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

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Abstract
Development of resistance traits in agricultural crops is often a critical component of integrated disease management strategies. In contrast, tolerance traits—traits that reduce disease symptom severity without reducing pathogen populations in a host plant—may actually increase the risk of pathogen spread through a host population. At the same time, vector preference for hosts based on host infection status can have strong impacts on disease spread. Theoretical work predicts that the precise form of defense—whether resistance or tolerance—will influence the spread of a vector-borne pathogen depending on whether vectors prefer or avoid feeding on diseased hosts. The vector-borne bacterial pathogen *Xylella fastidiosa* causes Pierce's disease (PD) in grapevines and has led to reduced productivity and increased insecticide use in vineyards in California. In response, new defensive traits have been developed to improve management of PD. We investigated the biology of vector transmission from grapevines with one of two novel defensive traits: (1) transgenic grapevines expressing the *rpfF* gene and producing Diffusible Signaling Factor (DSF) and (2) conventionally bred grapevines expressing the *PdR1* dominant locus from native *Vitis arizonica*. Our results indicate that while transmission from the insect vector *Graphocephala atropunctata* (blue-green sharpshooter or BGSS) was reduced in DSF-producing grapevines relative to susceptible plants, the difference was not statistically significant. For *PdR1* grapevines, our results were more nuanced; transmission rates depended both on the genotype of the plant (resistant or susceptible) and on the time since infection of the source plant, and were mediated by vector feeding preference. Specifically, our results suggest delayed or induced resistance in *PdR1* plants 12 weeks after infection but high rates of transmission prior to this. Moreover, by integrating our experimental data into a mathematical epidemic model, we predict high infection prevalence of vectors in both *PdR1* and susceptible vineyards but lower prevalence of PD in resistant vineyards.

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 and mitigate insecticide use to suppress insect vectors. 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 have conducted transmission experiments with the insect vector *Graphocephala atropunctata*

(commonly known as blue-green sharpshooter or BGSS) of *X. fastidiosa*. We then integrated these data to explore pathogen spread in simulated vineyards using mathematical models. Our results suggest that BGSS is capable of acquiring and transmitting *X. fastidiosa* from the resistant grapevines but at overall lower rates relative to susceptible grapevines, sometimes at significantly lower rates depending on the resistance trait. In particular, conventionally bred grapevines expressing the *PdR1* resistant allele appear to exhibit a promising form of delayed or induced resistance that could significantly reduce *X. fastidiosa* spread within a vineyard.

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 *rpfF* gene, the PdR1 major locus, and the HxfB protein (Meredith et al 2000; Walker and Tenscher 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 HxfB-producing plants as a case study, we found that while HxfB 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 was 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 assessed PD epidemiology in two defended lines: transgenic grapevine lines expressing the *rpfF* gene (Lindow et al 2014) and conventionally bred grapevine lines with the *PdR1* dominant locus (Walker and Tenscher 2014). The research consisted of three specific objectives:

1. Estimate transmission of *X. 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.*

Transmission from *rpfF* transgenic grapevines

We investigated differences in transmission of *X. fastidiosa* by *Graphocephala atropunctata* (BGSS) between transgenic *rpfF* grape (producing diffusible signaling factor, or DSF) and wild-type control grapevine plants (cv. 'Freedom'). Nine weeks after needle-inoculating DSF and WT plants with *X. fastidiosa* (STL strain), we caged two BGSS adults on each plant—one near the point of inoculation and one between 60 and 90 cm above the point of inoculation along the main stem. Both vectors were caged on petioles. The vectors were allowed to acquire the pathogen for four days and then were placed on WT test plants for four additional days. We cultured *X. fastidiosa* from source and test plants 10 weeks after initial infection, and estimated *X. fastidiosa* populations inside vectors using qPCR. We obtained sample sizes of 42 and 37 for vectors caged on DSF and WT plants, respectively. We analyzed the data with generalized linear mixed-effects models (GLMM) with either log or logit link functions, for Poisson or binomially distributed data, as appropriate.

