

**Title of report:** Renewal Progress Report for CDFA Agreement Number 15-0217-SA

**Title of project:** Assessing Pierce's disease spread in grape lines with novel defensive traits

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**Reporting Period:** The results reported here are from work conducted between October 2016 and March 2017.

**Introduction**

This proposal expands on previous work funded by this program to develop PD-resistant grape lines. Previous projects have successfully developed grapevine lines with promising traits conferring resistance against *X. fastidiosa*, including plants expressing the *rpjF* gene, the *PdR1* major locus, and the HxjB protein (Meredith et al 2000; Walker and Tenschler 2014; Lindow et al 2014). All these grape lines exhibit low symptom severity when inoculated with *X. fastidiosa*. We propose to expand upon previous work by testing the potential of PD-defended grapevine lines to reduce the spread of *X. fastidiosa* using a multi-disciplinary combination of vector transmission experiments and mathematical modeling. Using this approach and HxjB-producing plants as a case study, we found that while HxjB plants are unlikely to eliminate PD in the field, spread would nonetheless be significantly reduced. Further study will allow us to assess the impacts of these reductions on large-scale and long-term PD spread in resistant grape lines.

**Objectives**

The overall goal of this project is to assess the potential for novel defensive traits in grapevine lines to reduce the transmission of *X. fastidiosa* by insect vectors and the prevalence of Pierce's disease (PD) within and among heterogeneous vineyards. We will assess PD epidemiology in two defended lines: transgenic grapevine lines expressing the *rpjF* gene (Lindow et al 2014) and conventionally bred grapevine lines with the *PdR1* dominant locus (Walker and Tenschler 2014). The research consists of three specific objectives:

1. Estimate transmission of *Xylella fastidiosa* and vector feeding behavior on novel PD-defended grapevine lines.
2. Assess large-scale and long-term PD prevalence in defended grapevine vineyards.

3. Inform vineyard managers on the efficacy of novel PD defenses.

*Objective 1. Estimate transmission of Xylella fastidiosa and vector feeding behavior on novel PD-defended grapevine lines.*

As reported in our October 2016 Interim Progress Report, we are investigating the influence of the PdR1 major locus on vector feeding behavior and transmission. Sharpshooter vectors, when given a choice, avoid feeding on Pierce's Disease symptomatic plants. We are using measures of preference and transmission rates of the vector species *Graphocephala atropunctata* (blue-green sharpshooter; BGSS) to understand the progression of both infectiousness, disease symptoms, and ultimately transmission rates between PdR1 resistant plants and near-isogenic susceptible plants.

