

WHICH GRAPE VARIETALS ARE SOURCES OF PIERCE'S DISEASE SPREAD? DECOUPLING RESISTANCE, TOLERANCE, AND GLASSY-WINGED SHARPSHOOTER DISCRIMINATION

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ABSTRACT

Plant resistance to pathogens and the feeding behavior of the pathogen vectors can influence patterns of disease spread. This study is aimed at evaluating the two above ecological factors that could limit the negative impact of Pierce's disease (PD) on the economically valuable grapevine, *Vitis vinifera*. Eighteen commonly used varieties are being scored for symptom severity and quantified for *Xylella fastidiosa* (*Xf*) infection level. We will investigate the vector, glassy-winged sharpshooter (GWSS), feeding-choice behavior for infected versus healthy plants. In addition, we will compare the vector transmission efficiency among different grape varieties. Our preliminary results for symptom severity, in weeks eight and 12 post-inoculation, indicates that Rubired has the least symptom development score among 17 other tested varieties. Chardonnay and Chenin Blanc also show a relatively slow symptom development. Our study on vector feeding behavior showed that, while GWSS does not discriminate against water stressed plants (which mimic PD symptoms); they had a greater tendency to position themselves on lower and woody parts of the stem due to its response to brown colors. Our experimental setup and preliminary finding will be described and discussed.

LAYPERSON SUMMARY

The degree of resistance (and tolerance) to Pierce's disease (PD) is under evaluation for 18 commonly used grapevine varieties. We also quantify feeding and host-choice behaviors of glassy-winged sharpshooter, a key vector for the pathogenic bacteria. This project has been just started. Our preliminary results showed that the variety Rubired has the least symptom development score among 18 varieties. Chardonnay and Chenin Blanc are also showing slower symptom development relative to the other tested varieties. The insect vector did not discriminate against water stressed plants (with similar symptoms as PD) though showed a distinct preference for the brown coloration of the lower main shoot. Our experimental setup and preliminary finding will be described and discussed.

INTRODUCTION

Plant resistance to pathogens is important for limiting disease in many agro-ecosystems (e.g. Kolmer 1996; Leung et al. 2003). The levels of resistance and tolerance (infected with limited or no symptoms) exhibited among host genotypes typically vary continuously (Kover & Schaal 2002). The used definition of resistance and tolerance is important because each may differ in its effect on disease spread, especially plant pathogens that are transmitted by insect vectors (see below).

Pierce's disease (PD) is caused by the bacterial pathogen *Xylella fastidiosa* (*Xf*). This bacterium is xylem-limited and pathogenic to a wide range of agricultural crops, such as alfalfa, almond, citrus, coffee, peach, plum, and also infects dozens of native, ornamental, and weedy species in the U.S. (Purcell 1997, Hopkins and Purcell 2002, Costa et al. 2005, Wistrom and Purcell 2005). PD symptoms include leaf scorch, irregular maturation of the cane, and dieback of the apex of the plant (Krivank et al. 2005). Typically, infected susceptible vines die within two to three years. Xylem sap-feeders are known to be the vectors for the bacterial pathogen. In the 1990s severe outbreaks of PD occurred in southern California that was attributed to the invasion by the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) (Sorensen and Gill 1996, Blua et al. 1999).

Vector preference for infected versus uninfected plants can dramatically impact disease prevalence (McElhany et al. 1995). For example, vector preference for healthy plants (i.e. discrimination against infected plants) should limit pathogen spread. Under this scenario, relatively tolerant varieties that are infected but yet have no (or limited) symptoms may potentially act as a source for the bacteria. Needless to say that this can potentially promote disease spread in nearby healthy vines. Likewise, a lack of discrimination against infected plants would also increase the pathogen spread. Whether susceptible varieties can act as a source for *Xf* spread also depends on GWSS behavior and acquisition efficiency. Two aspects of GWSS feeding behavior may be important for *Xf* acquisition and spread: 1) preference for feeding on certain grape varieties (e.g., Purcell 1981), and 2) discrimination against infected vines of a given varietal (e.g., McElhany et al. 1995, Marucci et al. 2005). Thus, understanding vector feeding-choice behavior and varietal resistance versus tolerance are directly relevant to understanding the epidemiology of PD in vineyards.

In general, *Vitis vinifera* cultivars are susceptible to *Xf* infection (Krivaneck and Walker 2005). While anecdotal field observations (Raju and Goheen 1981, A.H. Purcell and J. Hashim-Buckey, personal communication) are indicative of

differences in symptoms severity among varieties, studies quantifying *Xf* infection levels have also documented substantial variability among cultivars (Raju and Goheen 1981, Krivanek and Walker 2005). Thus, in order to predict which grape varieties are most likely to act as *Xf* sources for vectors we need to independently measure both the varietal infection level (i.e. resistance) as well as symptom severity for that particular variety in relation to vector transmission efficiency.

