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Estimating *Xylella fastidiosa* transmission parameters: decoupling sharpshooter number and feeding period

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Abstract

Compared to human- and wildlife-transmitted pathogens, less emphasis has been placed on developing models of plant pathogen transmission by insects. Here, we describe the transmission ecology of the bacterium *Xylella fastidiosa* Wells et al., the causal agent of Pierce's disease in grapevines, by its leafhopper vectors. First, we performed a meta-analysis of transmission studies of *X. fastidiosa* by its two most important vectors in the Western USA, the invasive glassy-winged sharpshooter, *Homalodisca vitripennis* Germar, and the native blue-green sharpshooter, *Graphocephala atropunctata* Signoret (both Hemiptera: Cicadellidae). The importance of vector number, pathogen acquisition period, and inoculation access period (IAP) for transmission differed between the two species. We fit these transmission datasets to two biologically derived transmission models, i.e., a binomial and a Poisson probability model. The Poisson model provided substantially better fit and produced estimates of *H. vitripennis* transmission efficiency that were dramatically lower than for *G. atropunctata*. We also conducted a separate pair of experiments that decoupled vector number from IAP. These experiments supported the results of the meta-analysis. Interestingly, high vector loads not only increased transmission rate, but also shortened *X. fastidiosa* incubation period in grapevines. This work provides quantitative estimates of transmission of an economically important pathogen that is analogous to risk models for arthropod-vectored human and wildlife diseases. In addition, this work suggests that heterogeneous vector loads may accelerate the disease cycle, increasing the potential for secondary spread in vineyards.

Introduction

Numerous ecological contingencies exist for the dynamics of vector-borne pathogens, stemming from interactions among pathogens, vectors, and hosts (e.g., Figure 1 in Irwin & Thresh, 1990). Nonetheless, underlying all aspects of a pathosystem is the transmission process, which is a fundamental determinant of epidemiology (Jeger et al., 1998; Ng & Perry, 2004), and for which a precise understanding is required for effective disease management (Almeida et al., 2005). Because of the importance of transmission for disease dynamics, much effort has been expended to document aspects of the transmission process, including descriptions of vector biting rate (Kilpatrick et al., 2006), the latent period of infections (Purcell & Finlay, 1979), and the competence (i.e., transmission efficiency) of various vector species (Palermo et al., 2001). These studies are critical for parameterizing models of disease dynamics (Jeger et al., 1998).

Studies of transmission efficiency, especially, are also valuable for quantifying disease risk metrics, such as the 'entomologic inoculation rate' for malaria (Killeen et al., 2000), or risk-assessment measures for West Nile Virus (Kilpatrick et al., 2006). These 'snapshot' methods seek to relate vector abundance, feeding behavior, and infectivity to a relative measure of risk of transmission to a focal host. Such methods have been used widely to provide quantitative measures of the probability of transmission to humans among locales or sampling periods. Much less effort has been devoted to developing a quantitative framework for describing vector transmission of plant pathogens,

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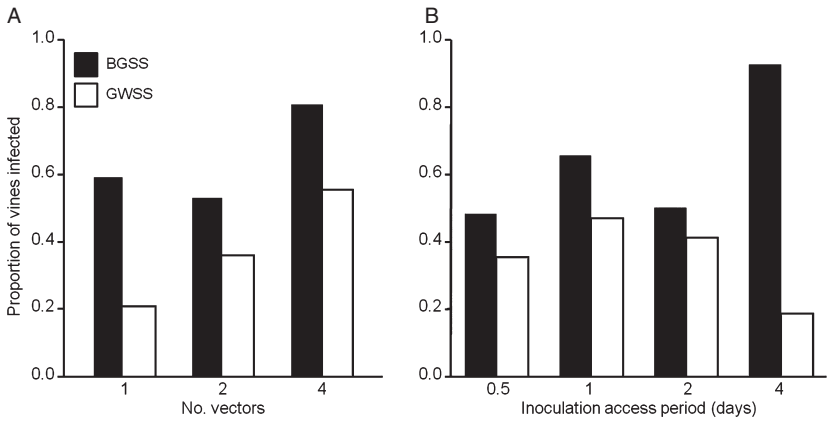

Figure 1 *Homalodisca vitripennis* (GWSS) and *Graphocephala atropunctata* (BGSS) transmission rate (proportion of vines infected) in the two greenhouse experiments among (A) vector number and (B) inoculation access period treatments. BGSS was consistently more efficient.

especially considering the large number of studies that have investigated aspects of arthropod efficiency in transmitting plant viruses (e.g., Irwin & Thresh, 1990; Ng & Perry, 2004) and bacteria (e.g., Severin, 1949; Purcell & Finlay, 1979; Palermo et al., 2001; Almeida & Purcell, 2003). Here we describe efforts to quantify the transmission of an important plant pathogen by its leafhopper vectors.

