Grapevine Genotype Susceptibility to *Xylella fastidiosa* does not Predict Vector Transmission Success

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ABSTRACT For vector-borne diseases, interactions between vector, host, and pathogen can influence patterns of disease spread. In particular, previous studies suggest that host genotype may influence disease dynamics because of differences in susceptibility to the pathogen and, therefore, subsequent vector transmission efficiency from these plants. We tested this hypothesis by using the pathogenic bacterium *Xylella fastidiosa*, the etiological agent of Pierce's disease in grapevines, and its leafhopper vector *Homalodisca vitripennis* (Germar). Pathogen infection level and transmission efficiency among several widely cultivated red and white wine, table, and raisin grape cultivars, were compared with the expectation that vector transmission rate would differ among cultivars, because of underlying differences in susceptibility to infection. The 14 grapevine genotypes evaluated showed significant differences among cultivars in the populations of *X. fastidiosa* that developed in petioles. 'Flame seedless' hosted the highest bacterial populations, between 1.81 and 2.05 times higher than the least susceptible 'Merlot', 'Crimson seedless', 'Grenache Noir', and 'Rubired'. Although the transmission rate of *X. fastidiosa* by *H. vitripennis* varied substantially (zero to 33%), it was not significantly different among cultivars. These results suggest that either the relationship between vine infection level and transmission is weaker than previously reported, or innate differences in vector preference among cultivars confounded any effects of vine susceptibility to infection.

KEY WORDS glassy-winged sharpshooter, Pierce's disease, transmission efficiency, grape, susceptibility

Host-plant susceptibility to pathogens has long been recognized as a factor that may limit disease epidemics in agricultural ecosystems (Kolmer 1996, Leung et al. 2003). Although among-genotype variations in degree of susceptibility to pathogens have traditionally been quantified by the extent of associated visual symptoms (Parker 1985, Simms 1993), infection levels may not always be correlated with symptom severity—some host types may show little to no symptoms in spite of harboring high pathogen populations (Kover and Schaal 2002). In the case of vector-borne pathogens where complex interactions among pathogen, hosts, and vectors are expected, variation in source plant infection levels may play a definitive role in patterns of pathogen spread. Indeed, spatial and temporal distribution of pathogens (James and Oliver 1990, McElhany et al. 1995, Shaw et al. 2003), within-host pathogen population (Irwin and Thresh 1990), and within-host incubation time (Gildow and Frank 1988, Kilpatrick et al. 2006) are examples of factors that can affect vectors' exposure to pathogens at the source plant. The relationship between within-host pathogen populations and transmission efficiency of vectors has been previously shown for the plant pathogenic bacterium *Xylella fastidiosa* Wells et al. (Hill and Purcell 1997, Daugherty et al. 2010).

Xylella fastidiosa is a xylem-limited bacterium (Davis et al. 1978) transmitted by xylem sap-feeding insects, most notably sharpshooter leafhoppers (Hemiptera: Cicadellidae) (Houston et al. 1947; Severin 1949, 1950). During vector sap feeding on a healthy host, bacteria are inoculated into the plant, multiply, and spread throughout the host (Chatterjee et al. 2008). *Xylella fastidiosa* has been reported from a wide range of host plants (Purcell 1997) and is of economic importance to several agricultural crops, ornamental plants, and trees. Prominent examples of diseases caused by this bacterium are almond leaf scorch, citrus variegated chlorosis, and Pierce's disease in grapevine (Hopkins and Purcell 2002). For the latter, symptoms include leaf scorch, wilting and drying of the fruit, irregular maturation of the cane, and dieback of the plant apex (Hewitt et al. 1942, Krivanek et al. 2005).

The Pierce's disease epidemic in Southern California vineyards in the late 1990s followed the establishment and spread of the invasive leafhopper *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) (Sorensen and Gill 1996, Blua et al.1999, Purcell and Feil 2001). Although *H. vitripennis* is known to be a less

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Table 1. Grapevine cultivars and number of replicates for the susceptibility experiments and transmission trials

