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Context-dependent transmission of a generalist plant pathogen: host species and pathogen strain mediate insect vector competence

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Abstract

The specificity of pathogen–vector–host interactions is an important element of disease epidemiology. For generalist pathogens, different pathogen strains, vector species, or host species may all contribute to variability in disease incidence. One such pathogen is *Xylella fastidiosa* Wells et al., a xylem-limited bacterium that infects dozens of crop, ornamental, and native plants in the USA. This pathogen also has a diverse vector complex and multiple biologically distinct strains. We studied the implications of diversity in this pathogen–vector–host system, by quantifying variability in transmission efficiency of different *X. fastidiosa* strains (isolates from almond and grape genetic groups) for different host plants (grape, almond, and alfalfa) by two of the most important vectors in California: glassy-winged sharpshooter [*Homalodisca vitripennis* (Germar)] and green sharpshooter (*Draeculacephala minerva* Ball) (both Hemiptera: Cicadellidae). Transmission of isolates of the almond strain by *H. vitripennis* did not differ significantly, whereas transmission varied significantly among isolates from the grape strain (15–90%). Host plant species did not affect *H. vitripennis* transmission. Conversely, *D. minerva* efficiency was mediated by both host plant species and pathogen strain. No acquisition of an almond isolate occurred regardless of plant type (0/122), whereas acquisition of a grape isolate from alfalfa was 10-fold higher than from grape or almond plants. These results suggest that pathogen, vector, and host diversity impose contingencies on the transmission ecology of this complex disease system. Studies aimed at the development of management strategies for *X. fastidiosa* diseases should consider the complexity of these interactions as they relate to disease spread.

Introduction

One of the dominant paradigms for classifying the transmission characteristics of vector-borne plant pathogens is to categorically describe vector–pathogen relationships according to retention time and development of infectivity in vectors: non-persistent, semi-persistent, circulative, and propagative (Nault, 1997; Ng & Falk, 2006). An equally important component of vector-borne diseases is associated with specificity within a disease system. For cases where a pathogen is transmitted by only one vector species, the interaction is considered specific (Graca, 1991). At the other extreme are pathogens that lack vector specificity, and are transmitted by a large number of vectors within the same feeding guild (Redak et al., 2004). Intermediates also exist, where a pathogen may be transmitted by a few vector species but with varying efficiency (Bar-Joseph et al., 1989). An understanding of vector–pathogen specificity is required for identifying which vectors are most important for disease spread.

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A second aspect of pathogen specificity relates to interactions between pathogens and host species. For example, the bacterium *Spiroplasma kunkelii* Whitcomb et al. is notable for having a narrow host range, infecting only maize and its wild relatives under natural conditions (Nault, 1980). Conversely, *Tomato spotted wilt virus* can infect and cause disease in hundreds of host species (German et al., 1992). For generalist pathogens, host species can vary dramatically in their susceptibility to infection (Tooley & Kyde, 2007). Knowledge of pathogen host range is needed for determining which species are competent reservoirs vs. dead-end hosts (LoGiudice et al., 2003).

Designing effective disease management practices requires documenting vector–pathogen and pathogen–host specificity. However, the epidemiological significance of these two sources of specificity can be further complicated by genetic differentiation within pathogens. Most pathogens exist as a complex of distinct strains. This is important epidemiologically, because different strains may influence both transmissibility (Power, 1996) and virulence (Almeida & Purcell, 2003a). Thus, diversity in pathogen strains, vector species, and host species may all contribute to variation in disease prevalence (Brisson et al., 2008). One system where all three of these sources of variability may be important is for the plant pathogen, *Xylella fastidiosa* Wells et al.

