

MODELING SHARPSHOOTER TRANSMISSION OF *XYLELLA FASTIDIOSA*

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ABSTRACT

The dynamics of vectored diseases are governed by the interplay of a variety of biotic and abiotic factors. We studied some of these factors that are expected to be important to the epidemiology of Pierce's disease (PD) in California vineyards. We conducted a series of greenhouse transmission experiments to quantify how sharpshooter species, sharpshooter number, plant inoculation access period, and temperature affect transmission of *Xylella fastidiosa* (*Xf*) to grapes and PD symptom development. For the blue-green sharpshooter (BGSS), vector number and inoculation period had similar strong effects on infection rate. Interestingly, larger numbers and longer inoculation periods increased the onset of PD symptoms. However, while the number of glassy-winged sharpshooters (GWSS) increased infection rate, inoculation access period did not. In a final experiment looking at the relationship between temperature and transmission efficiency, high temperature (30°C) resulted in low BGSS survival yet marginally higher infection rate. Moreover, *Xf* population in vines was positively related to the number of vectors that tested positive for *Xf* using realtime PCR at the time of plant inoculation. These results suggest that high sharpshooter numbers may not only increase transmission rate but also decrease incubation period – presumably because of a larger inoculum. We expect that high vector densities and temperatures will increase the rate of disease cycling, which is particularly relevant to disease prevalence in this system.

INTRODUCTION

Pierce's disease (PD) epidemiology is complicated by the potential for several insects to vector *Xylella fastidiosa* (*Xf*) (Severin 1949), by having many alternative reservoirs of the pathogen (Wistrom and Purcell 2005), by *Xf* strain- or grape cultivar-specific differences in pathogenicity or resistance, and by seasonal acquisition and vine recovery (Hill and Hashim 2006). These factors likely contribute to variability in disease spread, hampering efforts to quantitatively describe disease dynamics in this system.

An understanding of transmission biology is critical to predicting PD epidemiology (Almeida et al. 2005). Fortunately experiments on vector transmission have been a major focus of PD research for over 50 years – contributing a vast amount of biological data on important vectors and their ability to transmit *Xf* (Severin 1949, Purcell 1981, Hill and Purcell 1995, 1997, Almeida et al. 2005). None-the-less little effort has been made to synthesize this work into a quantitative framework. To date the only quantitative description of *Xf* transmission is that of Purcell (1981). This statistical model predicts the probability a plant becomes infected (**P**) as a function of vector number (**n**), inoculation access period (**t**), vector infectivity (**i**), and inoculation efficiency (**E**): $P_{nt} = 1 - e^{-nEt}$. Two aspects of this model are important to note. First, any environmental (e.g., temperature, humidity) or ecological factors (e.g., vector species, pathogen virulence, plant abundance) that affect vector or pathogen abundance, vector feeding behavior, or efficiency will influence transmission. Second, the model assumes that vector number and inoculation access period are algebraically equivalent. In other words, 10 vectors for one day should result in the same infection rate as one vector for 10 days.

Our goal was to test and further refine this model via experimental estimation of the parameters explicitly included in this model as well as certain other ecological or environmental factors which may influence transmission of *Xf* to grapes. To this end, we first tested the assumption that vector number and inoculation period have equivalent effects on transmission rate. We also tested for effects of ambient temperature on transmission efficiency, as sharpshooter survival, growth, and feeding rate depend on temperature (Johnson et al. 2006). These two experiments were conducted with two important vectors of *Xf*, the BGSS; *Graphocephala atropunctata* and the GWSS; *Homalodisca vitripennis* to compare species-specific differences. Finally, in order to better understand how these factors ultimately contribute to disease dynamics, we tracked symptom development and infection level in vines after insect transmission occurred.

OBJECTIVES

1. Decouple estimated effects of sharpshooter number and inoculation period on transmission.
2. Quantify effects of temperature on transmission efficiency.
3. Link transmission to disease progression in the plant.

MATERIALS AND METHODS

In the fall of 2006 we conducted two greenhouse transmission experiments that varied independently sharpshooter species, number, and inoculation access period (IAP). In the first experiment we confined *Xf*-free BGSS adults on infected (STL strain) grapevines for a four day AAP. We then caged one, two, or four of these BGSS on a healthy grape seedling (var. Cabernet Sauvignon) for half, one, two, or four days. There were at least nine replicate plants for each of the 12 bug number-IAP treatment combinations. Beginning one month later we visually inspected plants one to two times per week to determine when they first showed PD symptoms. After three months, seedling infection status was determined by culturing (Hill and Purcell 1995). We compared the onset of symptoms among treatments using a Cox proportional hazards survival analysis with bug number and IAP as continuous variates. We followed up significant effects with a logistic regression of IAP and bug number versus plant infection status.