Cultivar	FPS Clone selection[a]	Infection level statistical subsets[b]	Infection level trials (no. of plants per sampling date)	Transmission trials (no. of plants across sampling dates)
Rubired	BKN A R17.00 10.00	a	14	20
Grenache Noir	NYL C R11.00 24.00	a	13	33
Crimson seedless	NYL D R4.00 3.00	a	13	23
Merlot	NYL C R11.00 1.00	a	10	24
Cabernet Sauvignon	NYL D R20.00 9.00	b	14	27
Pinot Noir	NYL C R13.00 17.00	b	9	22
Syrah	NYL H R4.00 5.00	b	14	25
Barbera	NYL D R8.00 6.00	b	10	21
Colombard	BKN B R9.00 9.00	b	9	23
Ruby seedless	BKN B R17.00 4.00	b	13	21
Chardonnay	NYL D R18.00 5.00	b	10	19
Red Globe	NYL I R2.00 5.00	b	12	19
Thompson seedless	NYL C R1.00 21.00	b	8	23
Flame seedless	NYL E R9.00 8.00	c	13	28
		Total	162	357

Cultivars are listed based on infection levels in ascending order.

[a] The presented coding system is used by Foundation Plant Services of University of California, Davis to label plant material.

[b] Cultivars were placed in three statistically distinct subsets (Tukey HSD) based on petiole infection levels. Grape cultivars labeled by similar letters belong to the same homogeneous subset.

efficient vector of *X. fastidiosa* compared with the native *Graphocephala atropunctata* Signoret on grape hosts (Almeida and Purcell 2003; Daugherty and Almeida 2009), its occurrence in large numbers in Southern California is thought to be one of the main driving factors for the Pierce's disease outbreak (Purcell and Feil 2001).

Grape species (*Vitis* spp.) show variation in their resistance to *X. fastidiosa* infection, with *V. vinifera* being among the most susceptible (Fritschi et al. 2007). Despite their relatively high susceptibility to bacterial infection (Krivanek and Walker 2005), cultivars of *V. vinifera* also exhibit differences in Pierce's disease symptom severity (Hewitt et al. 1942, Purcell 1974, Raju and Goheen 1981, Krivanek et al. 2005), a progressive phenotypic signal of the host infection level (Fry and Milholland 1990, Alves et al. 2004, Krivanek and Walker 2005). Transmission efficiency of *X. fastidiosa* by sharpshooter vectors is also a function of infection level of the source plant (Hill and Purcell 1997). The role of infection level becomes even more pronounced in the absence of severe symptoms (i.e., relatively tolerant cultivars, early season infections, or both) because the vector exploits resources indiscriminately; sharpshooter leafhopper vectors tend to visit asymptomatic vines more often than symptomatic ones (Daugherty et al. 2011). Differential susceptibility of *V. vinifera* cultivars is associated with differences in bacterial populations and the rate of xylem-occlusion in the stem tissue (Fry and Milholland 1990, Krivanek et al. 2005, Baccari and Lindow 2011). Likewise, in this study, bacterial population level within tissue of the infected grapevine was used as a measurement of host 'susceptibility'. Cultivar susceptibility may affect the rate at which vectors are exposed to *X. fastidiosa* infection and, subsequently, their transmission rate. This contention is supported by the observed low transmission efficiency in almond, [*Prunus dulcis* (Mill.) D.A.Webb], which maintains relatively low infection levels compare with grape and alfalfa (*Medicago sativa* L.) hosts (Lopes et al. 2009). Recent evidence appears to support the conclusion of Hill and Purcell (1997) by demonstrating a tendency for greater exposure to *X. fastidiosa* at feeding sites to positively affect vector acquisition and overall transmission efficiency (Daugherty et al. 2010).

Although substantial evidence indicates variation in susceptibility to *X. fastidiosa* among different *V. vinifera* cultivars, no study to date has evaluated how this among-cultivar variability may impact pathogen transmission efficiency by *H. vitripennis.* Here, a greenhouse study was conducted to estimate within-host *X. fastidiosa* populations, at two incubation times, in 14 commonly used *V. vinifera* cultivars. Then, *H. vitripennis* transmission success was evaluated for each of the cultivars, in relation to their observed infection levels. This allowed examining whether any potential among-cultivar variation in transmission efficiency of *X. fastidiosa* by *H. vitripennis* is determined by host genotype susceptibility to bacterial infection. Because each cultivar was obtained from the same clone, hereafter the terms 'cultivar' and 'genotype' are used interchangeably.

