THE EFFECT OF DORMANT SEASON SURVIVAL OF *XYLELLA FASTIDIOSA* IN GRAPEVINES ON PIERCE'S DISEASE EPIDEMICS IN CALIFORNIA

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ABSTRACT
The two California Pierce's disease (PD) epidemics associated with population outbreaks of glassy-winged sharpshooter, at Temecula in the mid 1990s and in Kern County peaking in 2002, differed dramatically in the number of vineyards lost and the grapevine varieties affected. It is postulated that vine-to-vine (secondary spread) of infections occurred throughout all vineyards in both areas but the survival and progression to disease of these infections differed between the two areas. In Temecula, many of the resulting infections survived vine dormancy and progressed to chronic disease resulting in the loss of half or more of the area's vineyards of all varieties. In Kern County only some of the infections in only two varieties, Redglobe and Crimson Seedless, survived vine dormancy and progressed to disease, and vineyards of all other varieties were unaffected. A hypothetical explanation of this epidemiological pattern is presented and experiments are begun to test this hypothesis. The benefit to grape growers in the southern San Joaquin Valley will be to provide reliable ways to reduce risk of loss by PD epidemics.

INTRODUCTION
Following the appearance in the mid 1980s of the glassy-winged sharpshooter (GWSS) in California, there have been two major epidemics of Pierce's disease (PD) associated with large populations outbreaks of GWSS, first in Temecula in the mid 1990s, and second in the General Beale area of Kern County peaking in 2002. The patterns of PD incidence and vineyard loss differed dramatically between these two epidemics. In Temecula, the site with the milder winter climate and shorter dormant season, more than half of the region's vineyards were lost, and most or all the varieties had substantial losses resulting in removal of vineyards. In Kern County (which has a colder winter climate and longer dormant season), only a small percentage of the vineyards were lost, and all of the lost vineyards were in only 2 of the 6 varieties in the area, Redglobe and Crimson Seedless. The losses to vineyards of the other 4 varieties were very small, in most cases less than 1 in 10,000 vines. By contrast, all 12 of the Redglobe vineyards in the General Beale area were significantly damaged with from 2% to more than 50% of the vines lost (Hashim, et.al., 2003), and most of these vineyards were ultimately removed.

Grapevines acquire new *Xylella fastidiosa* (*Xf*) infections either by primary spread or secondary spread. Primary spread occurs when vector insects acquire the bacterium from source plants outside the vineyard, then fly into the vineyard to infect vines. Secondary spread occurs when vector insects acquire *Xf* from an infected vine in the vineyard and then spread the infection to other vines, vine-to-vine spread. The risk associated with these two kinds of spread is different. The patterns of spread associated with primary spread are linear, that is a typically small and relatively constant number of vines per year become infected, and the accumulation of infected vines increases additively. The result is usually small but manageable losses each year. The patterns of spread associated with secondary spread are typically logarithmic, and the accumulation of infected vines increases as a multiple of the infected source vines that are present. The result can be the rapid loss of entire vineyards within just a few years.

Secondary spread can not begin to occur until that time in the growing season when the bacterial cells in diseased vines have multiplied and moved within the vine from the refuge site where they survived the dormant season, up into the new growth where vector insects can feed and acquire them. Secondary spread of infection can then continue until the end of the growing season. However infection does not equal disease. The phenomenon of over-winter curing of *Xf* infections is well documented in most viticulture areas of California (Fiel et.al., 2003). Early season inoculations can result in infections that survive the dormant season and progress to chronic disease and vine death. However later season infections do not become sufficiently established to survive the dormant season, and the vines are free of infection the following year (Fiel et.al., 2003). In most viticulture areas of California (Napa Valley, for example) secondary spread of infection regularly occurs, but it cannot begin early enough in the season such that the infection can survive vine dormancy and progress to chronic PD. In these areas secondary spread occurs but does not result in disease.