Xylella fastidiosa is an economically important plant pathogen, causing disease in many agricultural crops, ornamental plants, and trees (Purcell, 1997; Hopkins & Purcell, 2002). The only means of *X. fastidiosa* dispersal is via insect vectors, specifically, xylem-sap sucking sharpshooter leafhoppers (Hemiptera: Cicadellidae: Cicadellinae) and spittlebugs (Hemiptera: Cercopidae) (Redak et al., 2004; Almeida et al., 2005). There is no vector transtadial or transovarial transmission of *X. fastidiosa*, and there is no observable latent period (Freitag, 1951; Purcell & Finlay, 1979; Almeida & Purcell, 2003c). While these characteristics are consistent with non-specific vector systems, other aspects of this pathogen's biology are more typically associated with specific interactions. *Xylella fastidiosa* forms a biofilm in the foregut of vectors (Almeida & Purcell, 2006) and is persistent in adults (Hill & Purcell, 1995a), which are suggestive of intricate insect–microbe interactions, as evidenced by the fact that no other xylem-limited bacteria are transmitted by sharpshooters (Barbehenn & Purcell, 1993) and other plant-associated bacteria do not adhere to insect surfaces (Killiny & Almeida, 2009).

Because it has been shown that different vector species are capable of transmitting different strains of *X. fastidiosa*, it is generally believed that there is no specificity in this system. Frazier (1965) hypothesized that 'the ability to transmit Pierce's disease is a group characteristic of the Tettigellinae [currently Cicadellinae] and that each species of the group should be a suspected vector until proven otherwise'. For example, the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), has been demonstrated to transmit isolates belonging to all four main *X. fastidiosa* genetic groups (Purcell & Saunders, 1999; Purcell et al., 1999; Costa et al., 2006; Damsteegt et al., 2006). In other words, all *X. fastidiosa* isolates are assumed to be transmitted by all sharpshooters and spittlebugs. However, that does not mean that efficiency (i.e., competence) will be the same for different vector–pathogen–host plant combinations (Severin, 1949; Freitag, 1951; Purcell, 1980).

Xylella fastidiosa transmission efficiency is dependent on bacterial populations within plants (Hill & Purcell, 1997). The higher the infection level, the higher the probability a vector will feed on an infected xylem vessel, acquire *X. fastidiosa*, and inoculate it into a healthy susceptible host. Independent studies on pathogen and transmission biology with different *X. fastidiosa* source host plants suggest this is a general characteristic for this system. For example, citrus (Almeida et al., 2001) and almond (Almeida & Purcell, 2003a) plants harbor lower bacterial populations than grape (Hill & Purcell, 1995b), and corresponding transmission rates are lower than when grape is the source host (Purcell & Finlay, 1979; Purcell, 1980; Almeida & Purcell, 2003b; Marucci et al., 2008). In addition, because different strains of *X. fastidiosa* have different host ranges and different population growth rates within the same host, the *X. fastidiosa*–host plant interaction may strongly mediate vector acquisition efficiency. For example, *X. fastidiosa* isolates from the grape strain (or subspecies *fastidiosa*) cause disease in grape and almonds, whereas those in the almond strain (or subspecies *multiplex*) do not cause disease in grape (Almeida & Purcell, 2003a). Both, however, cause disease in alfalfa, but populations within that host differ between these strains (JRS Lopes, unpubl.). Lastly, host plant–vector interactions may also influence competence. Although sharpshooters as a group tend to be polyphagous, species in the group do not equally prefer all plants. Certain species are primarily grass-feeders, whereas other prefer woody plants (Redak et al., 2004). Thus, vector feeding behavior on host plants is variable and probably affects transmission rates, assuming all other experimental conditions are equal (Purcell, 1980). Therefore, *X. fastidiosa* transmission rates are the outcome of complex vector–plant–pathogen interactions.

We conducted two multifactorial transmission experiments to quantify variability in *X. fastidiosa* transmission stemming from different pathogen strains, host plant species, and vector species. Specifically, we measured the transmission efficiency of two of the most important vectors in California's Central Valley, *H. vitripennis* and green

sharpshooter, *Draeculacephala minerva* Ball (Hemiptera: Cicadellidae), on three economically important hosts (grape, almond, and alfalfa) infected with different *X. fastidiosa* isolates from the almond and grape strains.

Materials and methods

We used two sharpshooter vectors that differ in behavior and ecology: *H. vitripennis* (tribe Proconiini), a highly polyphagous, mobile, and invasive species observed on trees and shrubs in a variety of habitats in southern USA (Hoddle et al., 2003), and *D. minerva* (tribe Cicadellini), primarily a grass-feeder and abundant on grasses, sedges, and other herbaceous hosts that are common in weedy alfalfa fields, orchard cover crops, perennial irrigated pastures, and moist areas surrounding irrigation ditches in California (Hewitt et al., 1946; Purcell & Frazier, 1985; Cabrera-La Rosa et al., 2008). Unlike *H. vitripennis*, *D. minerva* is rarely found on woody plants such as grape and almond trees (Purcell & Frazier, 1985).