Materials and Methods

***X. fastidiosa* Infection Level in *V. vinifera* Cultivars.** Dormant cuttings of 14 different healthy grapevine cultivars were obtained from Foundation Plant Services at the University of California, Davis (Table 1). Two-bud cuttings were planted in trays filled with a 1:1 mixture of perlite and vermiculite and placed in a mist chamber. After root development, these cuttings were transplanted into 10-cm square pots filled with Supersoil (Rod Mclellan Company, San Mateo, CA). *Xylella fastidiosa* (STL isolate; Hendson et al. 2001) cells were grown on PWG medium, a modification of Periwinkle Wilt medium (Davis et al. 1981), and suspended in

SCP buffer following Hill and Purcell (1995). Twenty μl of this suspension was used in early July 2009 to inoculate 2-mo-old grape cuttings. To inoculate, two 10-μl drops were placed on the base of the main shoot, each 2 cm apart. Then, an insect pin (no. 1) (BioQuip Products Inc., Rancho Dominguez, CA) was used to poke the stem surface—through each drop— until the suspension was absorbed by the plant. Because water deprived plants absorb the suspension more readily than well-watered plants, cuttings were not watered 24 h before the inoculations. Experimental plants were regularly pruned to ≈30–40 cm in height. Average greenhouse temperature ranged from 17 to 26°C and was affected by outdoor thermal fluctuations. Each sampling involved removing one petiole from >10 cm above the inoculation point. Plants were sampled 8 and 12 wk postinoculation (hereafter referred to as 'incubation time'). All samples were stored in −80°C for later bacterial quantification.

DNA was extracted from petioles by using QIAcube (a robotic work station) and Qiagen extraction kit (Dneasy plant mini with Qiashredder 2007) (Qiagen, Hilden, Germany). The lysis buffer was supplemented with 0.5% lauryl sarcosine and 10% PVP-40 (polyvinylpyrrolidine). The absolute infection level quantification was performed with SYBR Green Mix (Applied Biosystems, Foster City, CA) on a 7500 real-time thermocycler (Applied Biosystems). Cell numbers were estimated based on the standard curve established by Daugherty et al. (2009). This standard curve was obtained by extracting *X. fastidiosa* DNA from suspensions of cultured cells while portions of these suspensions were used to plate serial dilutions to correlate the number of cells with the DNA content. Primers used, HL5 and HL6, were designed by Francis et al. (2006).

A two-way repeated measure analysis of variance (ANOVA) with plant cultivar and incubation time as categorical variables was used to compare infection levels among examined cultivars. Estimated infection levels were normally distributed within genotypes (Kolmogorov–Smirnov, $Ps > 0.05$), with the exception from 'Colombard' on week 8 ($P = 0.04$) and Crimson seedless on week 12 ($P < 0.01$). Plants with unsuccessful mechanical inoculations were excluded from susceptibility and transmission rate analyses (see Table 1).

***H. vitripennis* Transmission of *X. fastidiosa*.** A colony of vectors was established from ≈200 field-collected *H. vitripennis*, from Riverside, CA. To initiate colonies, sharpshooters were placed into four bugdorm insect rearing tents (75 by 75 by 115 cm) (MegaView Science Co. Ltd., Taichung, Taiwan). Insects were maintained in the Oxford Tract facility at the University of California, Berkeley. Each bugdorm contained four basil plants [*Ocimum basilicum* (L.), Lamiaceae] as feeding and oviposition hosts. Basil plants were replaced every 2–3 wk. Adults from the second generation were used in transmission experiments and were free of *X. fastidiosa*, as this pathogen is not transovarially transmitted (Freitag and Frazier 1954). Transmission experiments were conducted in the fall of 2009 and 2010.

In September and October of 2009, transmission experiments were performed on the 14 grape cutting cultivars used in susceptibility experiment (Table 1) on weeks 8 and 12 postinoculation. Source plants in this set of transmission experiments were inoculated with the STL strain of *X. fastidiosa*, as described. Insects were caged individually on each of the source plants for 48 h (acquisition access period). A second set of transmission experiments were performed with seedlings from 'Cabernet Sauvignon' ($N = 28$), 'Pinot Noir' ($N = 30$), and 'Cabernet Franc' ($N = 12$), in October of 2010. In this experiment plants were grown from seeds and thus genotype variability was expected within each of the three above listed cultivars. Two-month-old seedlings were inoculated with the Temecula strain of *X. fastidiosa* (Van Sluys et al. 2003) and were used for transmission experiments 10 wk postinoculation. Using mesh cages, insects were caged in groups of 10–15 on each of the source plants for 48 h (acquisition access period). Unlike previous set of experiments, experimental insects were caged on two source plants per cultivar. Source plants used for the transmission experiments were tested positive for *X. fastidiosa* via culturing with the exception of one of the source plants of the Cabernet Franc cultivar.