In this project our goal is to evaluate: *i* the differential response of *V. vinifera* to *Xf* infection, and *ii* feeding-choice behavior of the pathogen vector GWSS.

OBJECTIVES

1. Measure the relative levels of both resistance and tolerance for important California grape varieties.
2. Evaluate GWSS feeding behavior in grapevine and measure discrimination against infected vines and *Xf* spread for different grape varieties.

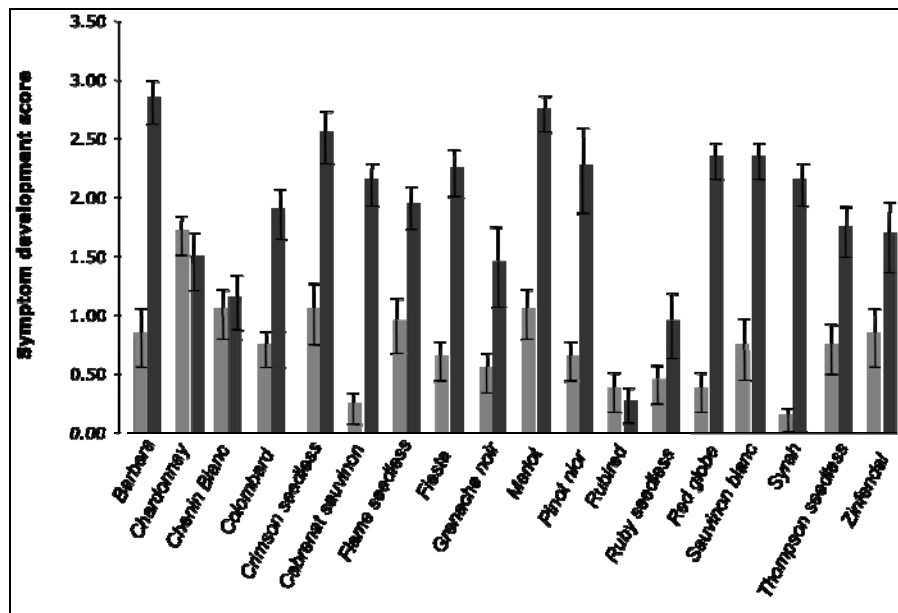
RESULTS AND DISCUSSION

The project currently is at the beginning and therefore this report presents some preliminary results and experimental setup and preparation for upcoming time blocks scheduled for mid-October and mid-December 2009.

In order to quantify the levels of resistance and tolerance we have obtained and planted dormant cuttings of 18 different commonly used varieties of *Vitis vinifera* in March 2009. We now have an estimated 100 plants per genotype for each of those varieties. We have mechanically inoculated *Xf* into 22 plants of each variety in two different time blocks within 24 hrs. All plants were inoculated at the base of the main shoot.

Inoculated plants were quantified for symptoms on the weeks eight and 12 post inoculation. The same plants will again be quantified on weeks 16 and 20. The disease symptoms were scored following Guilhabert and Kirkpatrick (2005). The PD symptom development appears to be most aggressive for the varieties Flame Seedless, Red Globe, Crimson Seedless, Cabernet Sauvignon and Syrah consistently across the two time blocks. The varieties Chardonnay and Chenin Blanc should a weaker symptom development compare to the other tested varieties Red Globe seem to show a slight but non-significant recovery in its symptom severity (see **Figure 1**). However, PD symptoms are sometime hard to distinguish from water stress or nutrition stress symptoms. Therefore, a final conclusion needs to be made following culturing and qPCR, which verify the bacterial presence and quantify the infection level, respectively.

On weeks eight and 12 post-inoculation we sampled leaf petioles of all inoculated varieties at +10 cm above the inoculation point. In addition, we took one sample at +30 cm above the inoculation point (per plant) to also compare the bacterial movement within different grape varieties. All samples are stored in -80°C freezer for later processing. Plants will also be sampled on weeks 16 and 24. For the sake of consistency, we will start qPCR after the labor-intensive greenhouse experiments are finalized. This approach would provide us with a sufficient measure of bacterial populations at the three different stages of disease progress, which allow us to track within host disease spread over time for each variety and enables us to compare population growth of bacteria among our varieties.



a.