Healthy *D. minerva* adults (1–2 weeks old) were obtained from a laboratory colony reared on sweet basil plants (*Ocimum basilicum* L.; Lamiaceae). *Homalodisca vitripennis* adults used in the experiment were collected with sweep nets on citrus trees in an experimental orchard of the University of California (UC), in Riverside, California (33°58′N, 117°20′W), on 1–2 August 2007. The insects were immediately transported to Berkeley, California, inside closed cages (BugDorm-2 rearing cage; Bioquip, Rancho Dominguez, CA, USA) containing potted bell bean (*Vicia faba* L.; Fabaceae), and then kept for 6 days on a mixture of potted grapevine (*Vitis vinifera* L.; Vitaceae), sweet basil, and mugwort (*Artemisia douglasiana* Besser ex Hook.; Asteraceae) plants inside plexiglass cages, in a secured greenhouse at UC Berkeley, before the experiment.

We used four grape *X. fastidiosa* isolates (subspecies *fastidiosa*: Pavich, Traver, Buena Vista, and M35) and five almond isolates (subspecies *multiplex*: Butte, Dixon, ALS4, ALS6, and Glenn) in this study. These isolates were collected in the Central Valley of California from diseased grapes in Kern (Pavich and Buena Vista) and Tulare (Traver) counties, and from diseased almond trees in San Joaquin (ALS4, ALS6), Butte (Butte), Solano (Dixon), and Glenn (Glenn) counties. M35 was isolated from alfalfa in Fresno County. Here we use 'grape strains' and 'almond strains' as synonyms to the proposed '*fastidiosa* subspecies' and '*multiplex* subspecies', respectively (Schaad et al., 2004; Schuenzel et al., 2005). We triply cloned all isolates, which were stored at –80 °C. Frozen cells were plated on solid Periwinkle wilt gelrite (PWG) medium (Hill & Purcell, 1995b) and grown for two passages at 28 °C before inoculation in the source plants. These isolates have been previously typed as grape and almond strains based on genetic and biological differences (Hendson et al., 2001; Almeida & Purcell, 2003a). Both grape and almond *X. fastidiosa* strains colonize and cause disease (i.e., stunting) in alfalfa (JRS Lopes, unpubl.).

To produce source plants for the transmission studies, we mechanically inoculated seedlings of alfalfa (*Medicago sativa* L.; Fabaceae) cv. WL625HQ grown in 10-cm-diameter pots (0.5 l) and showing 5–6 stems (2 weeks after first cut) with a cell suspension of each *X. fastidiosa* isolate in two points of each stem (Hill & Purcell, 1995b). The inoculation was done at 5 and 10 cm above soil level by pipetting 5 µl of the cell suspension on the young stem and pricking through the drop with a no. 0 entomological pin. Turbid *X. fastidiosa* suspensions with estimated concentrations of 10^8–10^9 colony forming units (CFU)/ml, were prepared for inoculation by suspending cells in 0.5 ml of succinate-citrate buffer (Hopkins & Thompson, 1984) after growth of the second passage on PWG medium for 1–2 weeks. Similarly, we pin-inoculated healthy potted plants of grapevine (*V. vinifera* cv. Cabernet Sauvignon) and almond [*Prunus dulcis* (Mill.) D.A. Webb, cv. Sonora-Hansen grafted on peach, *Prunus persica* (L.) Batsch, cv. Lovell (Rosaceae)] with turbid (10^8–10^9 CFU ml^{-1}) suspensions of Traver (grape) and Dixon (almond) isolates in two points of the young stem. The source plants were used for vector acquisition at 3–5 months after pin-inoculation, when leaf scorch symptoms were visible in grape and almond plants, or after infection had been confirmed by culturing in the case of alfalfa.