In both sets of experiments, insects had access to any plant tissue as the whole plant was contained within a mesh-cage. After 48-h acquisition access period elapsed, insects were removed from the infected plants and caged individually on healthy rooted cuttings of the corresponding cultivar for 6 d of inoculation access period. Insects were then removed and plants were grown in the greenhouse (17–26°C). After 3 mo, petioles of the experimental plants were cultured in PWG for detection of bacteria.

Binary logistic regression model with cultivar and incubation time (repeated measure) as categorical co-variates and infection level as a continuous covariable was used to determine whether the probability of a successful transmission event was predicted by any of the variables in the model. In this experiment each transmission event was an independent replicate as insects were caged on different infected hosts individually. The second set of transmission experiment in 2010 did not include a repeated measure category (incubation time), thus, a χ^2 goodness-of-fit was used to detect any heterogeneity in transmission rates among the three seedling cultivars evaluated in 2010. Yates' correction was applied, as there were expected values <5.

Results

***X. fastidiosa* Infection Level in *V. vinifera* Cultivars.** ANOVA results revealed a significant variation in *X. fastidiosa* populations within petioles of *V. vinifera* cultivars ($F_{13, 148} = 2.72$, $P = 0.002$). Bacterial populations were not significantly affected by incubation time ($F_{1, 148} = 0.14$, $P = 0.70$), and there was not a significant interaction between genotype and incuba-

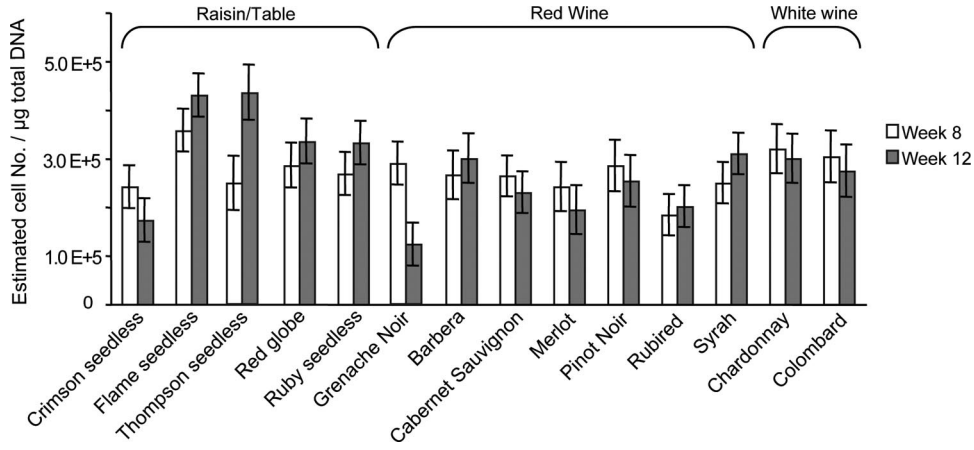

Fig. 1. *X. fastidiosa* infection levels (estimated cell no./µg total DNA) within petioles of 14 different grapevine cultivars measured eight (empty bars) and 12 (filled bars) wk postinoculation. Error bars represent ± 1SE.

tion time ($F_{13,\ 148} = 1.71$, $P = 0.06$). Further Tukey comparisons revealed that the overall significant cultivar effect (Fig. 1) was driven by differences in bacterial populations between Flame seedless and cultivars Rubired ($P = 0.002$), Grenache Noir ($P = 0.008$), Crimson seedless ($P = 0.009$), and Merlot ($P = 0.04$). These four cultivars (Group a in Table 1) were the least susceptible to *X. fastidiosa* infection. Flame seedless (Group c in Table 1) proved to be the most susceptible among the 14 evaluated *V. vinifera* cultivars. The remaining nine cultivars (Group b in Table 1) formed an intermediate statistically homogenous subset. Red and white wine, and raisin and table grapevines cultivars evaluated in this study showed differential susceptibility to *X. fastidiosa* infection, as confirmed by a separate model ($F_{2,\ 159} = 5.61$, $P = 0.004$; Fig. 1). Post hoc comparisons revealed that, overall, table and raisin grapes had significantly higher infection levels (week 8: mean number of cells per µg of DNA (± SE) = 2.80E + 5 (2.08E + 4); week 12: mean (± SE) = 3.41E + 5 (4.74E + 4)) than red wine cultivars (week 8: mean (± SE) = 2.54E + 5 (9.60E + 4); week 12: mean (± SE) = 2.30E + 5 (8.70E + 4)) (Tukey Honestly Significant Difference (HSD), $P = 0.005$).