The bacterial pathogen *Xylella fastidiosa* Wells et al. is endemic to the Americas, and is widespread throughout the western and southeastern USA. This xylem-limited bacterium is pathogenic to numerous agricultural crops, such as alfalfa, almond, citrus, coffee, peach, plum, and grapes, as well as forest tree species, ornamentals, and weeds (Hopkins & Purcell, 2002; Wistrom & Purcell, 2005). In grapevines [*Vitis vinifera* L. (Vitaceae)], *X. fastidiosa* causes Pierce's disease, which results in leaf scorch symptoms, crop loss, and eventual death of infected vines.

Vectors of *X. fastidiosa* in the western USA include several species of xylem sap-feeding insects, the most important being sharpshooters (Hemiptera: Cicadellidae: Cicadellinae; Severin, 1949). The dominant native vector of *X. fastidiosa* in coastal California is the blue-green sharpshooter (*Graphocephala atropunctata* Signoret). This leafhopper uses riparian plants for breeding and overwintering, therefore Pierce's disease outbreaks in this region follow a pattern of primary spread, with infectious adult vectors inoculating grapevines with *X. fastidiosa* along vineyard margins in the spring as they move inward from surrounding vegetation (Purcell, 1975). The invasion and spread of the non-native glassy-winged sharpshooter (*Homalodisca vitripennis* Germar) into southern California altered dramatically Pierce's disease incidence and dynamics (Hopkins & Purcell, 2002). *Homalodisca vitripennis*-associated Pierce's disease outbreaks occurred in Temecula valley vineyards in the 1990s, then a few years later near Bakersfield (Hill & Hashim, 2004). The close association of citrus orchards with vineyards in both of these outbreaks, and the large numbers of *H. vitripennis* overwintering on citrus during the winter, has been hypothesized to be driving the current California Pierce's disease epidemic (Perring et al., 2001).

Over the last 70 years, substantial effort has been devoted to understanding the transmission biology of *X. fastidiosa* and its vectors in vineyards. Transmission of *X. fastidiosa* is limited to xylem sap-feeding insects, but within this group there is no clear evidence of *X. fastidiosa* specificity (Purcell, 1989). There is also no evidence of transovarial transmission (Freitag, 1951) or a latent period (Purcell & Finlay, 1979). Vectors acquire *X. fastidiosa* from tapping into infected xylem vessels. Microscopy studies suggest that *X. fastidiosa* attaches to and replicates in a specific region of the insect foregut, the precibarium (Almeida & Purcell, 2006). *Xylella fastidiosa* colonization of this region is strongly associated with successful transmission events. Because nymphs shed the foregut cuticle at each molt they lose infectivity, whereas adult sharpshooters persistently maintain the bacterium (Purcell & Finlay, 1979).

Purcell (1981) proposed a quantitative description of sharpshooter–*X. fastidiosa* transmission ecology, using a pair of general transmission models that are based on either the binomial or Poisson distributions. These models sought to predict the probability of transmission as a function of vector abundance, inoculation efficiency, and the time period over which transmission may occur. The efficiency with which sharpshooters acquire the bacterium is dependent on the pathogen population in acquisition plants (Hill & Purcell, 1997) and the length of the

acquisition period (Purcell & Finlay, 1979; Almeida & Purcell, 2003) – low population and short feeding periods reduce the probability of acquiring *X. fastidiosa*. Evidence also indicates that inoculation efficiency is positively related to inoculation period (Purcell & Finlay, 1979; Hill & Purcell, 1995; Almeida & Purcell, 2003). Moreover, as predicted by Purcell (1981), sharpshooter number appears to be positively related to *X. fastidiosa* transmission rate (Costa et al., 2000). Thus, measures exist for each of the factors identified as important for transmission by Purcell (1981). However, the relative importance of each of these factors has not been evaluated, nor have these measures been synthesized into a framework for predicting *X. fastidiosa* transmission by vectors.