Approximately one month later, we repeated this experiment with GWSS. *Xf*-free GWSS adults from a Riverside, CA colony were placed on *Xf*-infected (Temecula strain) grapevines for four days (AAP). Afterwards one, two, or four bugs were transferred to a healthy grape seedling for half, one, two, or four days. There were at least five replicate plants for each treatment combination. We did not inspect plants for PD symptoms, but after three months they were all cultured for the presence of *Xf*. We used logistic regression to quantify the effect of bug number and IAP on plant infection status.

In the summer of 2007 we conducted a third transmission experiment that varied BGSS number and ambient temperature. We caged BGSS adults on known *Xf*-infected source plants (STL strain) for four days (AAP), after which we caged either one or four of these BGSS on healthy grape cuttings. These insects and vines were then placed in one of three temperature controlled rooms at UC Berkeley's Jane Gray Research Greenhouse that were set to approximately 20, 25, or 30°C during the day and 20°C at night. The rooms received natural light filtered through a shade cloth, plus grow lights on a 12:12 (L:D) photoperiod. There were at least 10 replicates of each treatment. After two days (IAP) we removed bugs and froze them. Three, six, and nine weeks later we sampled randomly two petioles from each plant, and at 10 weeks we sampled the stem at the top of where the sleeve cage was. We quantified *Xf* populations in stems and petioles using realtime PCR (qPCR; Francis et al. 2006), and qPCR was used on each sharpshooter to estimate the total *Xf* inoculum potentially introduced into each vine. We analyzed effects of bug number and temperature on disease status using logistic regression. For all positive plants, we used multiple regressions to quantify the effects of temperature, number of positive bugs, and total inoculum on stem infection level.

RESULTS

For BGSS, both vector number and IAP were strongly related to infection rate. IAP had a slightly stronger effect (regression coefficient = 0.5718, SE=0.1857) than did bug number (0.3963, SE=0.1785), though not significantly ($P=0.4730$). Both variables also increased the onset of first PD symptoms (Figure 1). Conversely, GWSS was not only less efficient over-all (36% infection vs. 64% for BGSS), but there were substantially different effects of bug number and IAP. Number of GWSS strongly increased vine infection rate, while IAP did not (Figure 2).

Temperatures in the third experiment averaged 24.3, 21.4, and 17.4°C. Higher BGSS number increased infection rate and temperature had a marginally significant effect (Figure 3). The proportion of plants infected was the highest in the four BGSS-high temperature treatment, despite substantially higher sharpshooter mortality at this temperature (45% at 24.3, 5% at 21.4, and 20% at 17.4°C). Few petioles tested positive at weeks three and six, but several were positive by week nine – with

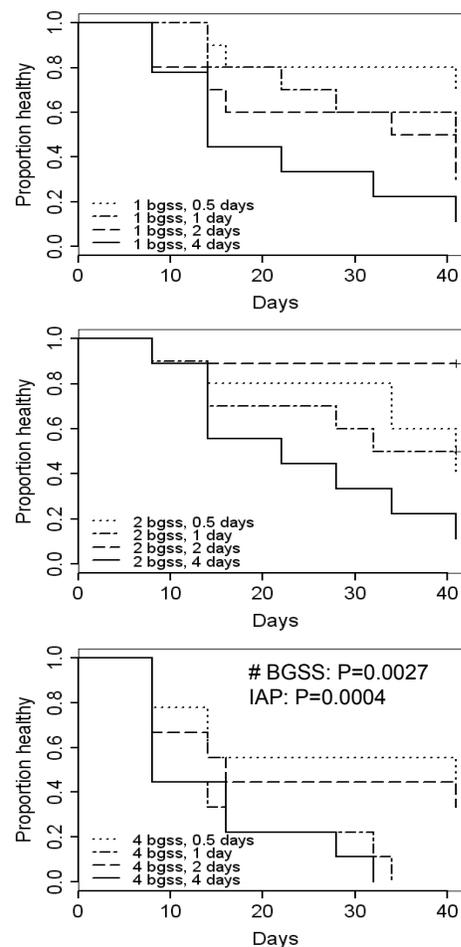


Figure 1. PD symptom onset as a function of BGSS number and inoculation period. Day 0 equates to approximately one month post inoculation.

