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The epidemiology of novel *PdR1* resistant grapevines: epidemic and vector movement models to support integrated disease management

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Reporting Period

The results reported here are from work conducted 4 October 2018 to 15 March 2019.

Introduction

Resistant cultivars of agricultural crops are integral to sustainable integrated disease management strategies. Our previous work indicated that grapevines expressing the *PdR1* gene exhibit resistance against *Xylella fastidiosa*, and are likely to slow the spread of *X. fastidiosa* among vineyards. In the current project, we are testing the generality of our previous results, by testing multiple *PdR1* resistant and susceptible genotypes into our vector transmission experiments and integrating greater biological detail into our epidemic modeling work. Our preliminary experimental results suggest that vector transmission from *PdR1* grapevines follows our theoretical predictions and exhibits non-linear dynamics. Specifically, while *PdR1* resistant grapevines provide promising resistance, under some conditions, we see greater transmission rates from *PdR1* resistant vines than from susceptible vines. This may be caused by an interaction between the resistance trait and vector feeding preference. These results, while preliminary, complicate integration of *PdR1* grapevines into Pierce's disease management strategies for growers. Moreover, growers may be able to benefit from *PdR1* resistant cultivars without planting all of their acreage to them. We are exploring tradeoffs between disease resistance and economic profit of *PdR1* plants through bio-economic modeling, with the ultimate goal of developing management recommendations for the optimal planting of *PdR1* grapevines. Finally, our modeling efforts rely on assumptions on insect vector dispersal within and among vineyards; yet our knowledge of sharpshooter dispersal has been limited by the difficulty of experimentally measuring dispersal. We are developing a spatio-temporal stochastic epidemic model that integrates spread of Pierce's disease and dispersal of the vector *Graphocephala atropunctata* in Napa and Sonoma vineyards. Taken together, our project will provide clearer recommendations for disease management strategies using *PdR1* and related resistant grapevines.

Objectives

The overall goal of this project is to assess the epidemiological consequences of managing Pierce's Disease (PD) with resistant grapevines expressing the *PdR1* locus (Walker and Tenscher 2016). Specifically, we ask, under what ecological conditions and spatial arrangements will the use of *PdR1* vines reduce *X. fastidiosa* spread and maximize economic benefits to growers? The research consists of three objectives:

1. *Test the effects of* PdR1 *resistant plants on vector feeding preference and transmission of* X. fastidiosa
2. *Model the optimal mixture of* PdR1 *and susceptible grapevines to reduce* X. fastidiosa *spread and maximize economic return*
3. *Estimate dispersal of insect vectors from field population data*

Results and Discussion

1. Test the effects of PdR1 *resistant plants on vector feeding preference and transmission of* X. fastidiosa

In 2017, we investigated the interplay between vector feeding preference and transmission of *X. fastidiosa* from *PdR1* resistant and susceptible grapevine genotypes. We inoculated two *PdR1* resistant genotypes (labeled 094 and 102) and two susceptible genotypes (007 and 092) with *X. fastidiosa* STL strain. At 2, 5, 8, and 14 weeks post-inoculation, we introduced eight blue-green sharpshooters (BGSS, *Graphocephala atropunctata*) into a cage with one inoculated plant (from one of the four genotypes) and one *Xylella*-free test plant, of either susceptible genotype. We included eight replicates of each combination of week since inoculation and genotype, and each replicate was independent—using different plants and vectors in each trial. We recorded which plant the vectors were feeding on at regular intervals over a 4-day period, estimated *Xylella* populations in the source plants using culturing, assessed Pierce's disease symptoms in the source plants, and assessed transmission by culturing from *Xylella*-free test plants 3 months after the trials. We are in the process of estimating *Xylella* populations in vectors using qPCR.

In previous Progress Reports, we reported on the effects of genotype (Resistant or Susceptible) and week post-inoculation on PD symptom severity, *Xylella* populations in source plants, feeding preference of vectors, and infectiousness of vectors, and transmission rate to test plants. We showed that transmission rate—the most important dimension of our experiment—exhibit non-linear dynamics with respect to week post-inoculation and that these dynamics differed between genotypes. Here we report results on explaining this variation in transmission rates. We combined all data from the experiment relevant to transmission and conducted an analysis using the elastic net algorithm. Elastic net is a form of statistical regularization, in which two penalty terms are added to a linear regression model (James et al. 2013; Hooten and Hobbs 2015). The elastic net is a preferred statistical method when covariates covary with each other as well as with the response variable, which we expected *a priori*. While elastic net does not calculate p-values, it reduces the coefficient estimates of unimportant covariates to zero. As such it acts as a model selection or variable selection routine; all non-zero coefficients can be interpreted as being important variables to some degree, with larger coefficient estimates being the most important.