Here we describe quantitatively the transmission ecology of *X. fastidiosa* by two of its most important sharpshooter species in California vineyards. First, we conducted a meta-analysis of *H. vitripennis* and *G. atropunctata* transmission studies that provides estimates of the relative importance of vector number, plant acquisition access period (AAP), and inoculation access period (IAP) on transmission rates. Then we used these data to evaluate the fit of two transmission models developed by Purcell (1981). Finally, we conducted two experiments that decoupled effects of vector number and inoculation period on *X. fastidiosa* transmission and quantified the onset of disease symptoms.

Materials and methods

Meta-analysis of *Xylella fastidiosa* transmission experiments

Researchers have conducted transmission experiments of *X. fastidiosa* by sharpshooters and other xylem-feeding insects since at least the 1940s (e.g., Severin, 1949). These experiments have taken two forms, either 'natural infectivity' tests of field-collected individuals of unknown pathogen status (e.g., Hewitt et al., 1946) or controlled transmission experiments with initially non-inoculative insects (e.g., Almeida & Purcell, 2003). Only the latter can be used to estimate the efficiency of a given vector species at transmitting *X. fastidiosa*. We conducted a meta-analysis of this second class of studies to estimate the relative importance of three commonly manipulated transmission variables of sharpshooters to grapes.

Controlled experiments of *X. fastidiosa* transmission by sharpshooters consistently use the same methodology. First, batches of sharpshooters (e.g., 20–50 insects of one species) are caged on known *X. fastidiosa*-infected host plants for a certain access period to acquire the pathogen (AAP). Next, a number of these potentially infectious vectors (ranging from one to several insects) are caged on individual healthy test plants. After some access period (IAP), during which inoculation may occur, all insects are removed from these test plants. Finally, at a later time, the infection status of each test plant is evaluated. Thus, individual test plants are the unit of replication, with three commonly manipulated variables associated with transmission: AAP, IAP, or vector number.

We compiled all available datasets, both published and unpublished, that relate to the transmission of *X. fastidiosa* to grapevines by either *H. vitripennis* or *G. atropunctata*. The minimal criteria used for inclusion in the analyses are that the data involve either *G. atropunctata* or *H. vitripennis* on grape test plants, they report precise numbers for AAP, IAP, and vector number, they report the total number of replicates for all treatments, and that they either report the number of infected plants or the percent transmission for all treatments. Using these criteria, we identified 20 studies, from laboratory and field, of *G. atropunctata* (12) or *H. vitripennis* (8) transmission. The complete datasets are available from the authors upon request.

We analyzed these combined transmission datasets using multiple logistic regressions on *H. vitripennis* and *G. atropunctata* data separately (Crawley, 2007). Multiple logistic regressions were used to estimate the combined effects of three variables (AAP, IAP, and vector number) on plant infection status, including interactions between them. Tests were conducted in the R programming language, using quasibinomial errors to cope with overdispersion in the data (Crawley, 2007). Stepwise deletion was used in conjunction with chi-square goodness-of-fit tests to simplify models when possible, which eliminates unnecessary variables or interactions to produce the most parsimonious adequate description of the data (Crawley, 2007). The end product of this analysis is a relative 'best-fit model' with estimates of the relative strengths with which AAP, IAP, and vector number affect the probability that a grapevine becomes infected.

We used these *H. vitripennis* and *G. atropunctata* transmission datasets in a second set of analyses to compare the predictive power of two biologically based models of sharpshooter transmission that were described by Purcell (1981). In this study, Purcell used some of the basic features of vector systems to derive two alternative non-linear functions relating vector number, efficiency, and transmission period to the probability of grapevine infection. One is derived from a binomial probability model (notation changed for clarity):

$$P_{NB} = 1 - (1 - ba)^{NB}. \quad (1)$$

The second is based on a Poisson probability model:

$$P_{NB} = 1 - e^{-baNB}, \quad (2)$$

where N is the number of vectors, 'a' is the probability that a vector is infectious, and b is the vector inoculation rate for a given IAP, B, and P_{NB} is the probability of infection (as a function of two variables, N and B). Both of these models were intended to describe a situation where populations of *G. atropunctata* sweep seasonally into vineyards from surrounding riparian vegetation. However, with a minor modification they can be applied to the assembled greenhouse transmission datasets.