Transmission Experiments. The rate of a successful *X. fastidiosa* transmission by *H. vitripennis* did not depend on grapevine genotype (Wald $X_{13}^2 = 8.55$, $P = 0.80$; Fig. 2); incubation time (Wald $X_1^2 = 1.42$, $P = 0.23$; Fig. 2); or infection level (Wald $X_1^2 = 0.03$, $P = 0.86$; Fig. 3). Although statistically nonsignificant, transmission rates showed variation among genotypes. Ruby seedless had the highest rate of transmission (33%) and no successful transmission was reported for Barbera, Grenache Noir, Pinot Noir, and Rubired (Fig. 2). A separate logistic model revealed a significant variation in transmission efficiency of *X. fastidiosa* among economic categories (Wald $X_2^2 = 9.25$, $P = 0.010$), with table and raisin cultivars having the highest rates of transmission (mean: week 8 = 7%; week 12 = 16.4%; Fig. 2).

Similar to the experiment with the STL strain of *X. fastidiosa*, no difference in transmission rates was detected among the seedlings of the tested Cabernet Sauvignon, Pinot Noir, and Cabernet Franc, inoculated with the Temecula strain of the bacterium ($X_2^2 = 1.12$, $P = 0.57$).

Discussion

Xylella fastidiosa transmission success by *H. vitripennis* vectors was compared among several grapevine cultivars in relation to their bacterial infection levels and incubation time.

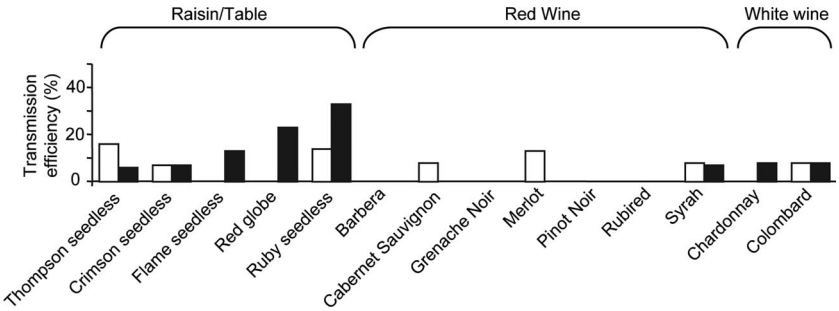

Fig. 2. *X. fastidiosa* transmission rates by *H. vitripennis* for 14 different grapevine cultivars eight (empty bars) and 12 (filled bars) wk postinoculation.

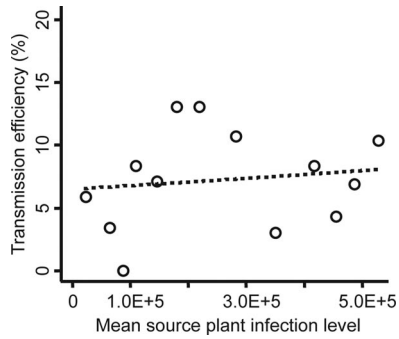

Fig. 3. Relationship between *X. fastidiosa* infection level (estimated cell no./μg total DNA) in source plants and *H. vitripennis* transmission rate, across all grapevine genotypes and incubation times. Because the response variable is binary, the figure was created by ranking according to infection level then calculating a mean acquisition rate for groups of 25–34 consecutive vectors, based on natural breaks in infection level. The dashed line denotes the fit of the logistic regression to the raw data {logit[y] = 4.398e-7($\pm$ 1.277e-6)*x − 2.664.($\pm$ 0.418)}.