The results of the elastic net analysis for both experiments conducted in 2016 and 2017 correspond closely (Fig. 1). The most important factor explaining whether *Xylella*-free test plants became infected (i.e., probability of transmission) was the proportion of vectors infectious with *Xylella* (Fig. 1). In both years, probability of transmission was positively associated with proportion of infectious vectors. Source plant genotype, i.e., whether the source plant was Resistant or Susceptible cultivar, had diverging effects between the two years. Transmission was much more likely from Susceptible genotypes in 2016, whereas in 2017 transmission was slightly more likely from Resistant genotypes (Fig. 1). Finally, in both years, probability was negatively associated with vector leaving rate from test plants. Interestingly, this was the most important component of vector preference for explaining transmission.

Our finding that transmission is closely related to the proportion of infectious vectors is perhaps not surprising and is in line with previous research on transmission biology of BGSS vectors. While genotype had varying importance, our elastic net results corroborate our previous analyses suggesting that transmission dynamics associated with *PdR1* resistant grapevines are qualitatively different from more susceptible plants but, importantly, transmission from *PdR1* vines are neither consistently lower or greater than from susceptible

vines. Finally, our results provide a novel insight into the mechanisms underlying the importance of vector feeding preference for transmission. Our work builds on previous studies examining preference of sharpshooters by decomposing preference into its component parts, namely attraction and leaving rates. Our result that leaving rates from the test plant was the most important for driving transmission is novel and unexpected; previous studies have that visual stimuli are important for sharpshooter host selection, suggesting that attraction rates should be most important (Daugherty et al. 2011).

