EVALUATING VARIATIONS IN RESISTANCE AND GLASSY-WINGED SHARPSHOOTER TRANSMISSION RATE AMONG GRAPEVINE VARIETALS

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ABSTRACT
We evaluated the susceptibility of different *Vitis vinifera* varieties to *Xylella fastidiosa (Xf)* infection under greenhouse conditions. We further compared *Xf* transmission efficiency by the glassy-winged sharpshooter in no choice trials for the tested varieties. Our results indicated that there is a great degree of variability in symptom development among the tested varieties. Furthermore, a significant variation in bacterial populations in leaf petioles was also detected among experimental varietals. While Crimson seedless and Grenache Noir had the lowest bacterial populations, Flame seedless had the highest bacterial population. Transmission efficiency was not influenced by grape variety or by the bacterial population in leaf petioles.

LAYPERSON SUMMARY
The degree of susceptibility to Pierce's disease is under evaluation for 18 commonly used grapevine varieties. Our results indicate that symptom severity and pathogen populations in leaf petioles vary among varietals. For example, while Crimson Seedless and Grenache Noir had the lowest pathogenic bacterial populations within petiole tissue, Flame Seedless possessed the highest bacterial population. The transmission efficiency of the plant pathogen by the glassy-winged sharpshooter did not differ among tested varieties. Vector transmission rate was also not affected by the pathogen's population in petioles. Establishing an objective categorization of the degree of susceptibility of grape varieties is currently ongoing.

INTRODUCTION
The degree of plant susceptibility to infectious pathogens is a measure, understanding of which is important for managing disease spread in agricultural systems (e.g. Kolmer 1996; Leung et al. 2003). Genetic variability among host plants may influence the level of plant resistance and/or tolerance to infections (Kover & Schaal 2002). Here, we consider a plant tolerant if it shows limited or no visual disease symptoms despite being infected by a large pathogen population. We refer to a host as resistant if the pathogen population (hereafter, 'infection level') remains low in the infected host. 'Resistance' and 'tolerance' are used as relative terms.

The xylem-limited bacterium *Xylella fastidiosa (Xf)* is the etiological agent of the epidemic Pierce's disease (PD) in grapevines (Purcell 1997, Hopkins and Purcell 2002). PD symptoms include leaf scorch, irregular maturation of the cane, and dieback of the apex of the plant (Krivanek et al. 2005). Although *Vitis vinifera* cultivars are generally susceptible to *Xf* infection (Krivanek and Walker 2005), anecdotal field observations (A.H. Purcell and J. Hashim-Buckey, personal communication) and a few experimental studies (e.g. Raju and Goheen 1981; Fry and Milholland 1990; Krivanek et al. 2005) indicate that differences exist in symptom severity among varietals. Indeed, symptom severity is correlated with the infection level of the host (Fry and Milholland 1990; Alves et al 2004; Krivanek and Walker 2004). In addition to variation in symptom severity, bacterial populations may also affect vector transmission efficiency among varietals as it has been shown that *Xf* transmission efficiency depends on the level of infection in the source plants (Hill & Purcell 1997). A greater exposure to bacteria can increase acquisition efficiency and, subsequently, the inoculation rate. This report includes data on evaluations of bacterial population growth and the transmission efficiency of glassy-winged sharpshooter (GWSS) among several commonly used grape varietals.

OBJECTIVES
1. Evaluating the degree of varietal susceptibility to *Xf* infections
2. Comparing *Xf* transmission efficiency by the glassy-winged sharpshooter among grape varietals
3. Measuring overwinter recovery from infection for different grape varietals

RESULTS AND DISCUSSION
Objective 1.
Variation in *Xf* populations colonizing different host plant species have been documented previously (e.g. Alves et al. 2004; Krivanek et al 2005). Our objective was designed to evaluate the extent of host colonization and symptom severity among eighteen varieties of *Vitis vinifera* that are commonly used in California. In March 2009 grape cuttings were needle-inoculated with the STL strain of *Xf* at the base of the main shoot (n=22 per variety). We reported the results of symptom development among several commonly used grapevine varieties in a previous report. We also quantified bacterial

populations in the petioles of respected varieties using quantitative PCR (**Figure 1a, b**). Our ANOVA results (repeated-measures) showed significant differences among varieties in bacterial populations within petioles ($F_{13, 166} = 2.4$, P = 0.005). There was no significant effect of sampling week 8 (**Figure 1a**) or 12 (**Figure 1b**) on the bacterial populations ($F_{1, 166} = 0.13$, P = 0.7). No interaction between variety and sampling date was detected ($F_{13, 166} = 1.55$, P = 0.10). Grenache Noir and Crimson seedless formed a statistically homogeneous subset with the lowest bacterial populations. Rubired, Merlot, French Colombard, Syrah, Pinot Noir, Cabernet Sauvignon, Thompson Seedless, Barbera, Ruby seedless, Red Globe, and Chardonnay were the 11 varieties forming a statistically homogeneous intermediate subset (varieties are arranged in an ascending order of infection level). Flame seedless was the single variety with the greatest bacterial population in petioles, which did not fall into any of the above subsets (**Figure 1a, b**). By comparing bacterial population growth and symptom severity it can be safely concluded that Rubired represents one of the least susceptible varieties tested in this study.

