines were surveyed, about 4.6% of the vineyard acres in Kern County.

There have been two recent major California epidemics of PD that have been vectored by GWSS: General Beale in Kern County and Temecula in Riverside County. However data about each of these was not obtained until the epidemic was well underway or had already peaked. Because the other five viticulture areas of Kern County did not yet have such high numbers of GWSS, it was thought that disease and insect data from those would provide baseline information in the event that another epidemic such as the General Beale and Northern outbreak might occur, and such an epidemic could be studied from the beginning. Among the other 5 viticulture areas, 4 (Central, South A, South B, and West) have had low numbers of GWSS present since sometime before 2000, and GWSS was discovered in the 5th (Hwy 65-Delano) after 2000. Thus this extensive project to monitor the PD disease incidence in these areas was intended to provide both an understanding of the effect of low populations of GWSS on the incidence of PD, as well as a complete epidemic profile over time if another one should occur in this county.

OBJECTIVES

Project 1: Epidemiological assessments of Pierce's Disease. (BLH)

1. Evaluate the importance of epidemiological factors such as GWSS population size, vine age, cultivar susceptibility, control practices, and GWSS control treatments in vineyards and nearby GWSS hosts or habitat.
2. Make all the epidemiological data obtained available in a commonly acceptable GIS format for analysis by other qualified researchers and epidemiologists.

Project 2: Monitoring and Control Measures For Pierce's Disease In Kern County. (JH)

1. Determine changes in the incidence of PD over time in seven distinct grape-growing areas in Kern County.
2. Develop PD monitoring and management techniques and strategies for use by growers to reduce risk and damage. Update and provide educational materials to assist vineyard managers, pest control advisors, other researchers and government agencies involved in advising growers in the area-wide pest management of the GWSS project.

RESULTS AND CONCLUSIONS

Vineyards were monitored by visually inspecting each vine for PD symptoms, and by collecting and testing (by ELISA) samples from symptomatic vines (2). Thus far in October 2004 all but 2 of the General Beale vineyards have been completed, but much of the other areas of Kern County are still in progress. The results thus far in the General Beale area indicate that the dramatic decrease in the number of infected vines is continuing. From 2002 to 2003 the number of infected vines decreased by 85%, and from 2003 to 2004 the decrease was an additional 68%. Following the survey of these vineyards in 2001 and 2002 the vines found to have confirmed *Xf* infections were removed. The continued decline of *Xf* infection in this area demonstrates that effective PD control can be obtained with a combination of GWSS control, monitoring for infected vines, and removal of infected vines. These projects have demonstrated that vineyard disease monitoring and vine removal is cost effective.

Throughout the county as part of this project vines found to be infected with *Xf* were removed at the end of that season. As a result the surveys in 2003 and 2004 are identifying vines that are newly infected. The rate of infection in all areas of Kern county outside the General Beale and Northern areas is very low, an overall rate throughout the county of less than one new infection per 10,000 vines. By contrast in the General Beale area some of the vineyards developed very high levels of disease within a 2 to 3 year period, peaking in 2002. Several vineyards were entirely lost.

Before the arrival of GWSS, primary spread of *Xf* from sources outside the vineyard accounted for most or all of the PD in California. The rates of new infections in Kern county may be the result of both primary spread and secondary spread, that is vine to vine spread. The low rates of new infections outside the epidemic area is consistent with primary spread, but the rapid rates of infection in many vineyards within the General Beale area is consistent with secondary, vine to vine spread. Perhaps the most startling epidemiological discovery of this project so far was that in 2002, 99% of the PD infected vines in the General Beale area were in Redglobe and Crimson vineyards, the 2 most susceptible of the 6 varieties surveyed. The following year, 2003, these same vineyards accounted for 97% of the diseased vines. These two varieties comprised only 18% of the acreage surveyed in the General Beale area. There were dramatic instances where Redglobe and Flame Seedless were growing in adjacent vineyards, and the susceptible Redglobe vineyards were heavily impacted or totally lost, whereas the more tolerant Flame Seedless vines growing just a few feet away were almost unaffected. The rate of infection in vineyards in General Beale of varieties other than Red Globe and Crimson in any of the three years was less than 14 infected vines out of 337,693 vines surveyed. In the worst epidemic area in Kern County the infection rate in varieties other than Redglobe and Crimson was essentially negligible. The Crimson loss in the General Beale area involved only one vineyard, and these vines were less than three years old. Younger vines are more susceptible to PD than older vines, and it is possible that the losses in the Crimson vineyard were primarily related to their more vulnerable age, rather than a varietal susceptibility. Older Crimson vines may not have been so heavily impacted.