It is biologically reasonable to assume that the probability that a vector is inoculative, 'a', is an increasing function of AAP, 'A'. But, because it is a probability, the value of 'a' should be bounded between 0 and 1. A simple and biologically reasonable way to meet these two assumptions is if:

$$a = 1 - e^{-A}. \quad (3)$$

If this term is substituted into equations 1 and 2, these modified models can be used to predict the risk of transmission (i.e., P_{NAB}) as a function of three variables (vector number, AAP, and IAP) and one constant, the vector inoculation efficiency, b.

We fit the modified versions of the binomial and Poisson models to the *H. vitripennis* and *G. atropunctata* transmission datasets, separately, using non-linear least-squares regression (Crawley, 2007). The proportion of plants infected at each unique combination of AAP, IAP, and vector number was used as a measure of P_{NAB}. This allowed us to estimate vector inoculation rate, b. We then compared the over-all fit of both of these models to each other and to the best-fit multiple logistic regression model from the previous analysis using Akaike's information criterion (AIC; Crawley, 2007). Lower AIC values (more negative values) reflect a combination of better description of the data and/or more parsimony. Because it is not possible to calculate AIC values for data with quasibinomial errors, the best-fit multiple logistic regression models with binomial errors were used in comparisons with the modified Poisson and binomial transmission models. This substitution did not markedly affect the estimates of regression coefficients for the logistic regression models, therefore it should not unduly influence the comparison of the three models.

Greenhouse experiments: decoupling vector number and IAP

As a second, independent, method of quantifying the relative importance of vector number and plant access period for sharpshooter transmission rates, we conducted two greenhouse studies that decoupled effects of vector number and IAP. In the first experiment, we confined *X. fastidiosa*-free *H. vitripennis* adults on known infected (STL isolate, originally from a symptomatic grapevine in Napa Valley, CA, USA) grapevines (var. Cabernet Sauvignon) for a 4-day AAP. The *H. vitripennis* were from a colony established from individuals collected in Riverside, CA (33°58′N, 117°20′W), and reared in cages on mixed plantings of basil [*Ocimum basilicum* L. (Lamiaceae)], grape, and mugwort [*Artemisia douglasiana* Bess. ex Hook (Asteraceae)]. After the acquisition period, we confined one, two, or four *H. vitripennis* on a healthy grape seedling for 0.5, 1, 2, or 4 days using fine-mesh sleeve cages (10 × 10 cm). There were at least six replicate plants for each 12 vector number-IAP treatment combinations. In addition, there were 15 control plants that were never exposed to insects. After removing *H. vitripennis*, we sprayed the plants with insecticide. Three months later, seedling infection status was determined by plate culturing of one or more ground petioles on standard growth media (methods described by Hill & Purcell, 1995). We analyzed the effects of *H. vitripennis* number and IAP on plant infection status using multiple logistic regression.

A second experiment was conducted with *G. atropunctata*, which we also caged on known infected grapevines for a 4-day AAP. These insects were from a colony established at the beginning of the summer from field-collected adults on wild grape plants at the UC Berkeley Botanical Gardens in Berkeley, CA (37°52′N, 122°16′W). These insects were raised in cages on basil plants, following similar methods as for the *H. vitripennis* colony. After the acquisition period, we caged one, two, or four of these *G. atropunctata* adults on a healthy grape seedling for 0.5, 1, 2, or 4 days. There were at least nine replicate plants for each of the treatment combinations. In this experiment, unlike in the *H. vitripennis* experiment, we regularly inspected the plants to determine when they first began to show Pierce's disease symptoms. Beginning 1 month after removing *G. atropunctata*, we visually inspected all plants 1–2 times per week, during which any of the characteristic Pierce's disease leaf scorch patterns were noted (Krivanek et al., 2005). For these visual inspections, symptoms of Pierce's disease were noted categorically for individual seedlings. We did not quantify symptom severity. After 3 months, seedling infection status was determined definitively by plate culturing. We compared the onset of symptoms among IAP and *G. atropunctata* number treatments using a Cox proportional hazards survival analysis with vector number and IAP as continuous variates (Crawley, 2007). As it is used here, survival analysis includes information about overall proportion of plants infected and symptom onset. Significant effects of the survival analysis were followed up with a logistic regression of IAP and *G. atropunctata* number vs. plant infection status at week