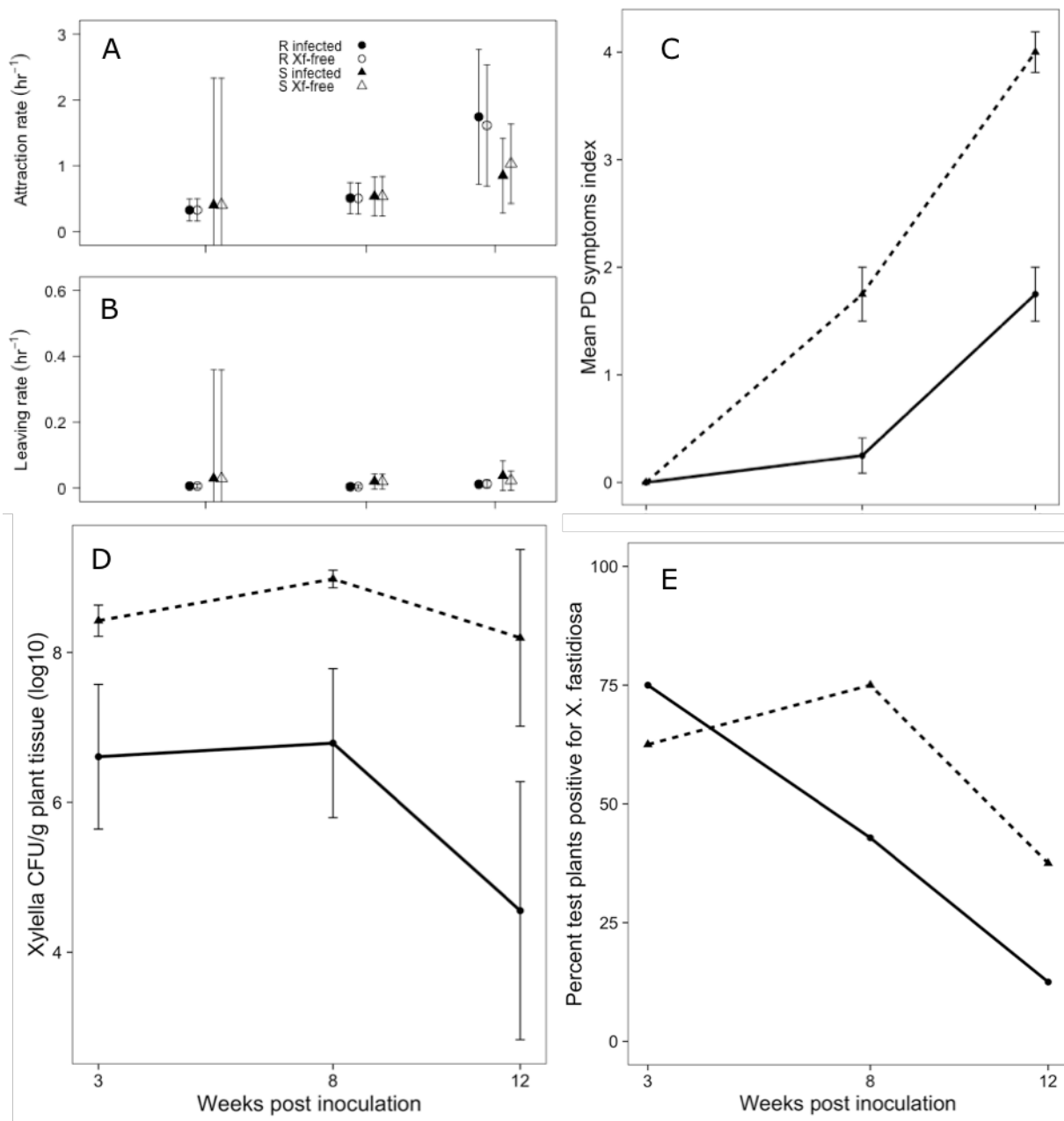
We inoculated "Resistant" plants that expressed the PdR1 allele and "Susceptible" plants that siblings from the same cross, and thus closely related, but did not have the PdR1 resistance allele. We then placed eight *Xylella*-free BGSS in a cage with two plants to choose from: a *Xylella*-free Susceptible test plant and a *Xylella*-infected source plant either of the Resistant or Susceptible genotype. We included eight replicates of each of the two treatments and repeated the experiment 3 weeks, 8 weeks, and 12 weeks after inoculating the source plants. We recorded which plant the vectors were feeding on at regular intervals over an 8-day period, estimated *Xylella* populations in the source plants, and assessed Pierce's disease symptoms in the source plants. We are currently estimating *Xylella* populations in the vectors using qPCR.

We estimated attraction rates and leaving rates of the BGSS by fitting data collected on the number of insects on each plant within a cage multiple times over a week-long period to the Consumer Movement Model described in Zeilinger et al. (2014). We used general linear models with quasi-Poisson link function to test for differences in genotype (Resistant vs. Susceptible) and time since inoculation (3, 8, and 12 weeks) in *Xylella* populations in source plants and in Pierce's Disease symptom severity, using the index described in Rashed et al. (2013). Similarly, we used logistic regression to test for differences in the percent of test plants infected with *Xylella*.

BGSS vectors showed no differences in attraction or leaving rates among plant choices until trials at 12 weeks. At this last time point, they showed increased attraction rates to Resistant source plants compared to Susceptible source plants; at the same time, they showed lower leaving rates from Resistant source plants compared to Susceptible source plants (Fig. 1A and 1B). Pierce's Disease symptom severity in source plants increased significantly over time ( $t = 4.97$ ,  $P < 0.0001$ ) and were significantly greater for Susceptible source plants than Resistant source plants ( $t = 2.59$ ,  $P = 0.013$ ; Fig. 1C). *Xylella* populations in source plants did not change significantly over time but were significantly greater in Susceptible source plants than Resistant source plants ( $t = 3.07$ ,  $P = 0.004$ ; Fig. 1D). Finally, the percent of test plants infected with *Xylella* did not differ between genotypes but significantly declined over time ( $t = -2.37$ ,  $P = 0.018$ ; Fig. 1E).

