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Vector preference for hosts differing in infection status: sharpshooter movement and *Xylella fastidiosa* transmission

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Abstract. 1. Epidemiological theory predicts that vector preference for hosts differing in infection status (i.e. healthy or infected) affects disease dynamics.

2. Numerous studies have documented strong vector preference for or discrimination against infected hosts. However, the significance of these behaviours for pathogen transmission and spread has been poorly described.

3. We conducted a series of choice assays to evaluate orientation preference, feeding preference, and movement rates of an important group of vectors, the sharpshooter leafhoppers, based on host infection status for the generalist plant pathogen, *Xylella fastidiosa* Wells *et al*.

4. Sharpshooters did not discriminate between healthy versus infected-but-asymptomatic grapevines, but they oriented preferentially to healthy grapevines more frequently than either symptomatic vines or those artificially coloured to mimic disease symptoms.

5. In a field trial three sharpshooter species showed different movement rates and preferences for feeding site, but all species exhibited similar and significant preference for healthy hosts.

6. Although there was no significant difference in acquisition efficiency among vector species, those individuals that spent more time on healthy hosts tended to be less likely to acquire the pathogen.

7. These results suggest that sharpshooters discriminate against infected grapevines, which are likely to be of poorer quality, with visual cues playing a role in host selection. Preference by these vectors may affect pathogen acquisition, which could affect disease spread in the field.

Key words. Disease dynamics, host choice, pathogen transmission, Pierce's disease, symptom status, vector behaviour.

Introduction

Predicting host–pathogen dynamics hinges on precise encapsulation of the transmission process (Wonham *et al*., 2006), as does the effective implementation of disease management (Lloyd-Smith *et al*., 2005). For vector-borne pathogens a potentially important factor is vector feeding preference, which may underlie transmission efficiency (i.e. competence; Daugherty *et al*., 2010a), rate of disease spread (Sisterson, 2008), and pathogen spillover (Kilpatrick *et al*., 2006).

One well-documented aspect of vector behaviour is the extent to which vectors show preference for host infection status (Jennersten, 1988; Blua & Perring, 1992; Altizer *et al*., 1998; Eigenbrode *et al*., 2002; Medina-Ortega *et al*., 2009; Mauck *et al*., 2010). For example, the settling frequency of aphids on *Barley yellow dwarf virus* infected wheat was up to two-fold higher than on healthy or sham-inoculated plants (Medina-Ortega *et al*., 2009). Conversely, aphid vectors are

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less likely to feed on *Zucchini yellow mosaic virus* infected versus healthy plants (Blua & Perring, 1992). Ecological theory suggests that such behaviour affects disease incidence, with rates of pathogen spread depending on both the magnitude of vector preference for or avoidance of symptomatic hosts (Kingsolver, 1987) and on pathogen prevalence (McElhany *et al*., 1995). Incidence is also predicted to be differentially affected by whether vectors exhibit preference during initial orientation to symptomatic hosts (i.e. orientation preference) versus time spent on hosts (i.e. feeding preference) (Sisterson, 2008).

Vector preference for host infection status has been documented for several plant pathogens, particularly plant viruses. These studies show that vectors exhibit orientation preference, feeding preference, or both – with the gamut represented from strong preference for infected hosts (Eigenbrode *et al*., 2002) to discrimination against hosts with severe symptoms (Blua & Perring, 1992). Preferences may stem from changes in host nutritional quality, which can improve (Castle & Berger, 1993) or decline (Mauck *et al*., 2010) following viral infection, with vectors using visual (Hodge & Powell, 2008) or chemosensory (Medina-Ortega *et al*., 2009) cues to orient to preferred hosts. For example, the green peach aphid (*Myzus persicae*) uses plant volatile organic compounds to orient to solanaceous plants infected with *Potato leafroll virus* (Eigenbrode *et al*., 2002). Yet change in plant attractiveness and nutritional quality following infection may be at odds with each other, which is hypothesised to facilitate pathogen spread in some cases (Blua & Perring, 1992; Mauck *et al*., 2010). Despite substantial research on this subject little information exists outside of vector-borne plant viruses – the exception being a handful of studies on pollinator-associated pathogens (Jennersten, 1988; Altizer *et al*., 1998). Moreover, the implications of vector preference for pathogen transmission and spread have been largely overlooked. We investigated these issues for a leafhopper-borne bacterial plant pathogen.