Table 1 Test statistics (t-value) and significance (P-value) for effects in the best-fit multiple logistic regression meta-analysis of *Homalodisca vitripennis* and *Graphocephala atropunctata* transmission datasets

	H. vitripennis		*G. atropunctata*	
Effect	t	P	t	P
Vector number	3.198	0.0053	1.590	0.1267
IAP	−1.292	0.2135	2.738	0.0123
AAP	−2.582	0.0194	2.134	0.0448
IAP*AAP	2.621	0.0179	−1.933	0.0669
IAP*vector number	−2.938	0.0092	n/a[1]	n/a[1]

[1]Not applicable.

12. Moreover, to estimate rates of symptom onset, we also compared the mean number of days to first symptoms among *G. atropunctata* number treatments and among IAP treatments using one-way ANOVA (Crawley, 2007).

Results

Meta-analysis of sharpshooter transmission datasets

Across all treatment conditions, *G. atropunctata* transmitted *X. fastidiosa* 62% of the time, whereas *H. vitripennis* transmitted 30% of the time. The multiple logistic regression analysis of *H. vitripennis* transmission indicated a best-fit model with significant effects of vector number, AAP, an IAP*AAP interaction, and an IAP**H. vitripennis* number interaction, but effects of IAP were not significant (Table 1). The IAP*AAP interaction showed a slight 'saddle-shaped' effect with the highest predicted transmission rates at the very high and very low combinations of both IAP and AAP. The IAP**H. vitripennis* number interaction showed a similar 'saddle shape', though with the highest predicted transmission rates at low IAP and high *H. vitripennis* number, and vice versa. However, it is important to note that for both the interactions these high transmission rates are predicted for regions in which there is a distinct lack of data in the meta-analysis dataset. Thus, these interactions are suspect and restraint should be used in drawing conclusions regarding their importance for *H. vitripennis* transmission. Pairwise comparisons of the strength of the main effects showed that neither AAP nor *H. vitripennis* number differed from the effect of IAP (AAP vs. IAP: $t = -1.517$, $P = 0.15$; vector no. vs. IAP: $t = -0.153$, $P = 0.88$), but AAP and *H. vitripennis* number differed significantly from each other, AAP having a strong negative slope and *H. vitripennis* number having a relatively strong positive slope ($t = -2.513$, $P = 0.021$; Table 2).

The logistic regression analysis of *G. atropunctata* transmission indicated a best-fit model with significant effects of AAP and IAP, but effects of *G. atropunctata* number and an AAP*IAP interaction were not significant (Table 1). The slopes of effects of AAP and IAP were both moderately strong and positive, whereas the effect of *G. atropunctata* number was weakly positive (Table 2). However, comparisons of these slopes did not indicate any significant differences among them (AAP vs. IAP: $t = -0.111$, $P = 0.91$; AAP vs. vector no.: $t = 1.824$, $P = 0.081$; IAP vs. vector no.: $t = -1.737$, $P = 0.096$; Table 2).

In the model fitting and comparison exercise, based on AIC rankings, the modified Poisson model provided better fit (i.e., had the lowest AIC score) to both the *H. vitripennis* and *G. atropunctata* transmission datasets than did the binomial model or the best-fit multiple logistic regression model (Table 3). Vector inoculation efficiencies (b) estimated by the Poisson and binomial models were similar and high for *G. atropunctata*. In contrast, *H. vitripennis* inoculation efficiency differed between the two models, with the Poisson model producing an estimate that was less than one-tenth that of *G. atropunctata*. Thus, according to this preferred model, *G. atropunctata* is substantially more efficient at inoculating grapevines with *X. fastidiosa* than is *H. vitripennis*.