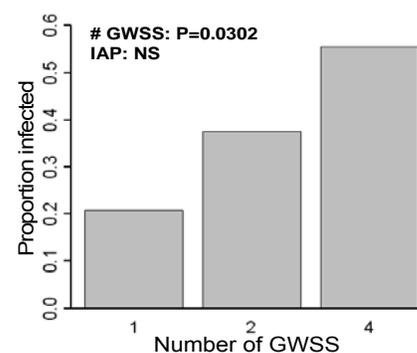


Figure 2. Infection rate increased with number of GWSS, while inoculation access period had no effect.

generally increasing trends in infection level through time. *Xf* population in the stems (excluding uninfected vines) was not significantly affected by temperature or total *Xf* from bugs, but there was a positive effect of the number of sharpshooters that tested positive for *Xf* from qPCR analyses (Figure 4).

DISCUSSION

Our first goal was to quantify the independent contributions of vector abundance and inoculation period on transmission rate, which is necessary to begin to parameterize models such as that of Purcell (1981). BGSS is clearly a more efficient vector of *Xf* than is GWSS, though the mechanism is not known. It may relate to feeding site preference or probing behavior. Regardless, our results suggest that sharpshooter species may differ in the relative strengths with which vector number and inoculation period contribute to transmission. Vector number and inoculation period appear to have equivalent effects on BGSS transmission, whereas IAP was weakly related to GWSS transmission (Figures 1, 2). This result suggests that GWSS inoculation, if it occurs, occurs soon after encountering the healthy plant (e.g., within 1 day) – a conclusion that is inconsistent with previous transmission experiments (Almeida and Purcell 2003).

In conjunction with these two experiments we are compiling the results of prior BGSS transmission experiments to conduct a meta-analysis on the role vector number, acquisition period, and inoculation period play in determining transmission of this efficient *Xf* vector. This work will provide more statistical power to precisely evaluate the fit of Purcell's (1981) transmission model.

The second set of results we collected relate to the effect of temperature on sharpshooter transmission efficiency. A study with GWSS indicated that its survivorship and feeding rates are an increasing function of temperature up to a point (maximum approximately at 30°C), above which mortality greatly increases and feeding shuts down (Johnson et al. 2006). Therefore, if transmission is related to feeding rate, sharpshooter transmission efficiency is expected to also be an increasing function of temperature. In our experiment, BGSS transmission was higher at the warmest temperature (mean=24.3°C) despite substantially higher sharpshooter mortality at this temperature (Figure 3). These results are consistent with the hypothesis that stressed sharpshooters may adjust feeding behavior, thereby affecting transmission rates. Testing this hypothesis would require estimates of BGSS feeding rates, movement on the plant, and probing behavior over a range of temperatures. We are currently conducting a similar experiment with GWSS to determine how temperature affects its transmission efficiency.

The third set of results we collected relate to the link between transmission and disease progression in the plant. In the first experiment grape seedlings with more BGSS or with longer inoculation periods had shorter incubation periods. We attribute this result to the higher sharpshooter loads favoring the introduction of larger *Xf* inoculum. In the last experiment we measured infectivity of individual bugs using realtime PCR. This provided us with two related metrics of potential *Xf* inoculum for each replicate – the number of positive insects that tested positive, and the total estimated *Xf* population among insects on a given plant. Quantitative measurements of *Xf* populations in insects were highly variable and therefore a poor predictor of the infection level in the plant. However, the number of positive insects was positively related to plant infection level (Figure 4). Together these results support the prediction that high vector loads increase PD onset because of larger initial inoculum. Based on previous research showing that temperature affects *Xf* growth (Feil and Purcell 2001), we expected to see higher *Xf* populations in infected vines at higher temperature. The weak affect of temperature on plant infection levels is probably the result of relatively low sample sizes of infected plants in all but the highest temperature, high bug number treatment.