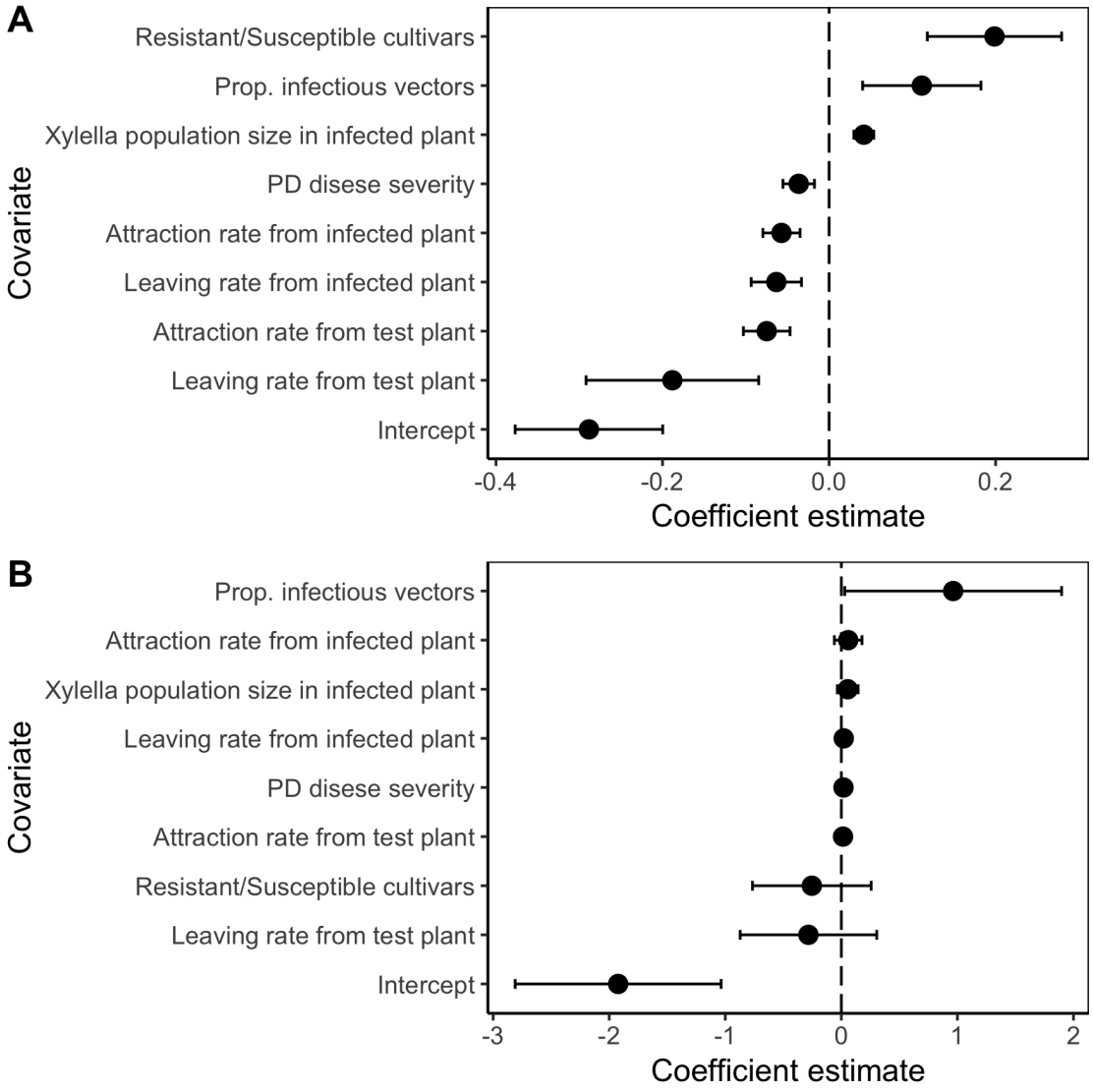

Figure 1. Coefficient estimates of the relationship between transmission-relevant covariates and infection status of *Xylella*-free test plant in *PdR1* transmission experiments conducted in (A) 2016 and (B) 2017. "Attraction rates" and "Leaving rates" are rates that BGSS vectors were either attracted to or left from infected source plants or uninfected test plants. "Prop. Infectious vectors" represents the proportion of vectors in each cage infectious with *Xylella* at the end of the trial. For "Resistant/Susceptible Cultivar", a positive coefficient estimate indicates that transmission was more likely from Susceptible genotypes, whereas the opposite is the case for negative estimates. Error bars represent ± standard error.

2. Model the optimal mixture of PdR1 *and susceptible grapevines to reduce* X. fastidiosa *spread and maximize economic return*

We have built a preliminary economic extension to our vector-SI epidemic model, described in our proposal. We consider a scenario where two vineyards are grown adjacent to each other—one composed of a grape cultivar susceptible to Pierce's Disease, Patch 1, and another composed of *PdR1* resistant grapevines, Patch 2. For the preliminary economic model, we followed the framework of Macpherson et al. (2017) and assumed that yield is proportional to the density of healthy or asymptomatic hosts at harvest time, *t*.

In our previous analyses of the bioeconomic model, we assumed that transmission was frequency-dependent. However, we have also explored a density-dependent form of transmission. Density-dependent transmission is generally thought to relate to pathogen systems where the pathogen spread beyond the immediate neighbors of an infected host is relatively common. As vector-borne pathogens often exhibit more frequent long-distance dispersals, adopting a density-dependent transmission term seems reasonable. Our model then takes the form:

$$H(\delta) = \delta L\left(1 - R_p\right) + I_A(t,\delta) + I_B(t,\delta)$$

$$\frac{dI_A}{dt} = \beta_A\left(\left(1-\delta\right)L - I_A\left(t,\delta\right)\right)\left(I_A\left(t,\delta\right) + \mu I_B\left(t,\delta\right) + \varepsilon\right)$$

$$\frac{dI_B}{dt} = \beta_B\left(\delta L - I_B\left(t,\delta\right)\right)\left(I_B\left(t,\delta\right) + \mu I_A\left(t,\delta\right) + \varepsilon\right)$$

Here, $H(\delta)$ represents monetary loss after harvest, as a function of the proportion of area planted to *PdR1* vines, δL, the value of *PdR1* grapes relative to susceptible grapes, $(1 - R_p)$, the density of diseased susceptible vines $I_A(t, \delta)$ and the density of diseased *PdR1* vines $I_B(t, \delta)$. These last two terms are then modeled dynamically and is modeled using density-dependent transmission. The within-patch transmission rate, β, differs between grape genotypes while we assume that cross-patch transmission, μ, and primary infection, ε, are equal between genotypes.

As a preliminary analysis, we investigated the optimal planting strategy depending on harvest time. In our simulations, we predict that growers who adopt a more short-term strategy—meaning that they are primarily focused on maximizing returns in a shorter time frame—should not plant *PdR1* vines but should only plant susceptible vines. However, growers who adopt a more long-term strategy should plant a mixture of *PdR1* and susceptible vines. Generally, the longer the harvest time, the greater the area that growers should plant to *PdR1* vines. These results should clearly depend on the epidemiological conditions experienced by growers. We are in the process of exploring how different epidemiological conditions, motivated by our experimental results, will change optimal planting mixtures as well.