Table 2 Regression coefficients (± SE) for the main effects of AAP, IAP, and vector number on transmission rate estimated by multiple logistic regressions for (1) meta-analysis of *Homalodisca vitripennis* and *Graphocephala atropunctata* datasets, or (2) *H. vitripennis* and *G. atropunctata* greenhouse transmission experiments

	H. vitripennis			*G. atropunctata*		
Source	AAP	IAP	Vector no.	AAP	IAP	Vector no.
Meta-analysis	0.9826[1]	0.4042	0.7719[1]	0.4951[1]	0.3772[1]	0.0906
	± 0.3805	± 0.3128	± 0.2414	± 0.2320	± 0.1378	± 0.0570
Experiments	n/a[2]	0.2880	0.4994[1]	n/a[2]	0.5718[1]	0.3963[1]
		± 0.2150	± 0.2250		± 0.2150	± 0.2150

[1]Coefficient is significantly different than zero.
[2]Not applicable.

Table 3 Vector inoculation rate [b (proportion/vector/day); ± SE] and over-all model fit (AIC score) for *Homalodisca vitripennis* and *Graphocephala atropunctata* meta-analysis datasets: (1) modified Poisson model, (2) modified binomial model, and (3) best-fit multiple logistic regression

	H. vitripennis		*G. atropunctata*	
Model	b	AIC[1]	b	AIC[1]
Poisson	0.025 ± 0.010	4.02	1.184 ± 0.387	12.23
Binomial	1.020 ± 0.168	74.23	1.039 ± 0.075	87.29
Logistic	n/a[2]	149.43	n/a[2]	202.77

The preferred model (Poisson) estimated large differences in efficiency between the two vectors.
[1]Lower AIC values represent better model fit.
[2]Not applicable.

Greenhouse experiments: decoupling vector number and IAP

The two greenhouse experiments generally supported the results of the meta-analysis. In the meta-analysis, *G. atropunctata* inoculation efficiency from the Poisson model was dramatically higher than for *H. vitripennis* (Table 3). This was supported qualitatively by the greenhouse studies, which showed 20–30% higher transmission by *G. atropunctata* than *H. vitripennis* (Figure 1). For the *H. vitripennis* transmission experiment, the best-fit model indicated a significant effect of *H. vitripennis* number ($\chi^2 = 5.268$, d.f. = 1, P = 0.022), but not IAP ($\chi^2 = 1.903$, d.f. = 1, P = 0.17) on transmission to grape seedlings (Figure 1). None of the control plants became infected in this experiment. As in the meta-analysis, vector number was strongly and positively related to transmission, whereas IAP was weakly and negatively related to transmission (Table 2, Figure 1).

For the *G. atropunctata* greenhouse experiment, rates of plant infection and symptom development of infected grape plants were both analyzed. All symptomatic plants in this experiment tested positive for *X. fastidiosa* according to culturing. Only one plant that was asymptomatic at the end of the experiment tested positive, and none of the control plants became infected. The results of the survival analysis (Figure 2) indicated significant effects of both *G. atropunctata* number ($\chi^2 = 8.730$, d.f. = 1, P = 0.0031) and IAP ($\chi^2 = 11.790$, d.f. = 1, P = 0.0006) on plant symptom status. The best-fit model for final infection rates (at week 12) indicated a significant effect of both vector number ($\chi^2 = 5.032$, d.f. = 1, P = 0.025) and IAP ($\chi^2 = 11.461$, d.f. = 1, P = 0.001) on transmission to grape seedlings (Figure 1). Again, as in the meta-analysis of *G. atropunctata* data, IAP had a slightly stronger positive effect on transmission rate than did vector number (Table 2), although a comparison of slopes indicates the difference is not significant (t = −0.718, P = 0.47). Comparison of symptom onset in infected grapevines showed a significant effect of *G. atropunctata* number ($F_{2,69} = 3.194$, P = 0.042), but not IAP duration ($F_{3,68} = 0.552$, P = 0.65). Large vector numbers shortened the time to first symptoms compared to small vector numbers (Figure 3A). Conversely, although there is a trend toward slower symptom development at the lowest IAP duration (i.e., 0.5 day), this effect was not significant (Figure 3B).