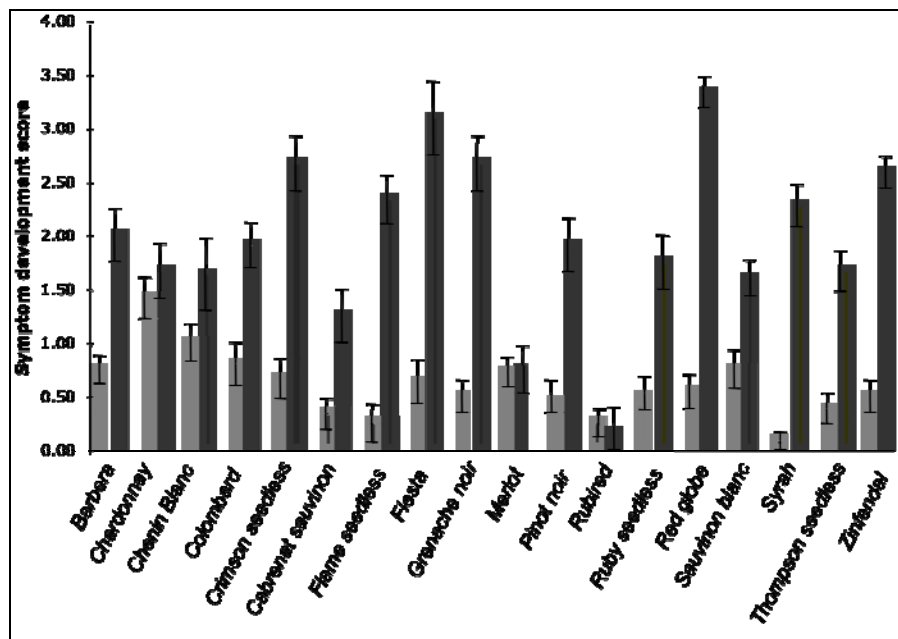


Figure 1: Symptom development scores for all tested grape varieties following a 0 to 5 scale (Guilhabert and Kirkpatrick 2005): a) time block 1; n= 10, b) time block 2; n= 12. Light-Gray fill marks the symptom development score on week eight and dark-gray marks the symptom development score on week 12.

By contrasting bacterial growth rate within the plant and visible plant symptoms we will provide an accurate classification for susceptible, tolerant and resistant varieties. Tolerance and resistance are used as relative terms within *V. vinifera* among its varieties.

No-choice transmission experiments (in progress)

Adult GWSSs were caged individually on the 22 mechanically inoculated plants (see above-block 1: n=10; block 2: n=12) per variety (18 varieties in total) for 48 hours (acquisition period). After 48 hours insects were gently removed from the infected plants and caged (again individually) on healthy plants of corresponding variety.

Insects were kept on healthy plants for six days (transmission period). Insects were removed and plants are currently being monitored for symptoms. Transmission rates will be calculated and compared among varieties. We stored the collected insects in -80°C freezer for future qPCR. Through this process we will be able to quantify the bacteria acquisition by insects across the 18 varieties.

The same procedure will be repeated on weeks 16 and 24 post-inoculation (using the original mechanically inoculated plants). Thus, the transmission/acquisition rates will also be compared across three different stages of bacterial growth within plants for each variety.

Feeding behavior of *H. vitripennis*- choice experiments

Morphological, physiological and nutritional changes in plants due to pathogen infection has been suggested to influence insect vector choice of the host plant (Hammond and Hardy 1988). For example aphids that are attracted to yellow color show preference to feed on yellow leaves caused by virus infection (e.g. Baker 1960). Although such behavior maybe an adaptation to detect higher amounts of nitrogen in plant resources (see Kennedy et al 1961), it also could lead aphids to plants that are more susceptible due to weakness.

In a previous study, however, Marucci et al. (2005) showed that sharpshooter vectors, *Dilobopterus costalimiae* and *Oncometopia facialis* (Hemiptera: Cicadellidae) both prefer to feed on healthy asymptomatic plants rather than symptomatic plants infected by the pathogenic *Xf*. Likewise, anecdotal observations are suggestive of a similar host choice pattern in GWSS. Based on these observations GWSS is expected to choose healthy hosts more frequently compared to a symptomatic plant. However, this proposed behavior has never been formally tested.

PD symptoms are similar to water stress possibly due to xylems being blocked by the *Xf* colonies. Thus, GWSS response to water-stressed plants is expected to follow that of *Xf* infected plants, if indeed GWSS chooses its host plant based on visual signals. We conducted this experiment as a preliminary test preparing for assays to be conducted as part of Objective 2, which we are preparing plants and insects for at this point.