Xylella fastidiosa Wells *et al*. is endemic to the Americas, and is widespread throughout the Western and Southeastern U.S.A. This xylem-limited bacterium is pathogenic to a wide variety of plants, including several important crop, native, ornamental, and weedy species (Purcell, 1997). In the Western U.S.A. the most economically important host is grapevine, in which *X. fastidiosa* causes Pierce's disease. Pathogen populations in grapevines increase over the course of several weeks following inoculation (Hill & Purcell, 1995). Multiplication of the bacterium in plants accentuates water stress by promoting vessel occlusion (McElrone *et al*., 2003). Thus plants only show disease symptoms at later time points, after pathogen populations have achieved high densities within the infected hosts. Pierce's disease symptoms manifest as progressive leaf scorch, including, reddening of leaves in from the margins, defoliation, 'match stick' petioles, uneven maturation of canes, and plant death (Purcell, 1997; Krivanek *et al*., 2005).

Historically, Pierce's disease prevalence in the Western U.S.A. was moderate, spread by certain native xylem sap-feeding insects (Purcell, 1997). Patterns of prevalence changed, however, following the invasion of the glassy-winged sharpshooter (*Homalodisca vitripennis* Germar), whose establishment coincided with dramatic Pierce's disease outbreaks in Southern California vineyards (Almeida *et al*., 2005). The impact of this invader, compared to native vectors, is likely attributable to high population densities near citrus plantings (Perring *et al*., 2001) or its ability to promote efficient secondary spread (Almeida *et al*., 2005). Whether innate differences in behaviour also contribute to *H. vitripennis*'s impact is not known.

Although *X. fastidiosa* is transmitted by several species of xylem sap-feeding insects, the most important group is the sharpshooter leafhoppers (Severin, 1949; Almeida *et al*., 2005). Vectors in this group are generally considered to be efficient at transmitting the pathogen to grapevines, although there is substantial variation in efficiency among species (Daugherty & Almeida, 2009). Sharpshooters are able to acquire and inoculate *X. fastidiosa* with no observable latent period in the insect (Purcell & Finlay, 1979). Studies have documented the time requirements for efficient acquisition and inoculation (Hill & Purcell, 1995, Daugherty & Almeida, 2009), with longer feeding periods tending to result in higher transmission rates. Notably, sharpshooter acquisition efficiency (i.e. proportion of vectors that become infectious during a given duration of feeding) is linked to *X. fastidiosa* populations in the grapevine (Hopkins, 1981) with threshold populations below which acquisition is unlikely (approximately 10^4 cells g^{-1} of plant tissue; Hill & Purcell, 1997).

Sharpshooters show orientation and feeding preference for different grapevine varieties, which may contribute to observed differences in transmission efficiency among varieties (Purcell, 1981). These insects also show fine-scale preferences for feeding sites, which in conjunction with heterogeneous infection levels in plants underlies differences in *X. fastidiosa* transmission efficiency among sharpshooter species (Daugherty *et al*., 2010a). However, it is not well known whether sharpshooters exhibit preference based on infection status. The only observations thus far come from South American sharpshooters on citrus, which show mixed results – one species exhibited no preference whereas the other discriminated against *X. fastidiosa* infected seedlings (Marucci *et al*., 2005). Given that *X. fastidiosa* disease symptoms mimic water stress (McElrone *et al*., 2003; Daugherty *et al*., 2010b), which is known to curtail sharpshooter feeding (Andersen *et al*., 1992), sharpshooters might be expected to discriminate against infected plants. If so, symptoms should reduce pathogen acquisition and spread. Thus, the expectation is that *X. fastidiosa* diseases should induce qualitatively different patterns of vector preference, due the degenerative nature of the pathogen (Purcell, 1997), relative to plant viruses that increase attractiveness (Eigenbrode *et al*., 2002; Medina-Ortega *et al*., 2009) or alternate between increasing and decreasing attractiveness over time (Werner *et al*., 2009). However, no studies have estimated *X. fastidiosa* transmission in a choice setting. Therefore, it is unclear to what extent differences in behaviour underlie ensuing patterns of Pierce's disease spread by different sharpshooter species.

We conducted a suite of behavioural assays to evaluate sharpshooter preference associated with hosts differing in infection status, and whether it differed among vector species. First, we measured orientation preference for different categories of grapevine infection and symptom classes, including the role of visual cues in orientation. Next we measured longer-term feeding preference in a field trial, which was used to determine whether species or individual variation in preference affected transmission efficiency.