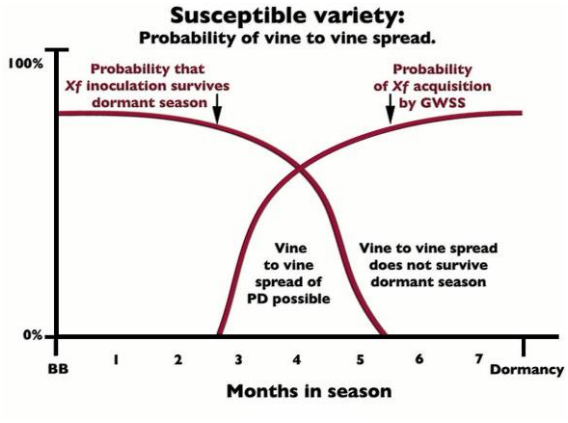

Figure 1

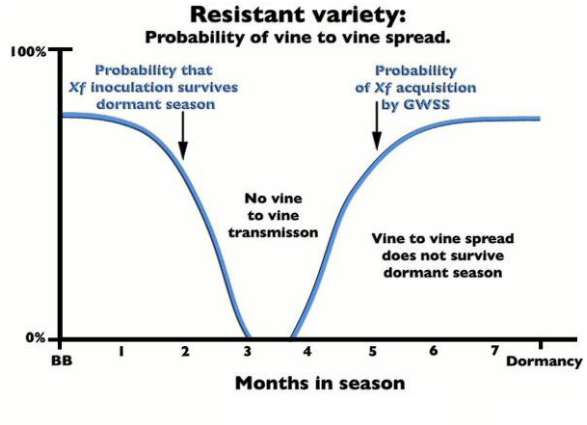

Figure 2

Our hypothesis is that in the General Beale area secondary spread of infection occurred in all varieties, possibly infecting large numbers of vines in every vineyard. The rate of *Xf* multiplication and movement varies within plant hosts (Hill and Purcell, 1995), and presumably varies between grapevine varieties. In the most susceptible varieties, Redglobe and Crimson, the rate of bacterial multiplication and spread was faster and the result was that the bacteria had a window of opportunity sometime in mid season when secondary spread could progress to disease. Secondary spread infections could not occur before this time window, and secondary spread infections after this time window did not survive vine dormancy. Thus in the two susceptible varieties some, but not all, of the secondary infections progressed to chronic disease. In the resistant varieties however, by the time secondary spread could begin, it was too late for the infections to become well enough established to survive vine dormancy, and virtually all of those infections died out leaving the vines free of disease the following year. This is illustrated in the two hypothetical figures below. The position and shape of the left hand curves in each of the figures, labeled "Probability that *Xf* inoculation survives dormant season," is affected by the rate of multiplication and movement of the bacterium as influenced by the characteristics of the variety. The position and shape of the right hand curves in the figures, labeled "Probability of *Xf* acquisition by GWSS," is also affected by the varietal's characteristic rate of multiplication and movement of the bacterium. The position and shape of these curves can also be influenced by the severity of winter climate and the length of the dormant season. A milder and shorter dormant season would move the curves for all varieties toward each other, resulting in a greater probability of overlap and thus a greater probability of a window of opportunity when secondary spread could result in chronic disease. A colder and longer dormant season would move the curves further apart, thereby reducing overlap and reducing or eliminating the possibility of secondary spread. This would account for the dramatic difference between the epidemiological patterns observed in the Temecula vs. the General Beale epidemics. In the General Beale area most of the varieties would be "resistant" to secondary spread of PD, and thus the vineyards were not lost to disease. Those same varieties, if grown in the Temecula area, would have a shift in their probability curves such that the curves would overlap, the varieties would then be "susceptible" to secondary spread, and the vineyards would be lost.

Current research efforts on PD being funded by the viticulture industry and by government are directed toward finding a solution to the threat of PD to viticulture in California, a cure if possible. While a cure is desirable, it is also likely to be a long-term effort, expensive, and possibly impractical. The risk from PD, even in the presence of GWSS is not uniform throughout the state because the epidemiology characteristics are different in various areas. If the epidemiological risk could be reliably defined for each area and effective control measures devised and adopted to reduce or eliminate risk, the threat could be reduced to economic unimportance. Ideally we could know enough specific epidemiology to provide the following advice to growers in each area: "Your risk of loss from primary spread is X, and by adopting these control measures at cost Y your risk can be reduced to Z. Furthermore your risk of loss from secondary spread is A, and by adopting these control measures at cost B, your risk can be reduced to C." This knowledge would satisfy the need of almost all California grape growers.

This project addresses the risk of loss from secondary spread in the southern San Joaquin area, and should identify a window of vulnerability when protections against secondary spread would be most effective. These experiments will provide actual data to help convert the hypothetical curves proposed here, to real curves for susceptible and resistant varieties in the southern San Joaquin Valley. If the timing and duration of the time window when susceptible varieties are vulnerable to secondary spread is identified, then chemical protections, such as systemic insecticides, may reduce the risk during that window of time to economic unimportance.

Based on historical experience the risk from primary spread appears to be negligible in Kern County and is confined to localized pockets in Tulare and Fresno Counties (pers. com. W. Peacock, J. Hashim). Primary spread during the General Beale GWSS/PD epidemic would have affected all the varieties, but there is no epidemiological evidence that this occurred

(Hashim et.al., 2003). Areas of southern Kern County where GWSS has been present in low numbers for more than 5 years have rates of new PD infections that are less than 1 vine in 10,000 in all varieties.