Overall, transmission to test plants was greater when the source plant was WT (Fig. 1A). However, the difference between genotypes was not statistically different ($Z = 0.822$, $P = 0.411$). We also found no significant relationship between distance from inoculation point and the probability of transmission to the test plant (Fig. 1B; $Z = 1.030$, $P = 0.303$). At the same time, we found a significant positive relationship between *X. fastidiosa*

populations in vectors and transmission (Fig. 1C; $Z = 2.039$, $P = 0.0414$). We also found a positive significant relationship between populations of *X. fastidiosa* in the source plant and transmission ($Z = 2.98$, $P = 0.003$), with a marginally significant interaction between source plant genotype (DSF or WT) and *X. fasitidiosa* population ($Z = -1.805$, $P = 0.071$). Transmission probability was higher for WT plants at low *X. fastidiosa* densities, but higher for DSF plants at high *X. fastidiosa* densities (Fig. 1D).

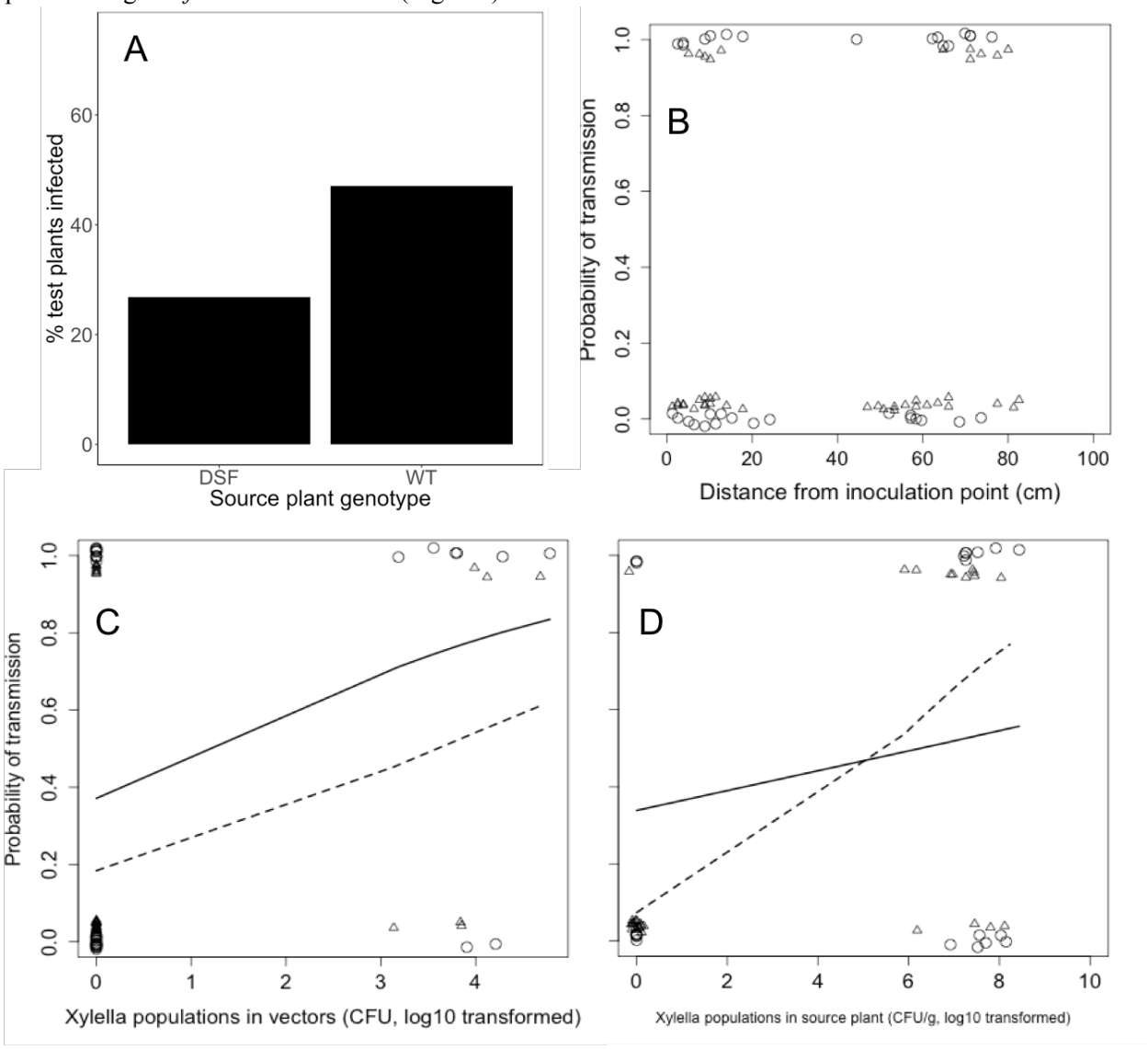


Figure 1. Results from transmission experiment with DSF-producing grapevines. Overall transmission (percent of test plants infected) was non-significant (A), and there was no effect of distance on transmission probability (B). However, there was a significant positive effect of *X. fastidiosa* populations in vectors (C) and source plants (D) on transmission probability. Circles and solid lines represent WT plants; triangles and dashed lines represent DSF plants.

We found a significant increase in *X. fastidiosa* populations in source plants with distance from inoculation point ($Z = 2.760$, $P = 0.011$), and no difference in *X. fastidiosa* populations between DSF and WT source plants ($Z = 1.685$, $P = 0.103$). However, the increase in populations with distance was driven entirely by a lack of live *X. fastidiosa* colonies close to the inoculation point—likely due to decline in plant quality after 10

weeks of infection; when we included only source plant petioles that tested positive for the bacterium, the relationship was significant and negative (t = -2165, P = 0.0215).

Overall, considering the limitations of the experimental design, we found little evidence that DSF-producing plants would reduce the transmission and spread of *X. fastidiosa* by BGSS vectors. Pathogen acquisition and estimates of bacterial populations in this study was performed on petioles rather than stems, a preferred feeding site for BGSS; however, it is possible that *X. fastidiosa* populations in petioles were not indicative of populations in stems. We found an intriguing interaction between genotype and *X. fastidiosa* density in plant tissues. However, the effect was only marginally significant, leaving it unclear how important such an interaction would be in a vineyard setting.

Transmission and vector preference with *PdR1* grapevines