Materials and methods

Latent infections

As a first test for vector orientation preference we evaluated whether sharpshooters distinguish between host plants based on infection status alone, in the absence of any visual symptoms. In July 2009 2-month-old, own-rooted grapevine (*Vitis vinifera* L.) cuttings (vars. Cabernet Sauvignon, and Rubired) growing in 10-cm pots were needle inoculated with *X. fastidiosa* (STL strain; Hill & Purcell, 1995) or were not inoculated (i.e. control plants). Twelve weeks later the infection status of inoculated vines was verified using quantitative PCR (Francis *et al*., 2006).

The following spring we conducted choice trials with *Graphocephala atropunctata* (Signoret) and *H. vitripennis*. Sharpshooters were from colonies established from insects collected originally in Riverside, California (for *H. vitripennis*) or near Forestville, California (for *G. atropunctata*). The progeny of the field collected *G. atropunctata* were reared on basil plants (*Ocimum basilicum* L.) and *H. vitripennis* were reared on a combination of basil, okra (*Abelmoschus esculentus* L.), and sunflower (*Helianthus annuus* L.). Plants were replaced approximately every 2 weeks to ensure that developing nymphs, which lose *X. fastidiosa* infection between moults (Purcell & Finlay, 1979), did not reacquire the pathogen.

Prior to the trials, grapevines were pruned to similar sizes (approx. 20 cm tall) and any symptomatic leaves were removed from the infected plants. At least some tissue was removed from all plants to limit any potential response by vectors to plant wounding. One control and one infected plant were then placed into a 60 × 60 × 60-cm mesh-and-clear-plastic cage (Bugdorm 2, Megaview Sciences Inc., Taiwan). Next an individual adult sharpshooter of one of the two species was released into the centre of the cage, after which they were observed every 20 min over 3 h, until the insect first chose one of the plants. A choice was recorded when the insect alighted on either one of the plants. After each trial the insect was discarded and the plants were replaced with a new pair. Thirty replicate trials were made for each of the vector species.

We compared the likelihood that sharpshooters first chose healthy or *X. fastidiosa* infected plants using a generalised linear model (GLM; procedure ‘glm()’ in the R programming language) with binomial error, for which vector species was a fixed effect (Crawley, 2009). This analysis tests the hypothesis that the two vector species respond differently to the two plant classes (i.e. healthy vs. infected-but-asymptomatic). In the event that there was no difference between the species, we pooled the two vectors and tested whether the overall proportion of insects that first chose infected plants was significantly less than 0.5 using a one-tailed binomial test (procedure ‘binom.test()’; Crawley, 2009). This second analysis tests the hypothesis that a significant minority of vectors alight on *X. fastidiosa* infected plants, which we assume represents orientation preference for healthy plants (Marucci *et al*., 2005).

Symptomatic and asymptomatic field cuttings

A second test for orientation preference consisted of a choice between field-collected symptomatic and asymptomatic cuttings. In October 2010 cuttings were taken from mature Cabernet Sauvignon grapevines from near Napa, California. For this collection we identified grapevines that showed evidence of Pierce’s disease, which were later verified to be positive based on plate culturing (Hill & Purcell, 1995). We collected one section from these cuttings that showed leaf scorch symptoms and another section that showed no foliar symptoms. Sections were returned to a greenhouse at the University of California, Berkeley, and stored at 4 °C. All samples were used in preference trials within 24 h of collection.

Symptomatic and asymptomatic sections were cut to 15–20 cm in length and then were placed in a 10-cm pot filled with wet sand. One symptomatic and one asymptomatic section, from the same grapevine, were placed into a cage and one adult *G. atropunctata* or *H. vitripennis* was added to the centre of the cage. As before, the position of each sharpshooter was noted every 20 min until they first landed on one of the two grapevine sections. Between trials pairs of plant sections were replaced. There were 24 replicate trials for *H. vitripennis* and 35 for *G. atropunctata*. We used a GLM with binomial error to test for species differences and a one-tailed binomial test to determine whether the pooled proportion of vectors first choosing symptomatic sections was less than 0.5.

Simulated symptoms

In a third test for orientation preference we used artificial colouring of plants to simulate Pierce’s disease. There is evidence that sharpshooters use colour cues for plant site selection (Rashed *et al*., 2011). Painted plants allowed us to evaluate the contribution of visual cues, alone, to vector preference. We painted 6-week-old healthy grape seedlings, grown in 10-cm pots, with water-soluble acrylic paint (Crayola LLC, Easton, Pennsylvania), such that they resembled advanced Pierce’s disease foliar symptoms. Specifically, seedlings were first painted with a 1.5-cm-wide stripe of red colour along the leaf edge. Then leaves were painted with a 0.5-cm-wide stripe of orange colour on the outer edge. This left approximately 2 cm^2 in the centre of most leaves that was not painted. In order to control for the effect of paint in our pairwise trials, all ‘asymptomatic’ seedlings were painted with a similar amount of green color.