As test plants for vector inoculation, we used grapevines obtained from healthy cuttings of *V. vinifera* cv. Cabernet Sauvignon, as well as seedlings of alfalfa cv. WL625HQ and almond cv. Peerless. The plants were grown in 10-cm-diameter pots (0.5 l) with Supersoil potting soil mix (Rod Mclellan, San Mateo, CA, USA). Throughout the experiment, test plants were kept in a heated (25 ± 5 °C) vector-proof greenhouse and irrigated 3–4 times a day; once a day, Peter's 20–20–20 (N-P-K) fertilizer with micronutrients was diluted in the irrigation water and applied on the plants. We determined transmission rates (i.e., proportion of infected plants) per treatment based on detection of *X. fastidiosa* infection in the test plants 3 months after inoculation by primary isolation on solid PWG medium (Hill & Purcell, 1995b). Alfalfa samples for culturing consisted of 0.1–0.15 g of stem sections removed from each test plant at 2–5 cm above soil level. Samples from test grape and almond plants were taken from leaf petioles located above and below the inoculation point. Random samples of recovered bacterial colonies on PWG medium were confirmed as *X. fastidiosa* by polymerase chain reaction using the diagnostic primer set RST31–33 (Minsavage et al., 1994).

Effect of *Xylella fastidiosa* isolate and host plant on *Homalodisca vitripennis* competence

We used the highly polyphagous *H. vitripennis* as a vector to determine how efficiently grape (four) isolates and almond (five) isolates of *X. fastidiosa* were transmitted from infected alfalfa to grape and almond test plants. Because *H. vitripennis* was field-collected, all adults used in the experiment were pre-tested for natural infectivity with *X. fastidiosa*, by confining them on healthy almond seedlings (30 insects per plant) for an inoculation access period (IAP) of 72 h. Both grape and almond *X. fastidiosa* strains induce leaf scorch in almond (Almeida & Purcell, 2003a). No symptoms of bacterial infection was detected by culturing in the pre-test almond plants up to 5 months after the IAP, indicating that all *H. vitripennis* adults used here were not infectious. After the IAP on pre-test plants, the sharpshooters were confined for a 96-h acquisition access period (AAP) on alfalfa plants infected individually with various *X. fastidiosa* isolates. The sharpshooters were then transferred to healthy almond seedlings for a 96-h IAP, followed by another 96-h IAP on healthy grapes. *Xylella fastidiosa* is persistent in adult insects and these transfers should not have affected results. For each isolate tested, 4–8 plants of each recipient host were inoculated by using four insects per plant, confined inside sleeve cages. As a negative control, six almond and grape test plants were not exposed to the sharpshooters, and none tested positive for *X. fastidiosa* by culturing.

Because no transmission of almond isolates to grape test plants was observed, we carried out two separate statistical analyses. First, transmission rates of the five almond isolates to almond seedlings were compared by running a one-way analysis of deviance with almond isolate as a fixed effect (Crawley, 2005). Second, transmission rates of the four grape isolates to both almond and grape test plants were compared by a two-way analysis of deviance with recipient plant type and grape isolates as fixed effects. For both of these analyses effects were evaluated at $\alpha = 0.025$ to control for multiple comparisons. In the second test model, simplification techniques were used to determine the most parsimonious adequate description of the data (Crawley, 2005). Significant main effects of isolate were followed up with pairwise χ^2-tests among grape isolates.

Effect of *Xylella fastidiosa* strain and host plant on *Draeculacephala minerva* competence

In a second experiment, we selected grape (Traver) and almond (Dixon) isolates to investigate how *D. minerva* transmission efficiency is influenced by pathogen strain, source host plant, and recipient (test) host plant. Laboratory-reared healthy adults of *D. minerva* were confined on one of three source hosts (i.e., alfalfa, almond, or grape) infected with one of these two isolates for a 48-h AAP. Afterward three insects were transferred to and caged on healthy alfalfa, almond, or grape test plants for a 48-h IAP. For the almond isolate (Dixon), which is not pathogenic to grape (Almeida & Purcell, 2003a), only alfalfa and almond were used as source and test plants. The experiment was repeated in three trials, with 7–12 replicate recipient plants for each isolate-source species-recipient species combination (Table 1). At least five non-inoculated test plants of each plant species were kept as healthy controls in each of the three trials. These plants were later shown to be negative for *X. fastidiosa* by culturing.