One water-stressed and one healthy *V. vinifera* (var. Zinfandel) plant was placed in an observational bugdorm cage. A plant was considered water stressed when at least two thirds of its leaves showed wilting and scorching symptoms. One adult GWSS was gently aspirated into the cage and its movement within the cage was monitored every 30 minutes until a choice was made. The choice was recorded and the individual's position was double-checked after two hours to make sure that no host plant switches occurred. None of the GWSS in our 20 trials moved to a different plant once they made their host-choice. Unexpectedly, GWSS did not discriminate against water stressed plants (Sign test (two-tailed): $P = 0.66$). Similar pairwise experiments will be conducted with inoculated plants on week 24 post-inoculation, when PD symptoms are visually detectable, to a considerable extent, to human eye.

In addition to between-plant choice, within-plant feeding site may also influence the transmission efficiency of an insect vector. Insect preference for a particular feeding site may coincide with the pathogen colonization and thus increase the acquisition and transmission rates, as a result. We have shown this to be the case for sharpshooter transmission of *Xf* from alfalfa plants (Daugherty, Lopes and Almeida, submitted). Anecdotal observations indicated GWSS preference for feeding on the lower parts of the grapevine stem. However, the exact reason for such a choice is yet to be confirmed. Here we tested the possibility of color matching in GWSS since the lower parts of grapevines turn into brown following maturation. Detecting a link between GWSS feeding site choice is significantly important because: *i*) cane color in grapevine changes in response to *Xf* infection (Krivaneck et al. 2005) and *ii*) different grape varieties may vary in their time of cane maturation and the amount of brown color of the stem. We hypothesize that this may explain the lower transmission rates of *Xf* from grape to grape by the GWSS (Almeida and Purcell 2003).

To test for this possibility we presented individual sharpshooters with grape cuttings *Vitis vinifera* (var Cab Sauv), 20-30 cm in length and with equal proportions of green and brown colors (to the experimenter eye). Our experimental data verified the fact that GWSS prefers to feed on the lower and brownish-colored parts of the stem (sign-test (two-tailed): $P= 0.011$; $n=20$).

To confirm the existence of background color preference, we also caged GWSS individually in a 30x30x30 cm cage covered with paper sheets of brown and green checkers and observed them for 15 minutes. We showed not only insects chose to jump to brown first (sign-test (two-tail): $P=0.04$; $n=30$), but also showed that they spend significantly more time on brown squares compared to the green squares (**Figure 2**). This additional test of the feeding site preference addresses questions directly associated with those Objectives related to GWSS feeding site preference and within-plant behavior and color attraction, which may explain the lower transmission rates observed in previous experiments by this species compared to other native CA sharpshooters (Almeida and Purcell 2003; Almeida et al. 2005).

We have ongoing experiments addressing the Objectives proposed. Monitoring and sampling of field experiments is also ongoing.

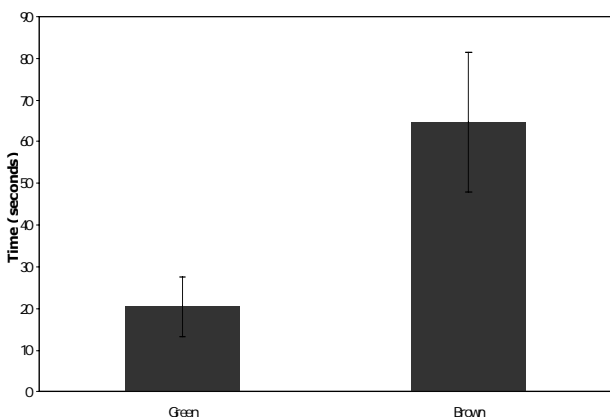


Figure 2. *H. vitripennis* individuals prefer to spend more time on brown colored- compared to green colored-squares (Wilcoxon brown-green: $Z = -2.407$; $n = 30$; $P = 0.015$).

CONCLUSIONS

This work directly addresses a recommendation by the PD advisory panel for more research to distinguish between *Xf*-resistant and *Xf*-tolerant varieties and the role of GWSS in spreading *Xf*. Our early visual inspection of the PD symptom development indicates that the varieties Flame Seedless, Red Globe, Crimson Seedless, Cabernet Sauvignon and Syrah show more susceptibility to *Xf* infection. The varieties Chardonnay and Chenin Blanc showed slower symptom development compared to the other tested varieties. Rubired seems to show a slight but non-significant recovery in its symptom severity. However, final conclusions cannot be made without further analysis upon a completed data collection. While, our finding, so far, indicates that GWSS does not discriminate against water stressed plants, which more-or-less mimic PD symptoms, they show a strong preference to feed on the lower parts of the stem where the colonization of the bacteria may be more difficult. Experiments are ongoing. Our final results will directly benefit growers by evaluating which varieties are most likely and least likely to promote spread of PD by GWSS. Targeted planting of more resistant varieties could be used as a vineyard management strategy to reduce PD incidence in high-risk areas.

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