Overall, *V. vinifera* cultivars showed a significant degree of variability in their susceptibility to infection, an expected outcome supported by previous studies (Raju and Goheen 1981, Krivanek and Walker 2005, Krivanek et al. 2005, Fry and Milholland 1990). Yet, host infection level was not a predictor of a successful transmission event by *H. vitripennis.*

Chardonnay previously has been shown to be one of the relatively susceptible grapevine cultivars (Raju and Goheen 1981, Krivanek and Walker 2005, Fry and Milholland 1990). Similarly, in the current study Chardonnay was among the four most susceptible genotypes. Merlot and Cabernet Sauvignon, however, were reported as relatively 'tolerant' cultivars in Raju and Goheen (1981), because they harbored lower bacterial populations in their petioles compared with other tested cultivars, including Pinot Noir and Chardonnay. A similar trend was observed in our study, as bacterial populations in Merlot and Cabernet Sauvignon were lower than both Chardonnay and Pinot Noir. Statistically, however, Cabernet Sauvignon, Pinot Noir, and Chardonnay were placed in the same category. Unlike Raju and Goheen (1981) who reported the highest *X. fastidiosa* populations in Colombard, using ELISA, this cultivar's infection level based on quantitative polymerase chain reaction (PCR) was lower than that of Chardonnay and Thompson seedless. Moreover, anecdotal observations indicate that Thompson and Flame seedless, the two most susceptible genotypes in this study, are relatively 'field resistant' cultivars (A.H.P., personal communication) Inconsistencies may have resulted from differences in the bacterial strains, environmental variation, or both, more particularly, thermal fluctuations, which can interact with both the bacterial and the host genotype (Feil and Purcell 2001). It is important to note that among study comparisons of cultivars' degree of susceptibility might not provide fully comparable results, as 'susceptibility' (or 'resistance') is a relative term that depends on the subset of the cultivars being evaluated in a given study under a defined set of conditions. Grouping cultivars based on their economic use indicated that red wine genotypes (N = 7), overall, had lower infection levels than table and raisin grape cultivars (N = 5). Although differences in infection levels between table and red wine cultivars may be relevant to differences in their biochemical properties (e.g., variations in phenolic groups; Cantos et al. 2002), more studies with a wider range of cultivars are required to confirm that such a pattern is nonrandom.

Baccari and Lindow (2011) demonstrated that differences in bacterial population and movement between resistant (vars. 'Roucaneuf' and 'Tampa') and susceptible (vars. Cabernet Sauvignon and 'Chenin Blanc') *V. vinifera* genotypes were more pronounced in the stem tissue rather than petioles. Structural differences between the larger stem xylem and the more narrow and clustered vessels of petioles have been proposed to explain this existing variation (Krivanek and Walker 2005, Baccari and Lindow 2011). Analysis of *X. fastidiosa* populations in petioles, however, was sensitive to differences in infection levels of the evaluated cultivars. Indeed, the quantitative PCR approach for estimating the infection levels previously had been proposed as an essential tool for distinguishing susceptible and resistant genotypes (Krivanek and Walker 2005). Despite the observed overall significant variation in bacterial populations, nine out of 14 evaluated cultivars formed a homogeneous subset. It should be acknowledged that quantifying infection levels in the stem tissue can provide a higher resolution picture (Krivanek and Walker 2005, Baccari and Lindow 2011), in particular within *V. vinifera*, where differences in bacterial populations are expected to be smaller than among different *Vitis* species. Such within-plant variability in pathogen distribution may be important when vector transmission is being considered, as it may have a large impact on *X. fastidiosa* transmission efficiency (Daugherty et al. 2010).

More susceptible genotypes of *V. vinifera* can potentially have an incremental role in *X. fastidiosa* spread; higher *X. fastidiosa* populations in a host plant can result in higher transmission rates, probably because vectors are more likely to probe into infected vessels while feeding (Hill and Purcell 1997). This expectation was not supported by this greenhouse study, as there was no significant difference in bacterial transmission rates by *H. vitripennis* among cultivars harboring different bacterial populations. This finding was not consistent with that of Purcell (1981), who reported a small but significant variation in transmission efficiency of *X. fastidiosa* by *G. atropunctata* among 11 European grape cultivars. Bacterial populations in the source plants were not evaluated by Purcell (1981). In that study, the observed among cultivar differences in transmission efficiency ranged from 80 to 100%; here it ranged from 0 to 33%. *Homalodisca vitripennis* is known to be a less efficient vector of *X. fastidiosa* compared with *G. atropunctata* on grapevines (Almeida and Purcell 2003, Daugherty and

Almeida 2009), and our results are consistent with those findings. Regardless, the overall transmission rate on grapevines varied little in studies with both efficient and inefficient vector species.