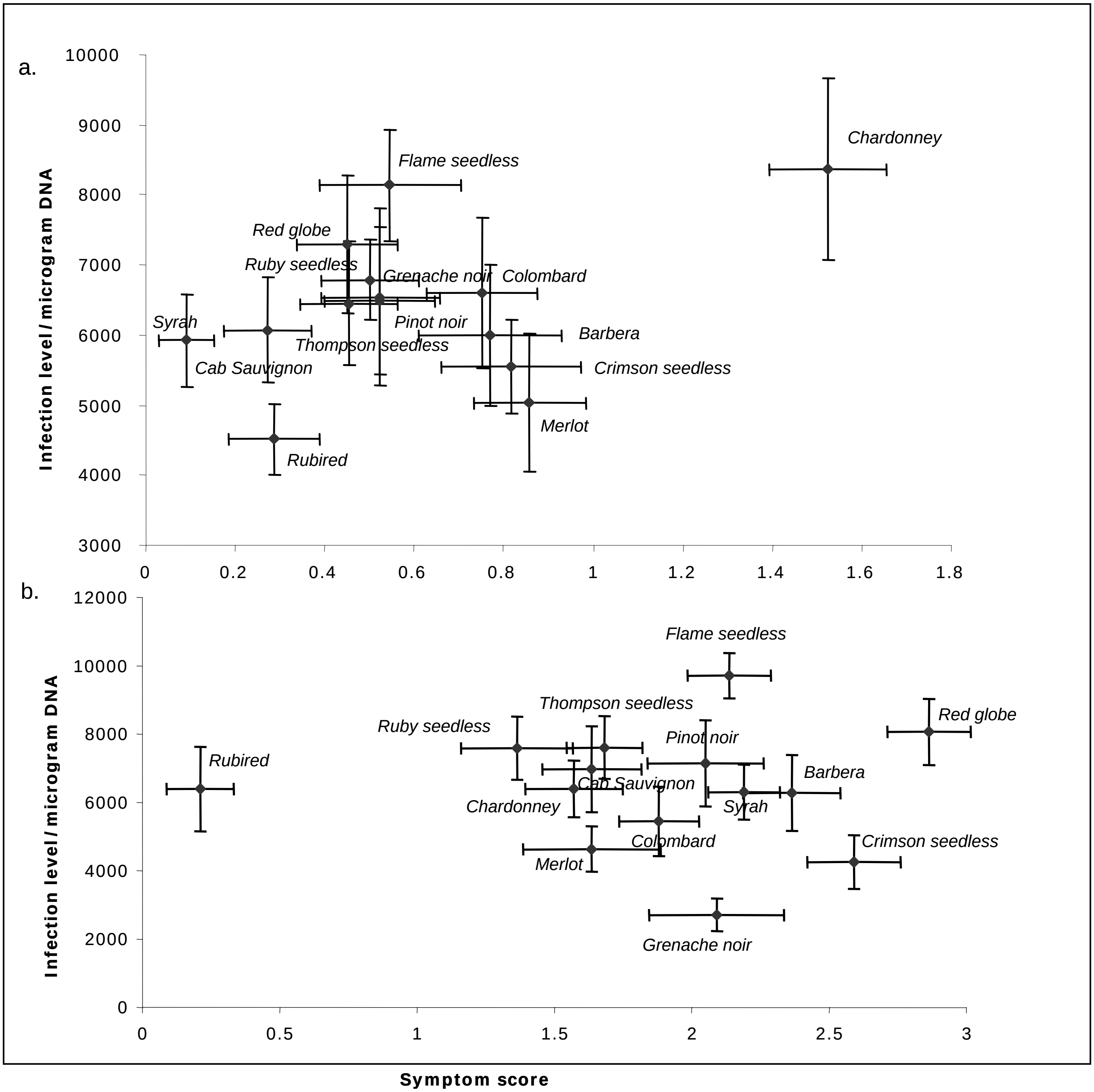

Figure 1. Scatter plots of mean infection levels (qPCR) versus mean symptom development scores (0 to 5 scale) for each of the 14 tested grape varieties (N=20 for most varieties); a) week 8, b) week12. Error bars represent ±1se.