We have developed a new hypothesis that would explain what might be causing this varietal difference. It is based on the timing of when in the season GWSS can acquire *Xf*, when in the season GWSS transmits *Xf* to new vines, and the phenomenon of over-winter curing of *Xf* infections. Over-winter curing of PD has been demonstrated to occur in many areas of California, including the San Joaquin Valley. Populations of *Xf* in grapevines are reduced during the winter dormant season. It has been experimentally demonstrated that if a vine is infected early in the season, the bacterium has enough time left in the growing season to multiply to high enough population levels and spread into areas of the vine where some of the bacterial cells find a refuge and can survive the winter dormancy. The vine then becomes chronically infected and usually eventually dies. Conversely, if a vine becomes infected later in the season, all the bacteria in the vine die over the winter, and the vine is free of disease the following year (1). Also pruning may play some role in over-winter curing. Vines that are inoculated late in the season when there is insufficient time for bacteria to move beyond the inoculated cane would, of course, lose the infection when that cane is pruned. However the bacteria in an un-pruned cane may die over-winter anyway. Our new hypothesis is predicated on the finding that *Xf* multiplies and spreads faster within a susceptible plant than it does in a more tolerant plant (3). It would reasonably follow that the bacterium would also multiply and spread more rapidly in the more susceptible grapevine varieties of Redglobe or Crimson than it would in the more tolerant varieties such as Flame Seedless or Thompson. The first part of our hypothesis is about when in the season a grapevine must become inoculated in order for the bacterium to survive the first winter dormancy in the plant thereby progressing to chronic Pierce's disease. We hypothesize that the tolerant varieties have to become infected with *Xf* earlier in the season than susceptible varieties in order for the bacterium to have enough time left in the growing season to multiply and spread sufficiently in the vine to be able to survive the winter dormancy period. In general it has been demonstrated that vines must be inoculated before some critical time in the season if the bacterium is to survive the winter (1). However the existence of differences among varieties regarding that critical necessary time of inoculation has not yet been experimentally demonstrated.

The second part of our hypothesis is about when in the growing season the bacterial cells, having over-wintered in a previously infected plant, multiply and spread from their winter refuge into the new growth and achieve population numbers great enough to be efficiently acquired by an insect vector, in this case GWSS. This growth and movement of the bacterium following winter dormancy has to happen before vine to vine spread can begin to occur. It is not possible to detect *Xf* in the new growth of an infected plant until sometime about mid-season, and it has been demonstrated that the bacterium must

multiply to relatively high (easily detectable population sizes) before acquisition becomes efficient (4). Because it multiplies and spreads faster, we hypothesize that bacteria become available for acquisition in an infected grapevine of a susceptible variety earlier in the season than in a vine of a tolerant variety.

Putting these two parts of the hypothesis together can explain why the varietal differences in disease rate were observed. In the most susceptible varieties inoculations occurring later in the growing season can result in infections that survive the winter to become chronic. Because of the faster bacterial multiplication and spread there is still enough time in the growing season to reach a threshold for survival. At the same time, the bacteria multiply in previously infected vines fast enough to become available for acquisition by GWSS earlier in the season. The timing of these two processes results in an overlap, that is a window of opportunity when GWSS can acquire *Xf* from an infected vine, transmit the acquired bacteria to a new vine, and the new infection has enough time to progress to chronic infection and disease. That window of time would close during the season, but vine to vine transmissions would still be occurring. However those later season transmissions, after the window of opportunity has ended, would be cured over the winter. So vine to vine transmission occurring within the window would become chronic, and vine to vine transmission occurring after the window would be winter-cured.