To verify that the paint treatments accurately represented healthy and diseased tissue, we quantified color reflectance of painted, symptomatic, and asymptomatic leaves using an Ocean Optics USB4000 spectrometer (Dunedin, Florida) with a P1000-2-UV-VIS reflection probe. All measurements were recorded under a Leica M205FA microscope. Spectra were recorded at 5 nm intervals from 200 to 800 nm.

Trials with painted symptomatic and asymptomatic plants were conducted in 20 × 30 × 50-cm clear acrylic and mesh cages. Two equal-sized seedlings with three to four leaves (all leaves painted) were placed in the cages, after which we inserted a 20 × 30-cm cardboard sheet on top of the pots so that only plant tissue was exposed to the insects. Individual *G. atropunctata* or *H. vitripennis* adults were then introduced into the middle of the cage. Observations took place between 10.00 and 13.00 hours. Each trial lasted 2 h or until the insect made a choice. Plant pairs and experimental insects were discarded after each trial. In the occasional case where the insect did not make a choice within 2 h it was replaced with a different sharpshooter. There were 32 replicate trials for *H. vitripennis* and 40 for *G. atropunctata*. Again, we used a GLM with binomial error to test for species differences and a one-tailed binomial test to determine whether the pooled proportion was less than 0.5.

Field cage trial

We conducted a test of feeding preference for infection status using mature 'Chardonnay' grapevines, planted at the University of California, Riverside, Citrus Research Center and Agricultural Experiment Station. The 9-year-old vines were enclosed in 10.7 × 4.8 × 2-m cages made from 60% shade cloth to exclude local sharpshooters. Some of the vines had been needle inoculated with *X. fastidiosa* whereas others were not infected.

In September 2009 we identified pairs of infected (i.e. leaf scorch and stunting) and healthy grapevines in these cages. Infected vines had tested positive to earlier plate culturing or ELISA tests (Yonce & Chang, 1987). We used 25 × 17-cm mesh and clear plastic sleeve cages, into which we inserted the end of one cane from a symptomatic vine and one cane from a nearby healthy vine. Cages were placed over a single node, with one leaf per cane. We then introduced one adult sharpshooter of one of three species: *G. atropunctata*, *H. vitripennis*, or *Homalodisca lacerta* Fowler (smoke tree sharpshooter, collected originally in Riverside, California). Insects were from laboratory colonies known to be free of *X. fastidiosa* infection. Cages were secured to the cane at each end with twist ties to contain the insects. There were 20 replicates for each vector species.

Insects were held in the cages for 3 days. During this period we censused cages hourly between 8.00 and 18.00 hours. At each census we noted the location of each sharpshooter on the healthy cane, infected cane, or cage wall and, if on the canes, the site on the cane (stem, petiole, or leaf blade). It was not possible to determine definitely whether all vectors were feeding at each of these censuses, although we did notice anecdotally that excreta on the leaf surface or insect abdomen was common. After 3 days we removed sharpshooters and cultured the heads of all insects alive at the end of the trial to determine whether they had acquired *X. fastidiosa* (Almeida & Purcell, 2003). Because of insect mortality and some contamination of cultured plates, we obtained acquisition results from 41 samples (9, 14, and 18 from *H. lacerta*, *G. atropunctata*, *and H. vitripennis*, respectively).

We evaluated feeding preferences of sharpshooters by comparing the cumulative proportion of all censuses for which sharpshooters were on the infected canes. This proportion, which ignored censuses where sharpshooters were on the cage wall, was compared among sharpshooter species using one-way ANOVA, for which vector species was a fixed effect (Crawley, 2009). This analysis is analogous to the GLM comparisons in the greenhouse trials in that it is testing for species-specific differences in preference (i.e. feeding preference) for infected versus healthy plants. In the event that there was no difference between the species, we tested whether the model intercept was significantly less than 0.5 using a one-tailed t-test. In other words, if the main effect of species was not significant it was dropped from the model and the value of the constant was compared relative to 0.5 (Crawley, 2009). This second analysis tests, in an analogous manner to the greenhouse trials, whether all of the vectors spent collectively less than half of their time on the infected cane, which we assume represents feeding preference for healthy hosts.