We also investigated the influence of the *PdR1* major locus on vector feeding behavior and transmission. Sharpshooter vectors, when given a choice, avoid feeding on PD symptomatic plants. We are using measures of preference and transmission rates of 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 were siblings from the same cross, and thus closely related, but did not have the PdR1 resistance allele. We then placed eight *X. fastidiosa*-free BGSS in a cage with two plants to choose from: a *X. fastidiosa*-free Susceptible test plant and a *X. fastidiosa*-inoculated source plant either of the Resistant or Susceptible genotype. We included eight replicates of each of the two treatments and repeated the experiment at 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 *X. fastidiosa* populations in the source plants and vectors using culturing and qPCR, respectively, assessed Pierce's disease symptoms in the source plants, and assessed transmission by culturing from *X. fastidiosa*-free test plants 3 months after the trials.

We estimated attraction rates and leaving rates of the BGSS by fitting data collected on the number of insects on each plant to the Consumer Movement Model described in Zeilinger et al. (2014). We used general linear models with quasi-Poisson or Poisson link function to test for differences in genotype (Resistant vs. Susceptible) and time since inoculation (3, 8, and 12 weeks) in *X. fastidiosa* populations in source plants, *X. fastidiosa* populations in vectors, and in PD symptom severity. For PD symptom severity, we used 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 *X. fastidiosa*.

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. 2A and 2B). Population densities of *X. fastidiosa* in source plants did not change significantly over time but were significantly greater in Susceptible source plants than Resistant source plants (Z = 3.07, P = 0.004; Fig. 1C). PD symptom severity in source plants increased significantly over time (Z = 4.97, P < 0.0001) and were significantly greater for Susceptible source plants than Resistant source plants (Z = 2.59, P = 0.013; Fig. 1D). Mean density of *X. fastidiosa* in vectors decreased over time (week main effect: Z = -2.04, P = 0.042) and showed a significant interaction between time since inoculation and genotype (Z = 3.013, P = 0.003; Fig. 1E). However, the proportion of vectors infected per cage showed no difference over time (Z = -1.667, P = 0.096) or between genotypes (Z = -1.094, P = 0.274; data not shown). Finally, the percent of test plants infected with *X. fastidiosa* did not differ between genotypes but significantly declined over time (Z = -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 *PdR1* 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. In other words, our results suggest that the decline in transmission from the Resistant and Susceptible lines likely occurred for different reasons.