These experiments quantitatively describe the effects of sharpshooter abundance, inoculation period, sharpshooter species, and temperature on transmission of *Xf* to grapes. The experiments also describe the consequence of these factors on disease progression in the plant. Collectively these results, along with other field and lab experiments, allow us to begin to link the transmission biology of *Xf* to the epidemiology of PD. Our ultimate goal is to begin to describe quantitatively how these

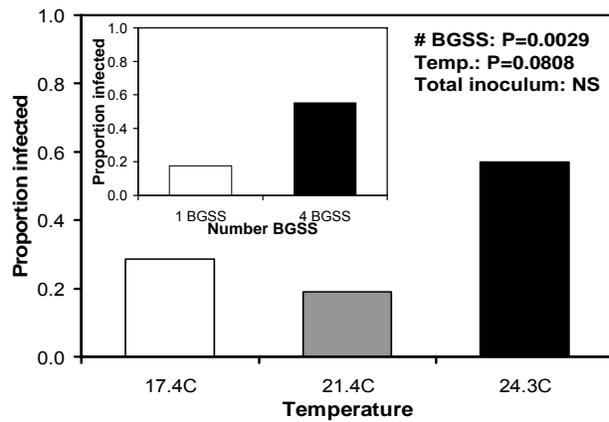


Figure 3. Infection rate as a function of temperature and BGSS number (inset).

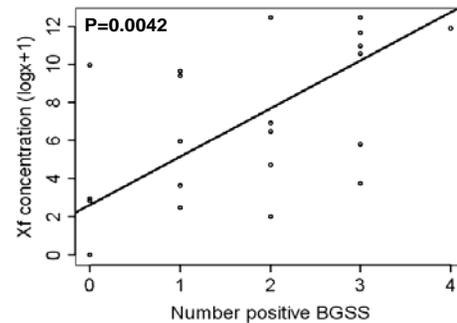
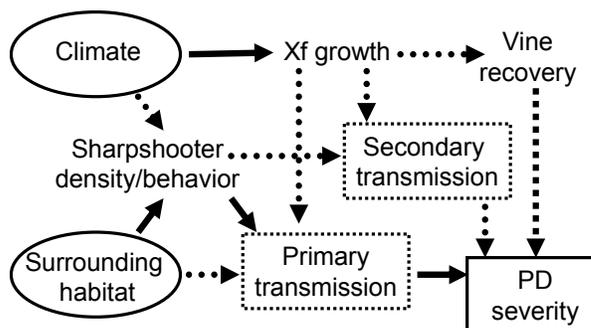


Figure 4. *Xf* population in the stems increased as a function of the number of BGSS identified as positive by qPCR

environmental and ecological factors that affect transmission interact with other elements of PD epidemiology to predict disease dynamics in different regions of California (Figure 5). We expect that high vector loads and warmer temperatures will increase the rate of disease cycling by shortening the time required for *Xf* populations in the plant to build up to the level where efficient re-acquisition can occur. The ultimate consequence of this would be to increase the likelihood of secondary spread, especially by the GWSS, and therefore increased PD severity.

Figure 5. Conceptual model for how climate and surrounding habitat drive PD severity in CA vineyards. Established links are denoted by solid arrows. Dashed arrows denote hypothesized effects that require more study.



FUNDING AGENCIES

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REFERENCES

- Almeida, R.P.P., Blua, M.J., Lopes, J.R.S. and Purcell, A.H. 2005. Vector transmission of *Xylella fastidiosa*: Applying fundamental knowledge to generate disease management strategies. *Annals of the Entomological Society of America* 98: 775-786.
- Almeida, R. P. P. and Purcell, A.H. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). *Journal of Economic Entomology* 96: 264-271.
- Feil, H. and Purcell, A.H. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. *Plant Disease* 85:1230-1234.
- Francis, M., Lin, H., Cabrera-La Rosa, J., Doddapaneni, H. and Civerolo, E. 2006. Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*. *European Journal of Plant Pathology* 115: 203-213.
- Hill, B. L. and Hashim, J. 2004. Epidemiological assessments of Pierce's disease and monitoring and control measures for Pierce's disease in Kern County. pp. 260-263 In: *Proceedings of the Pierce's Disease Research Symposium*. California Department of Food and Agriculture, Sacramento, CA.
- Hill, B. L. and Purcell, A.H. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. *Phytopathology* 85: 209-212.
- Hill, B.L. and Purcell, A.H. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. *Phytopathology* 87: 1197-1201.
- Purcell, S. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce' disease in European grape *Vitis vinifera* cultivars. *Phytopathology* 71:429-435.
- Severin, H.H.P. 1949. Transmission of the virus of Pierce's disease of grapevines by leafhoppers. *Hilgardia* 19:190-206.
- Wistrom, C. and Purcell, A.H. 2005. The fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts in California. *Plant Disease* 89:994-999.