Table 1 Transmission (number infected/total number of replicate plants) of *Xylella fastidiosa* isolates between host plants by *Draeculacephala minerva*

Isolate (host of origin)	Source host type[1]	Trial	Recipient host type[2]		
			Alfalfa	Almond	Grape
Traver (grape)	Alfalfa	I	4/7	4/9	6/10
		II	3/10	1/10	6/10
		III	7/10	9/10	8/11
	Almond	I	0/9	0/9	0/9
		II	1/9	1/9	0/9
		III	0/8	0/8	0/8
	Grape	I	8/10	2/9	2/9
		II	4/11	0/9	0/10
		III	0/8	1/7	0/8
Dixon (almond)	Alfalfa	I	0/10	0/10	–
		II	0/10	0/10	–
		III	0/10	0/9	–
	Almond	I	0/10	0/10	–
		II	0/12	0/12	–
		III	0/10	0/9	–

[1]Acquisition access period: 48 h.
[2]Inoculation access period: 48 h (3 insects/test plant).

We analyzed these transmission data using a blocked design to account for the three trials. Because the design was not fully crossed (i.e., grape was not tested as a source and recipient host for isolate Dixon), we ran two statistical analyses on the data set. In the first analysis, we excluded treatments involving grape either as a source or as a recipient host by running a blocked two-way analysis of deviance with source plant (i.e., alfalfa or almond), recipient plant (i.e., alfalfa or almond), and isolate (i.e., Dixon or Traver) as fixed effects (Crawley, 2005). A second blocked two-way analysis of deviance was performed only with data obtained for the grape isolate Traver, with source host and recipient host as fixed effects. Again, model simplification techniques were used to determine the most parsimonious adequate

model for each test. Significant main effects were followed up with pairwise χ^2-tests among isolates (Crawley, 2005).

Results

Effect of *Xylella fastidiosa* isolate and host plant on *Homalodisca vitripennis* competence

Homalodisca vitripennis did not transmit almond isolates to grape (0 out of 29 attempts), which was expected as the almond strain does not cause disease in grape if mechanically inoculated (Almeida & Purcell, 2003a). Transmission rate to almond seedlings ranged from 0 to ca. 50% among isolates (7 of 28 replicates, Figure 1). However, these differences among isolates were not significant ($\chi^2 = 6.99$, d.f. = 4, P = 0.137), presumably because of low statistical power associated with the relatively low number of replicates for some isolates (i.e., n = 4–8).

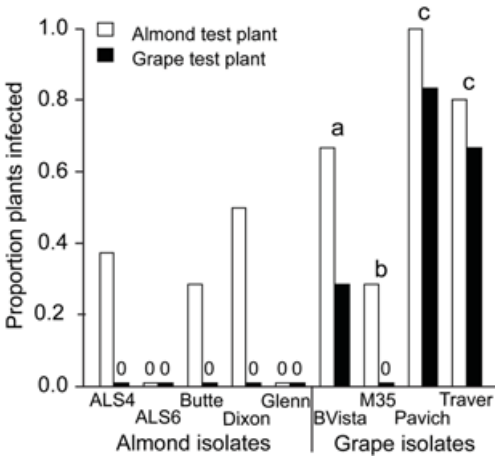

Figure 1 Effect of *Xylella fastidiosa* isolate and recipient host plant type on the transmission efficiency of *Homalodisca vitripennis*. Transmission of almond and grape isolates was evaluated in two separate analyses; different letters above bars denote significant differences between grape isolates across recipient host types. There were no significant differences among almond isolates.

Transmission of *X. fastidiosa* grape isolates by *H. vitripennis* was considered in a separate analysis. Across isolates, *H. vitripennis* transmitted successfully in 28 of 50 attempts. The best fit model included main effects of recipient host plant type and *X. fastidiosa* isolate. Transmission rate was slightly lower to grape than to almond seedlings (Figure 1), although not significantly so ($\chi^2 = 2.95$, d.f. = 1, P = 0.086). However, there were significant differences in transmission success among isolates ($\chi^2 = 21.54$, d.f. = 3, P = 0.0001), with the highest transmission occurring for Pavich and Traver and the lowest for the M35 isolate (Figure 1).