Conversely in the tolerant varieties infections must occur earlier in the season in order to have enough time, at the slower rate of multiplication and spread, to progress to chronic disease. At the same time bacteria from previously infected vines also multiply and spread slowly and do not become available for vector acquisition until later in the season. The result is that there is no overlap, no window of opportunity where GWSS can acquire *Xf* from an infected vine, transmit to a new vine, and have the newly infected vine progress to chronic disease. In this case all of the vine to vine transmissions occur too late in the season, and the result is that all the vine to vine infections are cured over the winter.

One question is why do epidemics that are vectored by GWSS result in vine to vine disease spread in susceptible varieties whereas no vine to vine disease spread seems to occur when the traditional native California sharpshooter vector species are transmitting the bacterium? The answer may be related to the feeding and inoculation locations of GWSS vs. other vectors. The GWSS will feed (and therefore inoculate vines) at the base of the canes, but the native vectors all feed almost exclusively at the tip of the cane. Inoculations at the tip of the cane probably require more time to move to an over-wintering refuge, so an early season inoculation is necessary for the infection to survive the winter and become chronic disease. Thus the window for vine to vine transmission leading to chronic disease would not exist. In this case only the early season primary spread from sources outside the vineyard would result in chronic disease, and because vine to vine transmission cannot begin until mid-season, these infections would be winter-cured.

If this hypothesis is correct, there are a number of possible consequences and conclusions that could improve PD management and control in areas where GWSS is present.

- The risk to growers of tolerant varieties is far less than has been previously assumed.
- There is a critical window of time somewhere in mid-season when susceptible vines need to be protected from vine to vine spread of PD. Chemical vineyard treatments early and late in the season, that is before and after this window, may be less effective than has previously been assumed.
- Economically important rates of secondary spread of PD may only happen in susceptible varieties and when large populations of GWSS are involved. Low but persistent populations of GWSS in Kern County do not appear to have resulted in appreciable losses from vine to vine spread.
- Better targeted and timed chemical treatments could result in lower costs and be more compatible with other IPM programs.
- Late season vineyard surveys and rouging of infected vines is an important and cost effective management tool.
- The GWSS monitoring programs could be tailored to critical parts of the season, thereby possibly reducing the overall cost of these programs.
- The GWSS population treatment thresholds could be based on better epidemiological information, again possibly reducing overall PD management costs.

Because of the beneficial implications for PD management, it is important to experimentally test this hypothesis. We will be proposing to conduct experiments over the next two years to test the components of this hypothesis. The best experimental protocol would involve experiments conducted in two adjacent working vineyards, one tolerant and one susceptible variety. Ideally the experimental site would be in southern San Joaquin valley with climatological conditions representative of the viticulture areas of Kern or Tulare counties. One experiment would involve inoculations of both varieties vines at intervals throughout the growing season to establish the probability curves for the over-winter survival of *Xf* as a function of time of inoculation. The hypothesis predicts that the probability curves would be significantly different. Another experiment, for year two, would involve acquisition of *Xf* by GWSS at intervals throughout the season from vines of both varieties that were inoculated the previous year. This would establish the probability curves for the acquisition of *Xf* by GWSS as a function of time. The hypothesis predicts that these probability curves would also be significantly different. Other components of the experiments would look for differences between the varieties in the rate of multiplication and spread of *Xf* in the vines. Again the hypothesis would predict differences. It is critically important to everyone involved that these experiments do not create any new local PD problems or outbreaks. We have considered extensive safeguards in the design of these

experiments. We intend for the risk to be very small, and the knowledge gained to be of great benefit in the practical control of PD in the southern San Joaquin and elsewhere in California. We would be happy to work collaboratively with other researchers and cooperators on various aspects of this research.

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