The observed low transmission efficiency of *X. fastidiosa* by *H. vitripennis* also was confirmed by a smaller set of experiment by using the Temecula isolate of the pathogen, and seedlings of Cabernet Franc, Pinot Noir, and Cabernet Sauvignon. Although the overall pattern indicated that vector transmission efficiency was higher in table and raisin grapes than the red wine grape cultivars, the existence of a true effect needs to be evaluated with more varietals in each economic groups. As discussed for pathogen-grapevine interactions, it may be possible that vector–grapevine interactions also are influenced by among-genotype differences in biochemical properties (Cantos et al. 2002).

In addition to the fact that low transmission frequency can constrain statistical power to detect a difference, other factors can explain the lack of among-cultivar differences in *H. vitripennis* transmission of *X. fastidiosa.* The estimation of *X. fastidiosa* cell numbers within source plants based on a single petiole may not be an accurate representation of host infection levels, as this pathogen is heterogeneously distributed within grapevines (Hopkins 1981). As mentioned above, differences in infection levels may be better reflected in the stem tissue (Baccari and Lindow 2011), which *H. vitripennis* shows a preference to feed on (Redak et al. 2004, Rashed et al. 2011). Thus, *H. vitripennis'* preference for feeding on stem tissue, which has lower bacterial populations (Krivanek and Walker 2005), or potential differences in feeding rates because of preference for certain grape cultivars (Purcell 1981), may have contributed to the overall observed nonsignificant correlation between source plant infection level and transmission rate. Direct observations of feeding behavior on different varietals, which were not made in the current study, may be an important avenue of future research for understanding how plant genotype affects pathogen transmission.

Although transmission of *X. fastidiosa* is known to be influenced by the host infection level (Hill and Purcell 1997), there has been no evidence indicating larger *X. fastidiosa* populations in vectors contributes to their transmission efficiency (Almeida and Purcell 2003, Hill and Purcell 1995, Jackson et al. 2008, Daugherty et al. 2009). This may be because only a small number of cells are required for a successful inoculation of a healthy host (Hill and Purcell 1995), as highlighted by the absence of a required latent period for transmission (Almeida et al. 2005). Vector host-choice behavior also could be a factor, but in the current study bacterial incubation time within source plants ranged from 8 to 12 wk and the infected grapevines were not showing severe disease symptoms. Asymptomatic yet infected hosts may play an important role in the rate of pathogen spread because *X. fastidiosa* vectors appear not to discriminate against them, whereas they seem to avoid symptomatic plants (Marucci et al. 2005, Daugherty et al. 2011). The use of plants that had been infected relatively recently and showed little to no symptoms may better reflect conditions in the field, especially for secondary infections that may happen during summer and fall (Hopkins and Purcell 2002).

The estimation of bacterial transmission rate by *H. vitripennis* was based on a single vector per grapevine. Indeed, *H. vitripennis* individuals tend to aggregate on their host, a behavioral function currently under investigation (Mizell et al., unpublished data). As the number of *H. vitripennis* on a plant increases, the possibility of a successful inoculation event increases proportionally (Daugherty and Almeida 2009). Likewise, the number of infectious *G. atropunctata* on a host plant has proven to be correlated with both the proportion of infected plants and their infection level (Daugherty et al. 2009). Importantly, however, the observed rates of infection and transmission in greenhouse experiments may differ from those observed under field conditions, as it has been shown for *X. fastidiosa* and other systems (Hooks et al. 2009, Purcell 1981). Environmental factors, such as temperature, have proven to affect *X. fastidiosa* transmission rate by *H. vitripennis* (Daugherty et al. 2009). In our study, although all the transmission experiments were performed in the greenhouse, average daily temperatures varied between 17 and 26°C. Such a variation is also expected under natural circumstances in the field, especially in northern California.

Overall, there was no evidence that *V. vinifera* genotype or populations of *X. fastidiosa* that developed in host petioles influenced pathogen transmission rate by *H. vitripennis.* This finding questions the existence of a strong relationship between infection level of the *V. vinifera* genotypes and transmission efficiency of associated vectors, potentially because differences in pathogen populations may not be large enough to result in higher transmission rates. Such correlation may be detectable, for example among *Vitis* spp., where differences in the infection levels are more pronounced (Krivanek and Walker 2005). Future studies should incorporate as much as possible current knowledge on the impact of *X. fastidiosa* and vector heterogeneous distribution within plants on transmission efficiency to address this question in more detail.

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