Effect of *Xylella fastidiosa* strain and host plant on *Draeculacephala minerva* competence

The best fit model for *D. minerva* transmission to alfalfa and almond test plants included main effects of trial, source host type, *X. fastidiosa* strain, and recipient host type. Effects of trial were marginally significant ($\chi^2 = 7.23$, d.f. = 1, P = 0.027; $\alpha = 0.025$) and recipient host type was not significant ($\chi^2 = 0.064$, d.f. = 1, P = 0.80), but there were significant effects of both *X. fastidiosa* strain ($\chi^2 = 55.62$, d.f. = 1, P<0.0001) and source host type ($\chi^2 = 30.15$, d.f. = 1, P<0.0001). *Draeculacephala minerva* failed to transmit the almond isolate Dixon in 122 attempts (i.e., replicate test plants) (Table 1, Figure 2). Meanwhile, although transmission rate of the grape isolate Traver was higher, it depended greatly on the source host type – with more than 10-fold higher transmission when acquired from alfalfa than from almond plants (Figure 2).

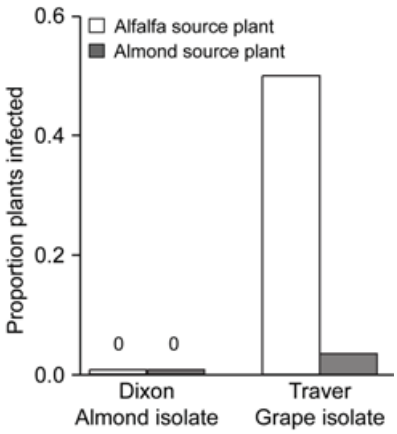

Figure 2 Effect of *Xylella fastidiosa* strain and host plant type on the transmission efficiency of *Draeculacephala minerva* to alfalfa and almond. Source host type affected significantly transmission of the grape isolate Traver; the almond isolate Dixon was not transmitted from any source plant.

The best fit model for *D. minerva* transmission of the grape isolate Traver included only main effects of trial, source host type, and recipient host type. Again, the effect of trial was marginally significant ($\chi^2 = 7.30$, d.f. = 2, P = 0.026; $\alpha = 0.025$), recipient host type was non-significant ($\chi^2 = 1.14$, d.f. = 2, P = 0.567), and source host type was significant ($\chi^2 = 86.62$, d.f. = 2, P<0.0001). Across recipient host types, the average transmission rate after acquisition on alfalfa was at least five times higher than on almond or grape source plants (Table 1, Figure 3).

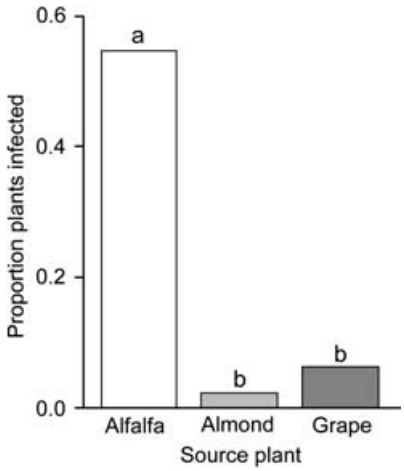

Figure 3 Differences in *Draeculacephala minerva* transmission efficiency of the grape *Xylella fastidiosa* isolate Traver acquired from alfalfa, almond, and grape source hosts. Different letters above bars denote significant differences between source hosts across recipient host types.

Discussion

The transmission of vector-borne pathogens consists of three steps: pathogen acquisition from an infected host, retention within vectors, and inoculation into a new host. The goal of this study was to estimate the extent to which variation in *X. fastidiosa* acquisition or inoculation results from different pathogen strains, host species, or vector species. Our results indicate that both genetic differentiation of *X. fastidiosa* and source plant species strongly affect the transmission efficiency of sharpshooter vectors. Interestingly, the identity of the plant species during the inoculation phase (i.e., test plant) did not play an important role.