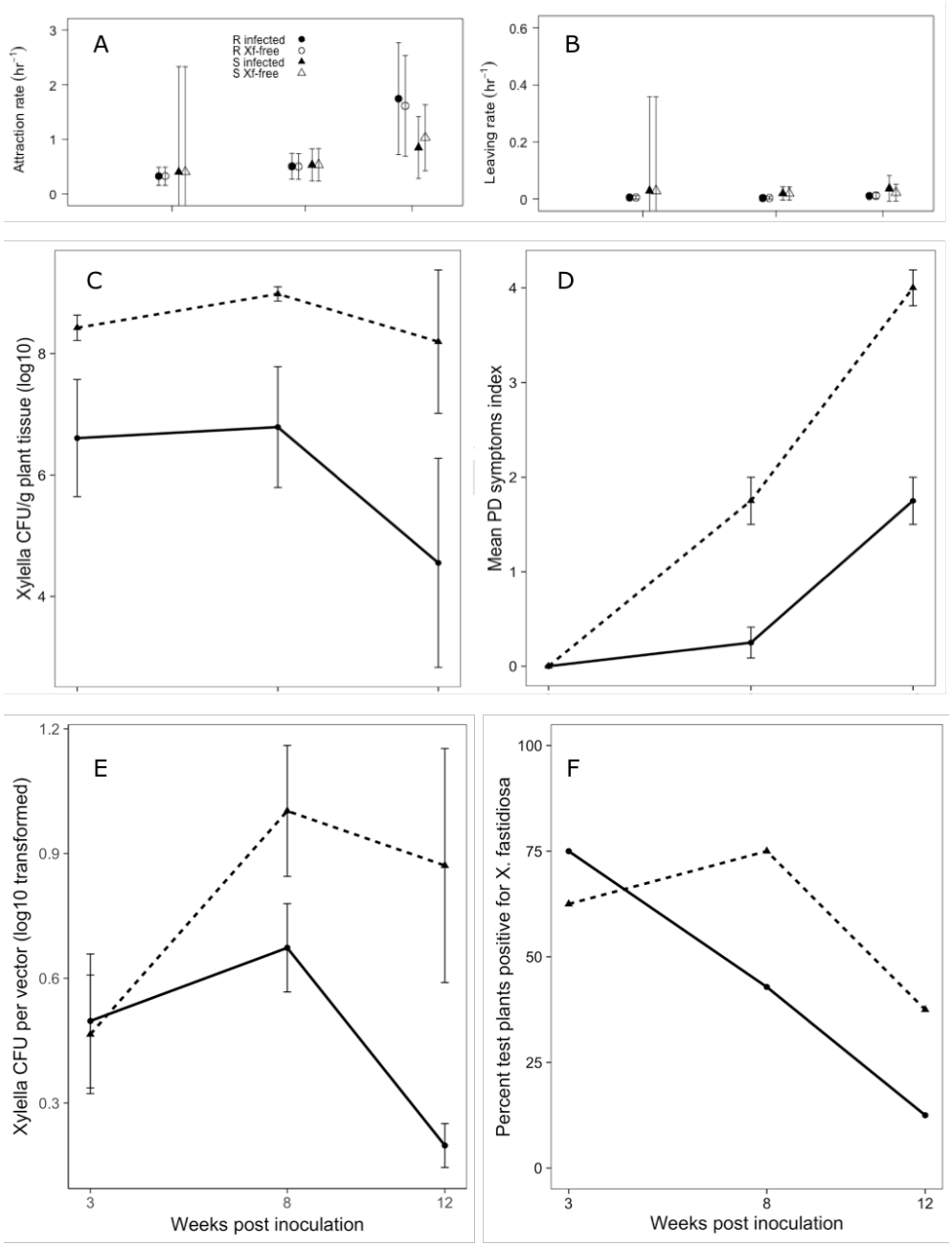


Figure 2. Attraction rates (A) were significantly greater for infected *PdR1* Resistant plants (filled circles) than infected Susceptible plants (open circles) at 12 weeks post-inoculation; leaving rates (B) were significantly greater from infected Susceptible plants than from infected Resistant plants. Mean *X. fastidiosa* population in infected plants (C) and mean PD symptom severity (D) were greater for Susceptible plants (dashed line) than Resistant plants (solid line). Mean density of *X. fastidiosa* in vectors showed a significant decline over time and interaction with genotype (E). Percent transmission, or percent Xf-free test plants testing positive for *X. fastidiosa* (F) was not significantly different between genotypes but significantly declined over time. Error bars in A and B represent 95% confidence intervals; error bars in C, D, and E represent ±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 (see Box 1 below).

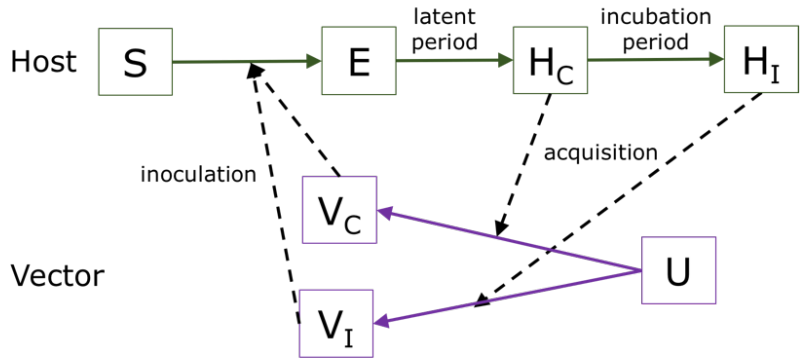
From our experimental results and our epidemic modeling, it appears that the *PdR1* gene confers a complex mix of tolerance and resistance to the hybrid plants, and that the nature of the defensive trait depends on the progress of the disease. Further study of vector transmission and disease progression 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 UCCE advisors and vineyard managers. However, because of the unexpected 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. We expect to present our results to grower groups during winter 2017-2018.

Conclusions