Overall, our results confirm earlier studies in that *PdR1* resistant grapevines showed lower populations of *X. fastidiosa* and PD symptom severity than sibling susceptible lines (Krivanek and Walker 2005). At the same time, BGSS vectors were able to acquire and transmit *X. fastidiosa* from the resistant lines. Importantly, however, we found a decline in vector

transmission over time from the *PdRI* vines that corresponds to an unexpected decline in *X. fastidiosa* populations. While transmission also declined from the susceptible line, this was likely due to severe PD symptoms and avoidance of infected plants by the vectors.

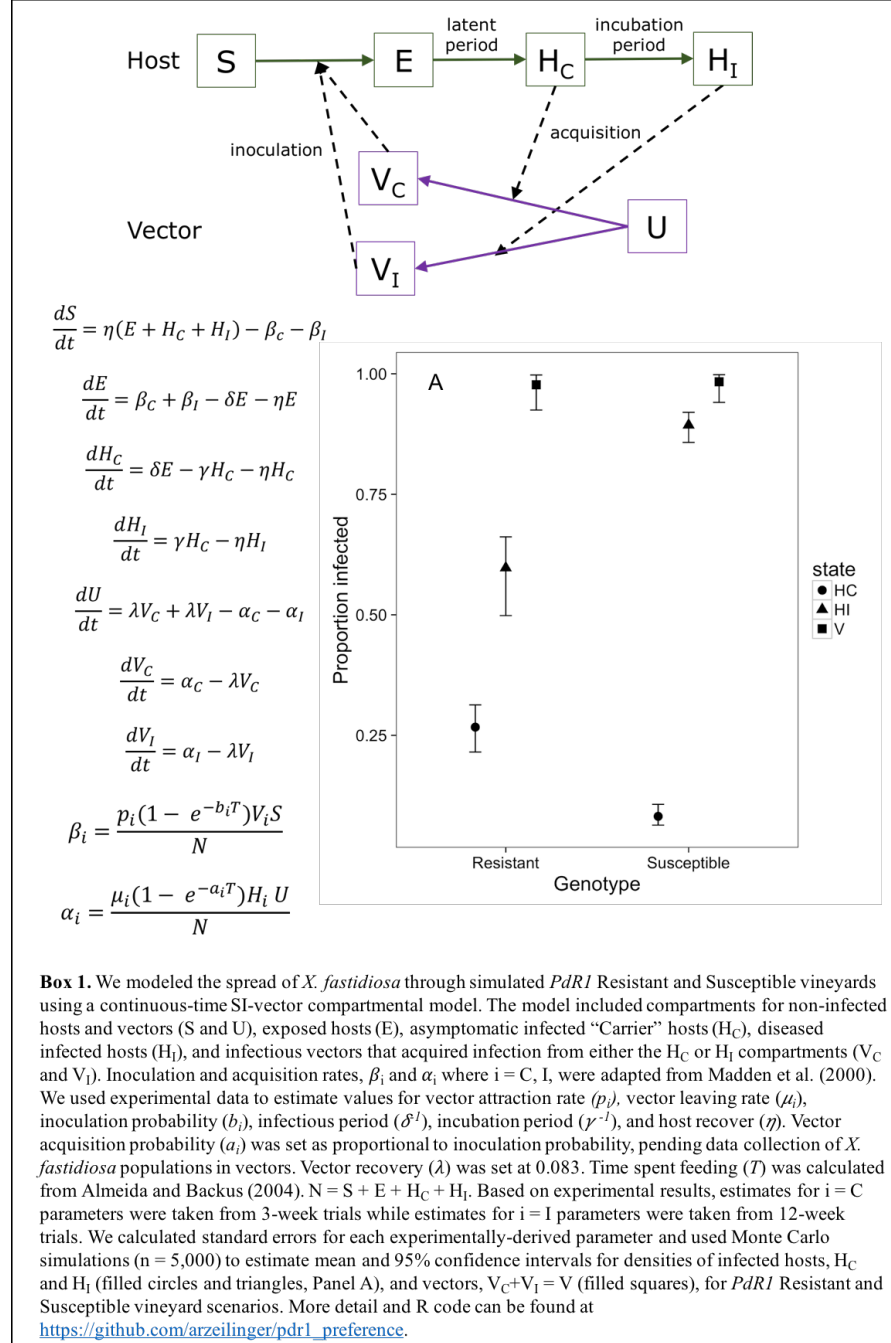


**Figure 1.** BGSS vector attraction rates (A) and leaving rates (B) for each plant choice in Resistant and Susceptible source plant cages. Mean Pierce's Disease symptom severity index (C), *Xylella* populations (CFU/g plant tissue) (D), and percent test plants infected with *Xylella* (E) for Resistant source plants (solid lines) and Susceptible source plants (dashed lines). Error bars in panels A and B represent 95% confidence intervals; error bars in C and D represent  $\pm$ SE.

## Objective 2. Assess large-scale and long-term PD prevalence in defended grape vineyards

We extended our previous modeling work, as described in previous progress reports and in our original proposal, to predict the spread of *Xylella* in vineyards in *PdR1* Resistant and Susceptible grapevines. We used our experimental results to estimate parameter values (Box 1).

From our experimental results and our epidemic modeling, it appears that the *PdR1* gene confers a complex mix of tolerance and resistance to the hybrid plants, and that the nature of the defensive trait depends on the progress of the disease. Further study of vector transmission and disease progression in *PdR1* plants will be critical to fully understand these promising but unexpected results and to assess their epidemiological significance.



### *Objective 3. Inform vineyard managers on the efficacy of novel PD defenses*

We are in the process of discussing our results with vineyard managers. However, because of the surprising results, we are emphasizing that these are preliminary findings and not publicizing them widely, until we are able to further verify the observed patterns with additional experiments.

### **Publications and Presentations**

Zeilinger, A.R., R.P.P. Almeida. Epidemiology of novel defensive traits against *Xylella fastidiosa* in grapevines. Modeling in Plant Health Symposium. European Food Safety Authority, Parma, Italy, 12 December 2016.