In both experiments, pathogen strain affected vector competence. For *H. vitripennis* this effect is apparent in the substantial differences in transmission efficiency of almond vs. grape isolates as well as with variability within the grape strain (Figure 1). For *D. minerva* this *X. fastidiosa* strain effect interacted with source host species – with the grape isolate more likely to be acquired from alfalfa than other hosts and far more likely to be acquired than the almond isolate from any hosts (Figures 2 and 3). Variation in acquisition efficiency can stem from differences in infection level for a given pathogen strain–host species combination (i.e., host resistance/pathogen virulence) or from vector species feeding behavior on source plant species. It has been well established that different host species are an important component determining *X. fastidiosa* populations within plants. For example, grape isolates occur at populations up to 100-fold higher within grapes than almonds, despite the fact that within almonds both strains occur at similar populations (Almeida & Purcell, 2003a). In this study, it was observed that source plants affected the efficiency with which *X. fastidiosa* is transmitted to plants. However, only the role of different isolates (in the same plant) was tested, and not host plant–vector interactions. Previous research has shown that transmission rates vary dramatically when the same host plant and isolate are used but multiple vector species are compared. Transmission efficiency of *X. fastidiosa* from almond sources to almond plants was vector species dependent (Purcell, 1980). *Draeculacephala minerva* was an efficient vector compared with *Graphocephala atropunctata* (Signoret) when alfalfa was used, but the opposite occurred on grapevines (Hewitt et al., 1946). Thus, source plants seem to be an important component on *X. fastidiosa* transmission, a process mediated by both pathogen interactions and vector behavior on different hosts.

The lack of transmission of the almond isolate Dixon by *D. minerva* is at one extreme of how pathogen strain can affect vector competence. Out of 122 attempts no transmission occurred on any of the host species (Figure 2). Based on this result, it is tempting to conclude that some vector species–pathogen strain specificity exists in this system, contrary to the current hypothesis and all other available data (Chatterjee et al., 2008). However, we caution against drawing this conclusion without explicit tests under conditions free of potential host plant–strain confounding effects. No other published studies have used almond isolates in the almond strain for transmission experiments, although Phony peach causing isolates, which belong to the same *X. fastidiosa* subspecies (i.e., *multiplex*), have been transmitted by *H. vitripennis* in previous work (Turner & Pollard, 1959). Other transmission studies of *X. fastidiosa* isolates originating from almond most likely used grape strain isolates (Purcell, 1980; Almeida & Purcell, 2003b). An alternative explanation for the lack of transmission may be rooted in the population growth characteristics of this particular isolate. A prior study showed that the almond isolate used (Dixon) does not reach high populations within alfalfa plants (JRS Lopes, unpubl.), which would hamper acquisition of the pathogen by *D. minerva*. However, this is unlikely to completely explain this result because *H. vitripennis* was able to transmit this isolate (Figure 1), despite being a relatively inefficient vector of *X. fastidiosa* (Almeida & Purcell, 2003c). To determine definitively whether vector–pathogen strain specificity exists, an artificial diet system is needed that delivers known quantities of different isolates to various vector species. In addition, more *X. fastidiosa* isolate and vector species combinations should be tested.

Unlike pathogen source host species, recipient host species did not significantly affect the competence of either *H. vitripennis* or *D. minerva* to transmit a particular *X. fastidiosa* isolate, except for the almond isolate Dixon, which was transmitted only to recipient almond plants by the former vector. Variation in inoculation efficiency can stem from differences in vector species feeding behavior on specific recipient plant species, or from differences in infective dose requirements for a given pathogen strain–host species combination. Results from a mechanical inoculation study that varied *X. fastidiosa* inoculum supply to coffee and citrus are consistent with there being a minimum infective dose (Prado et al., 2008). What the dose is for other host plants and how the required dose may vary among *X. fastidiosa* strains has not been described. Infective dose requirements are particularly important because vectors probably inoculate relatively low numbers of bacterial cells compared to mechanical inoculation (Almeida et al., 2005). For example, Almeida & Purcell (2003a) observed successful colonization of grape by mechanical inoculation of concentrated suspensions (10^8–10^9 CFU ml^{-1}) of various almond isolates of *X. fastidiosa*, although population levels were rather low and Pierce's disease symptoms were not observed in the grape plants. In contrast, we observed no colonization of grape plants by the almond isolate Dixon following inoculation by *H. vitripennis*, possibly because of the low number of bacterial cells deposited into the grape plants by the vector. Interestingly, the same groups of *H. vitripennis* successfully transmitted this isolate to almond before being transferred to the grape test plants (Figure 1). Considering that *X. fastidiosa* is persistently transmitted by sharpshooters (Hill & Purcell, 1995a; Almeida & Purcell, 2003c), we speculate that the required dose of the Dixon isolate for infecting plants after vector inoculation is lower in almond than in grapes. *Homalodisca vitripennis* was already shown to transmit a grape isolate of *X. fastidiosa* to almond (Almeida & Purcell, 2003b), indicating that vector–host plant interaction was not a factor limiting transmission in this particular case.