While grapevines expressing *rpfF* and *PdR1* can clearly alleviate Pierce's disease symptoms in infected plants, our results indicate that their effects on vector transmission and pathogen spread are not as clear. For *rpfF* plants, we found reduced transmission rates by BGSS vectors, but not significantly so. For *PdR1* plants, we found high vector transmission rates soon after inoculation but a significant decline in resistant plants after about three months of infection, suggesting a delayed, induced form of resistance conferred by the *PdR1* dominant locus. In both cases, we found some evidence of an interactive effect between genotype—resistant vs. susceptible—and the progression of disease—whether measured as distance from inoculation point or time since inoculation. Our modeling work suggests that *PdR1* confers a complex mix of resistance and tolerance traits. Most importantly, we predict that vector infection prevalence will be high in *PdR1* vineyards but that PD prevalence will be significantly reduced relative to susceptible vineyards. Additionally, our work with *PdR1* plants also highlights the importance of vector feeding preference for influencing transmission rates. Overall, our work indicates that *PdR1* grapevines show promise for improving the management of *X. fastidiosa*, but there remains significant risk of spreading *X. fastidiosa* through infectious vectors. *PdR1* resistant grapevines will need to be integrated carefully into more comprehensive disease management strategies. Additional work is necessary to better understand the impact of *rpfF* plants on *X. fastidiosa* epidemiology.