Discussion

Extensive research has documented the efficiency of insect vectors at transmitting (i.e., acquiring and inoculating) plant pathogens (Severin, 1949; Purcell & Finlay, 1979; Irwin & Thresh, 1990; Palermo et al., 2001). However, many of these studies test categorically whether vector species can or cannot transmit a given plant pathogen over a limited range of conditions. Less emphasis has been placed on developing a broad understanding of the factors

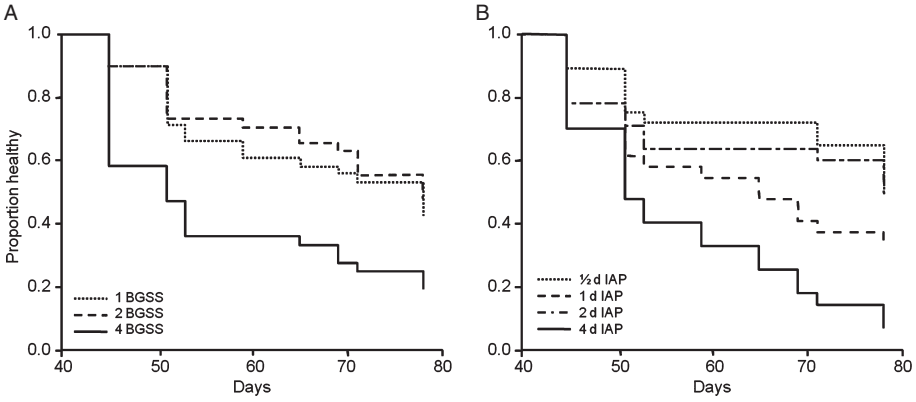

Figure 2 Onset of infection in grapevines among *Graphocephala atropuctata* (A) number and (B) IAP treatments. High vector loads increased both infection rate and accelerated disease onset.

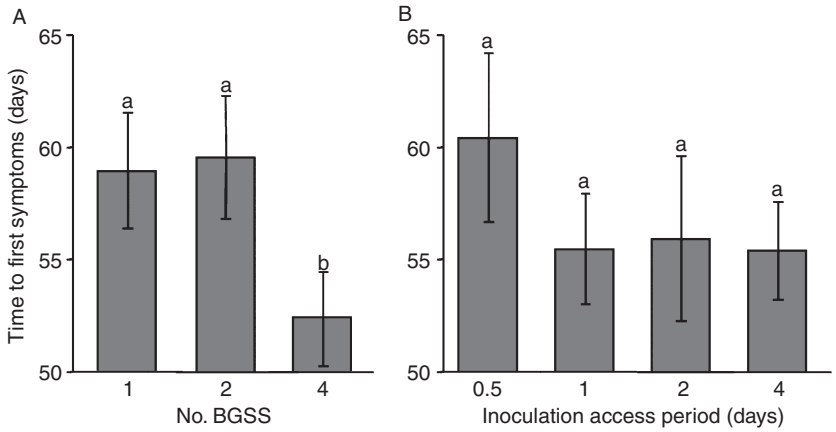

Figure 3 Mean time to first disease symptoms (± SE) among *Graphocephala atropunctata* (BGSS) (A) number and (B) IAP treatments. Bars capped with different letters differ significantly from each other (Tukey test: $P<0.05$).

contributing to transmission over a range of ecological conditions. In this study, we quantified differences in both overall vector species efficiency and the relative importance of common variables associated with *X. fastidiosa* transmission by two of its most important vector species in California.

Homalodisca vitripennis appears to be a relatively inefficient vector of *X. fastidiosa*. The efficiency of this vector as estimated by the best-fit transmission model, the Poisson, was substantially lower than for *G. atropunctata*. A similar pattern emerged from greenhouse transmission studies, wherein *G. atropunctata* was consistently more efficient than *H. vitripennis* across all treatment conditions. These results are also supported by the literature (Almeida & Purcell, 2003). More interesting, however, are the noted differences between the two sharpshooters with respect to the relative importance of vector number, acquisition period, and inoculation period for transmission. Distinguishing between these variables is important because each may be affected differently by specific management actions (Purcell, 1985). Of these three variables, only vector number was positively associated with *H. vitripennis* transmission in the meta-analysis. Neither aspect of transmission period increased *H. vitripennis* transmission rate. Conversely, for the *G. atropunctata* transmission datasets, both the aspects of transmission period contributed substantially to infection, whereas vector number had a weaker effect. These patterns are again supported qualitatively by the results of the greenhouse transmission studies. If *X. fastidiosa* transmission success is a function of the number of inoculation events more so than total inoculums supplied (M Daugherty, unpubl.), vector feeding period may be expected to be more weakly related to transmission rate than is vector number – especially for an inefficient vector. This hypothesis could also be invoked to explain a lack of positive relationship between AAP and *H. vitripennis* transmission. However, it cannot explain a strong negative effect of *H. vitripennis* acquisition period in the meta-analysis, which has no obvious biological basis. More likely, this effect is attributable to the nature of the meta-analysis data. The *H. vitripennis* dataset included 41 unique combinations of vector number, IAP, and AAP from eight studies and at least four research groups. Although the studies used similar methodologies, the statistical analysis could not control for all possible sources of variability among the studies including experiment location (e.g., field vs. laboratory), *X. fastidiosa* isolate, grape cultivar, plant age, source plant infection level, and ambient temperature. Thus, it is prudent to use some caution when drawing conclusions about the absolute effect size of each of the three transmission variables.