The significance of vector preference for certain host species on inoculation efficiency is not well known, and was not explicitly documented in our experiments. Although sharpshooters are generally polyphagous, there is large variability in plant preference within this group. For example, *D. minerva* is primarily a grass-feeder (Purcell & Frazier, 1985), whereas *G. atropunctata*, which is native to coastal California, prefers trees and shrubs, including grapevines (Purcell, 1976). *Draeculacephala minerva* was associated with Pierce's disease epidemics in Central California in the 1940s, but was never observed on grapevines. Transmission of *X. fastidiosa* by this vector is thought to have occurred accidentally during short dusk flights to this non-preferred host from adjacent alfalfa fields (Hewitt et al., 1946; Purcell & Frazier, 1985). It is possible, then, that the no-choice design used in this experiment and most classical plant pathology transmission studies may constrain natural vector feeding behavior and, therefore, underestimate the importance of recipient host species during the inoculation phase of the transmission process. However, this constraint should also minimize host species effects during the acquisition phase, which is not supported in the present study. We observed a strong influence of source plant type on vector competence.

The marked effects of source host species on transmission of *X. fastidiosa* by *D. minerva* may have important implications for Pierce's disease and almond leaf scorch spread. The fact that alfalfa is an optimum acquisition host for the grape isolate tested is consistent with previous observations that alfalfa fields serve as inoculum sources for primary spread of Pierce's disease (Hewitt et al., 1946). We have not detected transmission of the almond isolate (Dixon) by *D. minerva*; however, since grape isolates can cause almond leaf scorch (Almeida & Purcell, 2003a), alfalfa may still be a source of inoculum for pathogen spread to almond orchards. Moreover, it is likely that *D. minerva* promotes efficient within-field spread of grape strains of *X. fastidiosa* in alfalfa, particularly in weedy fields where this vector is abundant (Purcell & Frazier, 1985). Conversely, the low transmission efficiency of *X. fastidiosa* by *D. minerva* from grape or almond source plants, combined with the non-preference of this vector for woody plants, suggest that *D. minerva* is less likely to spread the pathogen between trees in almond orchards and vineyards, or from these woody hosts to alfalfa and other herbaceous hosts. In fact, roguing of diseased grapevines had little effect on Pierce's disease control in past epidemics in the Central Valley of California, suggesting that secondary spread of the disease in vineyards was unimportant (Hewitt et al., 1949).

Few studies have compared biological characteristics of multiple *X. fastidiosa* isolates in plants. Although there is genetic variation within each *X. fastidiosa* strain (Schuenzel et al., 2005), little is known about potential phenotypic variability in those groups. The finding that grape isolates were transmitted at different rates by *H. vitripennis* does not conclusively demonstrate that within-strain genetic diversity serves as a proxy for phenotypic variability, but suggests that not all isolates within a strain are biologically similar. Further research into this question is needed, if possible including other strains of *X. fastidiosa*, particularly those infecting citrus and coffee over a wide area in Latin America (Redak et al., 2004). This may be of importance as the gene pool of citrus isolates in Brazil is large, as evidenced by increments with more sampling (Silva et al., 2007).

Vector ecologists have long recognized that transmission is the end product of a series of pairwise interactions among pathogen and vector, vector and host, and pathogen and host (e.g., Irwin & Thresh, 1990). The results presented here suggest that for *X. fastidiosa* and its vectors and hosts, vector competence depends on the specific pathogen strain and host plant species. Specifically, pathogen strain interacts with host species to influence primarily sharpshooter efficiency during the acquisition phase of transmission. This is likely to be a general feature of other vector-borne diseases in which vector competence is tied to host infection level.

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