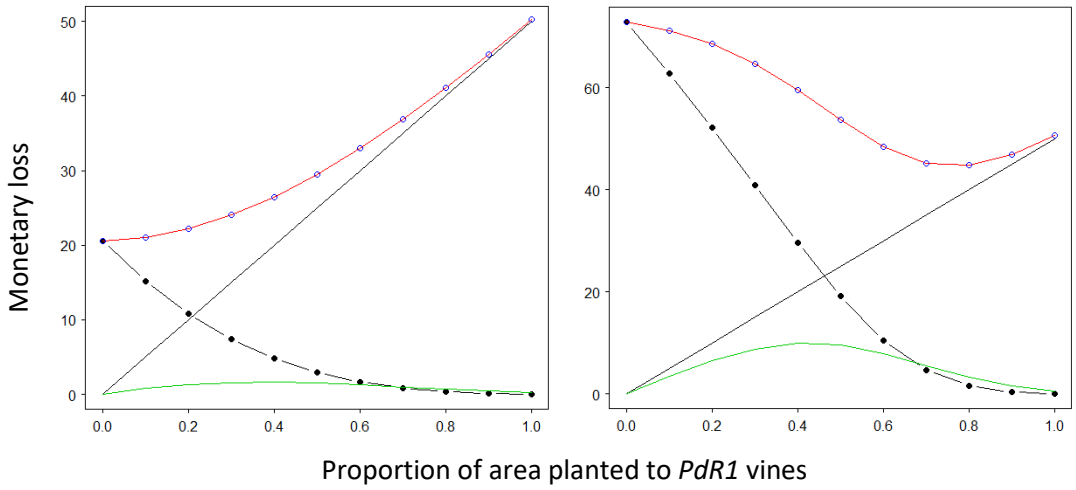

Figure 2. Optimal mixture of susceptible and *PdR1* vines corresponds to the minimum total monetary loss, represented by the red line. Black solid line: loss due to planting. Dashed line: loss due to infection for susceptible vines. Green line: loss due to infection for *PdR1* vines. Red line: total loss (blue points are calculated by subtracting a profit calculated from the final number of healthy plants from the maximum possible profit). Left panel: harvest at t = 50 time steps, right panel: harvest at t = 80 time steps.

3. Estimate dispersal of insect vectors from field population data

We are expanding our original vision of the vector dispersal models, and focusing our efforts on modeling dispersal and transmission by BGSS vectors. We are using data collected by our research group and colleagues of disease surveys and BGSS abundance in ~30 commercial vineyard sites across Napa and Sonoma from 2016 – 2018. We are currently working to fit a spatiotemporal stochastic epidemic model developed by Adrakey et al. (2017) to our Pierce's disease survey data. The model estimates three epidemiologically relevant parameters: primary infection rate, vine-to-vine transmission, and pathogen dispersal. In any vector-borne disease system, pathogen dispersal should be a function of vector dispersal. We plan to extend the model of Adrakey et al. to explicitly incorporate vector dispersal in the model of pathogen dispersal. From this work, we will be able to estimate vector dispersal directly from field data as well as the relationship between vector dispersal and pathogen spread. We will also be able to estimate the relative importance of vine-to-vine spread and primary infection rate, which Pierce's disease scholars have been debating for some time.

Layperson Statement of Relevance

Overall, our results confirm previous work in that *PdR1* resistant plants exhibit partial resistance to *X. fastidiosa*, resulting in reduced bacterial populations and reduced PD symptom severity. However, because *X. fastidiosa* is able to reach moderate population sizes in resistant plants, there is still significant vector transmission from these plants. Importantly, because of reduced symptom severity and vector feeding preference for healthy grapevines, transmission from resistant plants can be greater under some conditions. These results suggest that there may be a window of time where *PdR1* grapevines could act as reservoir hosts, amplifying vector transmission.

A critical question remains, under what ecological conditions, and for how long, could *PdR1* vines amplify transmission? We are working to address this question through epidemiological modeling. We also are working to describe conditions under which different mixtures of *PdR1* resistant and susceptible grapevines would maximize economic return for growers. So far, our bioeconomic modeling work suggests that a mixture of grapevines, with relatively more *PdR1* than susceptible would be optimal when 1) long-distance dispersal (but still within a vineyard) occurs and 2) when growers are interested in maximizing long-

term gain. Overall, while there is some concern that *PdR1* vines could enhance *X. fastidiosa* spread in the field, our results suggest that these partially resistant vines hold promise to greatly improve Pierce's disease management. The key question remains to develop strategies to optimize their use in vineyards under a variety of realistic conditions.

Publications and presentation

Zeilinger, A.R., D. Beal, A. Sicard, M.P. Daugherty, M.A. Walker, R.P.P. Almeida. Host defense and vector preference drive non-linear transmission dynamics for a plant pathogen. Bay Area Ecology and Evolution of Infectious Disease, Stanford University, Palo Alto, CA. 2 March 2019.

Status of funds

Funds are being spent as planned, and will finish just before the project's end date.

Intellectual Property

No intellectual property has been generated from this project.

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