Ideally the same kind of experiments should be conducted in various regions of California. However there are both practical and political impediments to conducting such experiments, and it is beyond the capacity of this laboratory to expand into other areas. The magnitude of these experiments requires plots with several hundred mature grapevines that are being cultivated as a commercial vineyard, and there are concerns about experimentally introducing PD into viticulture areas close to commercial production. This project was delayed due to these concerns and was eventually located in a mature vineyard in the Kerney Agricultural Field station near Parlier, California. Other similar safe and acceptable locations are yet to be located in other major viticulture areas.

OBJECTIVES

The hypothesis regarding differences among varieties regarding susceptibility to secondary spread will be experimentally tested by:

1. Determining the "Probability that *Xf* inoculation survives dormant season" curves for 4 different varieties, a resistant, a susceptible, and three unknowns, and
2. Determining the "Probability of *Xf* acquisition by GWSS" curves for the same 4 varieties.

Objective one will involve needle inoculations of 20 to 35 vines at a time, of each variety, at twice a month intervals for 4 months beginning at the end of April. Systemic infections will be confirmed by ELISA testing of each vine during the year that they are inoculated. The following year they will be tested to see whether the infections persisted over the dormant season. Objective two will involve inoculating 50 vines of each variety early in the season, then testing the vines at various time intervals the following year to determine when the bacterium appears in the new foliage such that GWSS could acquire the bacterium by feeding on the foliage. The experiments for objective one have been done previously, but not with sample sizes and frequencies that would allow the reliable depiction of bacterial survival curves. Objective two has not been done before, nor has the combination of the two curves been done together to determine the possibility and timing of a potential window of time when secondary spread would be possible.

RESULTS

The inoculation and monitoring experiments are being done at the University of California Kearney Research and Extension Center at Parlier, California on a 3.2-acre plot that had 1260 mature (ca.10 year old) Thompson Seedless vines. On 180 of these vines two grafts each of another variety (Selma Pete) were grafted 3 years ago on the mature Thompson roots. These 180 Selma Pete vines (now in their 4th season) and another 320 Thompson Seedless vines were needle-inoculated this year at twice per month intervals beginning the end of April through the middle of August, 8 total inoculations. The vines inoculated in May and June (4 inoculation times, 220 vines) have been tested so far, and 100% of the inoculations have resulted in *Xf* infections that have multiplied and moved beyond the inoculation site. The remaining vines will be tested before the vines go dormant this year.

The remaining 760 mature Thompson Seedless vines that were not involved in inoculation experiments this year were cut off about 30 cm above the soil and grafted with Redglobe, Thompson Seedless, or Princess cuttings in early April of this year. About 80% of these grafts were successful, and are therefore now near the end of their first year of growth. In three years these vines will be ready for the same kind of experiments that are being conducted this year with the currently mature Thompson's and Selma Pete vines. It was unfortunate that a site could not be obtained this year with sufficient mature Redglobe and Thompson vines to enable the experiments to be done now without waiting for three years, but the concerns of the PD control programs in the southern San Joaquin Valley prevented obtaining such a site.

Each needle inoculation introduced a droplet with at least 10,000 viable *Xf* cells into the plant xylem. Each plant was needle inoculated at two different sites, on shoots that were on different scaffolds or branches of the vine, and the inoculation sites were flagged so that they could be found again. The inoculations were near the base of the shoots, about 3 internodes (usually about 15 to 20 cm) from the mature wood. At each inoculation site both the stem and the closest petiole were inoculated. The intent was to make the inoculations with many thousands more cells than a vector insect would transmit, and at sites comparable to where a feeding GWSS might inoculate close to the old wood. The idea was to maximize the probability that the needle inoculation would result in infections that might survive the dormant season. If this intensive needle inoculation does not result in infections that survive the dormant season, then surely inoculations by GWSS would not result in infections that survive.

CONCLUSIONS

These experiments have just begun. We have established that the inoculation protocol is at or close to 100% effective at producing infections of *Xf*. There have been many speculative theories about why GWSS inoculations would be more likely than traditional California vectors to produce *Xf* infections that survived vine dormancy and progressed to disease. These experiments are even more likely than GWSS to produce infections that survive. If under these circumstances it is found that secondary spread in resistant varieties in the southern San Joaquin cannot begin until after the time when the new infections

can survive the dormant season (i.e. the curves do not overlap) then it could be asserted that the risk of secondary spread in this region in resistant varieties with GWSS as a vector is not economically significant.

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