$$\frac{dS}{dt} = \eta(E + H_C + H_I) - \beta_c - \beta_I$$

$$\frac{dE}{dt} = \beta_C + \beta_I - \delta E - \eta E$$

$$\frac{dH_C}{dt} = \delta E - \gamma H_C - \eta H_C$$

$$\frac{dH_I}{dt} = \gamma H_C - \eta H_I$$

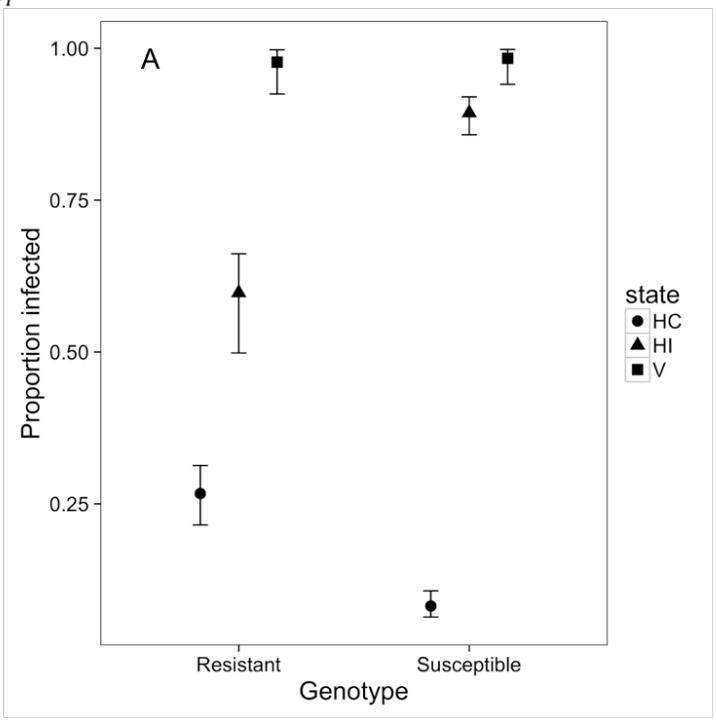
$$\frac{dU}{dt} = \lambda V_C + \lambda V_I - \alpha_C - \alpha_I$$

$$\frac{dV_C}{dt} = \alpha_C - \lambda V_C$$

$$\frac{dV_I}{dt} = \alpha_I - \lambda V_I$$

$$\beta_i = \frac{p_i(1 - e^{-b_i T})V_i S}{N}$$

$$\alpha_i = \frac{\mu_i(1 - e^{-a_i T})H_i U}{N}$$



Box 1. We modeled the spread of *X. fastidiosa* through simulated *PdR1* Resistant and Susceptible vineyards using a continuous-time SI-vector compartmental model. The model included compartments for non-infected hosts and vectors (S and U), exposed hosts (E), asymptomatic infected "Carrier" hosts (H_C), diseased infected hosts (H_I), and infectious vectors that acquired infection from either the H_C or H_I compartments (V_C and V_I). Inoculation and acquisition rates, β_i and α_i where i = C, I, were adapted from Madden et al. (2000). We used experimental data to estimate values for vector attraction rate (p_i), vector leaving rate (μ_i), inoculation probability (b_i), infectious period (δ^{-1}), incubation period (γ^{-1}), and host recover (η). Vector acquisition probability (a_i) was set as proportional to inoculation probability, pending data collection of *X. fastidiosa* populations in vectors. Vector recovery (λ) was set at 0.083. Time spent feeding (T) was calculated from Almeida and Backus (2004). $N = S + E + H_C + H_I$. Based on experimental results, estimates for i = C parameters were taken from 3-week trials while estimates for i = I parameters were taken from 12-week trials. We calculated standard errors for each experimentally-derived parameter and used Monte Carlo simulations (n = 5,000) to estimate mean and 95% confidence intervals for densities of infected hosts, H_C and H_I (filled circles and triangles, Panel A), and vectors, $V_C+V_I = V$ (filled squares), for *PdR1* Resistant and Susceptible vineyard scenarios. More detail and R code can be found at https://github.com/arzeilinger/pdr1_preference.

Publications and Presentations

Zeilinger, A.R., D. Beal, A. Sicard, M.P. Daugherty, M.A. Walker, R.P.P. Almeida 2017. Epidemiology of novel defensive traits against *Xylella fastidiosa* in grapevines. Hemiptera-Plant Interactions Symposium. Madrid, Spain. 5 June 2017.

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Status of Funds

Funds were used as proposed in submission. The project terminated at the end of the proposed period.

Status of Intellectual Property

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

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