We are currently in the process of analyzing petioles samples of another time block of the similar experiment with a subset of 10 varieties. We chose to perform a second time block as we thought some of the variations in symptom development during our summer experiment (**Figure 1a**) could have been affected by random environmental factors. Symptoms have been scored for the latest time block on weeks 8, 12, 16 and 20 (results not presented here) and following the completion of our quantitative PCR analysis our goal would be to establish an objective measure to categorize grape varietals base on their degree of susceptibility to *Xf* infection. This will be done by contrasting bacterial population level against symptom severity score in every single variety (currently in progress).

Objective 2:
Three hundred and ninety six GWSS were caged individually on 22 mechanically inoculated plants of each of the 18 varieties for a 48-hour acquisition access period. Insects were moved individually to a healthy host of the same variety and were allowed to feed for six days (inoculation access period). After four months petioles of the test plants were cultured on PWG medium to detect successful transmission events. Data from source plants, which tested negative for *Xf* presence based on the quantitative PCR data from 'objective 1', were not included in the transmission rate analyses. A binary logistic regression model with variety as a category, date as a repeated category, and infection level as a covariate (continuous) showed that transmission success of the glassy-winged sharpshooter was independent from plant genotype (Wald X_{13}^2= 8.13, P= 0.83), transmission date (Wald X_1^2= 0.89, P= 0.35) and infection level of the source plant (Wald X_1^2= 0.16, P= 0.68; **Figure 2**) (mean infection level (± SE): Successful transmissions, 6382.5 (700.6); failed transmissions, 6395.5 (212)). Our finding is also supported by Lopes et al (2009), who detected no association between host plant species (with different infection levels) and GWSS transmission. In contrast, Hill and Purcell (1997) showed that a relationship between the infection level of the source plant and the probability of a successful transmission is expected. Our failure to find a relationship may be the result of an overall low successful transmission incidence or the relatively low variations in bacterial populations among varieties. Indeed, with the exception of Flame Seedless, Crimson Seedless and Grenache noir, the rest of the tested varieties formed a statistically homogeneous group with respect to bacterial populations. In addition, the GWSS tends to prefer stem tissue rather than leaf petioles for feeding. Testing of stems to determine *Xf* populations in that tissue is more challenging and such large experiments would not have been possible. Ongoing studies are addressing this question in more detail.

Figure 2: An illustration of the overall relationship between the transmission rate and the bacterial infection level of the source plant.

Objective 3 (in progress):
Twenty cuttings of 11 commonly used grapevine varieties have been inoculated with STL strain of *Xf* in July 2010. All inoculated plants will be tested to confirm successful inoculations. Infected plants are scheduled to be transferred to an outdoor facility in November 2010. Starting April 2011, petioles of the experimental plants will be tested for *Xf* presence by PWG culturing. The goal of these assays is to determine overwinter recovery of varieties from *Xf* infections during the previous year.

In addition to the objectives listed in this report, our original proposal includes questions addressing GWSS feeding behavior and its role in transmission and pathogen spread. In particular, we are investigating host-choice as well as within-host feeding site preference of the GWSS. In previous reports we presented our findings on feeding site selection and its link to bacterial acquisition efficiency. Briefly, we showed that in spite their preference to feed on stem tissue, possibly due to background matching behavior, GWSS acquire more bacterial cells from petioles and leaves (although statistically non-significant). This part of the study is currently under review for publication. The GWSS's response to bacterial presence as well as visual PD symptoms is also under investigation.

CONCLUSIONS

This study follows a recommendation by the PD advisory panel and aims to objectively quantify *Xf*-resistant and *Xf*-tolerant varietals and the role of GWSS in spreading *Xf*. We showed the variability of symptom development and bacterial population growth among several grape varieties. Although our final results is pending upon completion of our second time block analysis, so far we showed that Flame seedless has the greatest bacterial population growth compared to the other tested varieties. The varieties Grenache Noir and Crimson Seedless had the lowest bacterial population growth. The transmission efficiency of the GWSS did not differ among our tested varietals. Likewise, the transmission efficiency was not affected by the variations in the bacterial population levels. Experiments are ongoing and our final results can be used to evaluate the feasibility of using existing *Vitis vinifera* cultivars to control PD spread by quantifying resistance, tolerance, and GWSS behavior for several important table and wine grape varietals. This work will provide recommendations to growers in affected areas on which varietals to use in order to minimize and contain the pathogen spread.

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