The results of the transmission model fitting and discrimination exercise suggest that both the modified versions of Purcell's (1981) transmission models outperformed the purely statistical logistic regression approach. This is one of the first attempts to fit such transmission models to insect-borne plant pathogen data, despite widespread application of similar methods to understand transmission risk for human pathogens (Killeen et al., 2000; Kilpatrick et al., 2006). These models are valuable in two ways. First, transmission models can be used to estimate vector efficiency from empirical data. This is how we applied them in this study. Alternatively, they may be used to predict the risk of pathogen spread in the field (P_{NAB}). This approach may be generally applicable for evaluating transmission risk for different plant pathogen systems, especially because these simple models require only a few commonly measured variables. Of the two

transmission models, the Poisson model provided the best fit indicating that it may be a promising tool for describing field patterns. For such applications, it should be noted that the modification made to Purcell's (1981) original model changes slightly its assumptions. In the original model, acquisition is presumed to have already occurred, in that the parameter 'a' (equations 1 and 2) is a measure of 'natural infectivity'. Thus, this formulation is most appropriate for situations of primary spread into focal habitats from surrounding habitats. This is the scenario envisioned by Purcell (1981) for *G. atropunctata* spread of *X. fastidiosa* into vineyards, and may be generally reasonable for describing spread of zoonotic diseases (e.g., West Nile virus, Lyme disease), for which alternative reservoirs play an important role. Conversely, the modified model makes no assumption regarding acquisition prior to vector spread into the focal habitat. Therefore, it should be more applicable to situations where spread occurs from host to host (i.e., secondary spread), as has been suggested for *H. vitripennis* vine-to-vine spread of *X. fastidiosa* (Almeida et al., 2005) or for anthroponotic diseases (e.g., malaria). Regardless of the formulation of the Poisson model, the next step is to use it in conjunction with laboratory-derived vector efficiency estimates and vector abundance in the field to predict disease incidence. Large differences in predicted vs. observed incidence would indicate that other epidemiological factors are playing an important role (e.g., plant recovery; Purcell, 1981), necessitating more sophisticated epidemiological models (Jeger et al., 1998) to describe disease risk.

A final observation from this study is that high vector loads not only increase transmission success, but they also drive disease dynamics. In the *G. atropunctata* greenhouse transmission experiment, higher vector numbers accelerated the onset of Pierce's disease symptoms. This decrease in incubation period (i.e., shorter pathogen latent period) likely occurs because higher vector densities increase initial plant infection levels (M Daugherty, unpubl.). However, such effects of vector density are not related to higher pathogen inoculum supply. Instead, they stem from more inoculation events increasing the potential for systemic infections to develop (M Daugherty, unpubl.). Thus, if the number of unique inoculation events is related more strongly to vector number than feeding period, this could explain the weaker effect of IAP duration on symptom onset.

Epidemiological models suggest that aggregation of vectors affects disease spread, typically slowing it (Zhang et al., 2000). These models, however, have not considered the effects of vector aggregation on disease incubation. Pierce's disease incubation period may be important epidemiologically because sharpshooter acquisition efficiency is dependent on plant *X. fastidiosa* infection level (Hill & Purcell, 1997), which in turn is associated with disease symptom expression (Krivanek et al., 2005). Thus, high vector loads may decrease the time to reacquisition, and therefore increase the likelihood of secondary spread of the pathogen. Further study is needed under field conditions to determine whether effects of high vector loads on disease incubation are ecologically important. If so, heterogeneous sharpshooter distributions in vineyards may promote spatial heterogeneity in plant infection, causing hotspots for disease. Such hotspots would be difficult to control with management actions that target absolute vector number rather than the spatial distribution of vectors.

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