### **Project Relevance**

While plants expressing *PdR1* show great promise for alleviating Pierce's disease in infected plants, it remains unclear whether the trait reduces the spread of *X. fastidiosa* within and among vineyards. By investigating the epidemiological effects of novel defensive traits, we will provide critical information on the efficacy to reduce the spread of *X. fastidiosa* and the potential role that defended plants could play in integrated disease management. Our work will also provide a general framework through which the efficacy of other novel plant defenses can be assessed.

In addition to the direct significance of our proposed work to PD management, the work will inform fundamental and long-standing questions in the field: (1) What are the epidemiological consequences of different forms of host defense against pathogens? (2) What are the most important components of the *X. fastidiosa* transmission process for determining the PD prevalence, and does this depend on the dominant vector species? (3) How can plant defense and vector management complement each other to reduce pathogen spread?

### **Lay Summary**

The Pierce's disease (PD) research community has developed grapevines that exhibit novel and promising defenses against *Xylella fastidiosa* and have the potential to reduce crop damage from PD. Yet it remains unknown if these novel defensive traits will increase or decrease large-scale spread of PD within and among vineyards, which is a critical dimension of sustainable disease management. We are conducting transmission experiments with important insect vectors of *X. fastidiosa* and using data from these experiments to explore pathogen spread using mathematical models. We will assess the efficacy of defenses by comparing simulated spread in defended and susceptible vineyards and use these data to inform vineyard managers of how to minimize disease outbreaks across California. So far, our results suggest that the blue-green sharpshooter (*Graphocephala atropunctata*)—an important insect vector—is capable of acquiring and transmitting *X. fastidiosa* from transgenic resistant grapevines and conventional susceptible grapevines at similar rates.

### **Status of Funds**

Funds are being used as originally proposed.

## Status of Intellectual Property

No intellectual property has been developed as part of this project.

## References Cited

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