To determine whether sharpshooters show different preferences for feeding sites we used MANOVA (Crawley, 2009) to compare the proportion of censuses for which insects were found on stems, petioles, or leaf blades, in which the three sites were the three response variables and vector species was a fixed effect. A significant effect was followed up with pairwise MANOVA between pairs of vector species, with Bonferroni correction for multiple tests (Crawley, 2009).

We evaluated differences in movement rates of the vectors in two ways. First, we compared the number of times an individual vector moved from healthy to infected canes, or vice versa, in adjacent censuses (i.e. number of switches). Next, we compared the number of censuses in which a sharpshooter moved onto the infected cane from either the healthy cane or the cage wall (i.e. visits to infected). Because we were not continually observing insects, these two metrics should be viewed as minimums. We compared the values of each of these metrics among vector species using two separate GLM with Poisson error (Crawley, 2009). Significant effects of vector species were followed by pairwise GLM tests among sharpshooters.

Finally, to determine whether vector preference affects transmission efficiency, we evaluated the likelihood that sharpshooters acquired *X. fastidiosa*. To do this we compared the proportion of culture-positive insect heads using GLM with binomial error, in which vector species was a fixed effect and the proportion of censuses a vector was found on the infected cane as a covariate.

Results

Latent infections

Sharpshooters did not differentiate between healthy and infected-but-asymptomatic vines. The likelihood that first visits were to infected vines did not differ significantly between the two vector species ($\chi_1^2 = 0.069$, $P = 0.7934$). *Graphocephala atropunctata* first oriented to infected plants in 12 of 30 replicates (40%), and *H. vitripennis* oriented to infected plants in 13 of 30 replicates (43%). The pooled proportion of first visits that were to infected plants, 0.417, did not differ significantly from 0.5 ($P = 0.1225$).

Symptomatic and asymptomatic field cuttings

As in the previous trial, the two sharpshooter species did not differ significantly in the proportion first visiting the symptomatic versus asymptomatic field-collected cuttings ($\chi_1^2 = 0.290$, $P = 0.5904$). However, both species were less likely to alight on the symptomatic plant. *Graphocephala atropunctata* first oriented to symptomatic plants in 11 of 35 replicates (31%), and *H. vitripennis* oriented to symptomatic plants in 6 of 24 replicates (25%). When the two species were pooled, the proportion of sharpshooters that first visited symptomatic cuttings was 0.288, which is significantly less than 0.5 ($P = 0.0008$).

Simulated symptoms

The mean peak reflectance (± SE) for the green paint ($n = 2$) and healthy leaf ($n = 5$) were recorded at 530.7 (± 7.9) and 551.9 nm (± 1.6), respectively (Fig. 1). Mean peak reflectance of the red paint ($n = 4$), orange paint ($n = 3$), and Pierce's disease leaf-scorch region ($n = 7$) were 598.7 (± 2.18), 591.5 (± 5.1), and 592.3 nm (± 4.6), respectively (Fig. 1). Thus, the painting treatments were reasonable reproductions of diseased foliage.

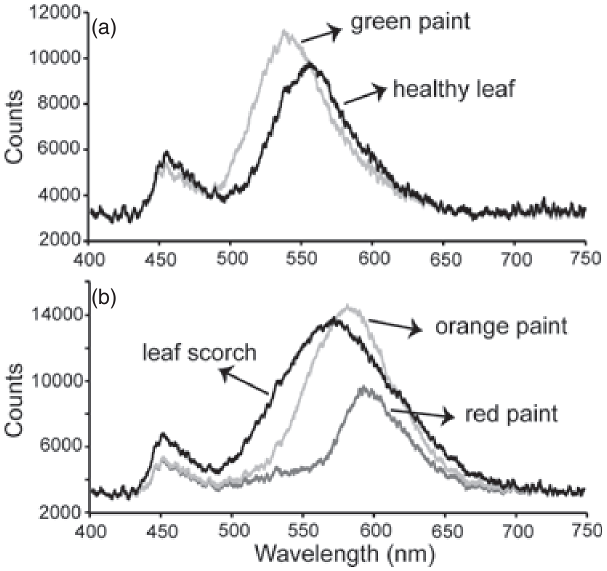

Fig. 1. Reflectance profiles of a healthy grapevine leaf or scorched region of Pierce's disease symptomatic leaves compared to (a) green or (b) orange and red paint treatments. Measurements made at 5 nm increments between 200 and 800 nm.

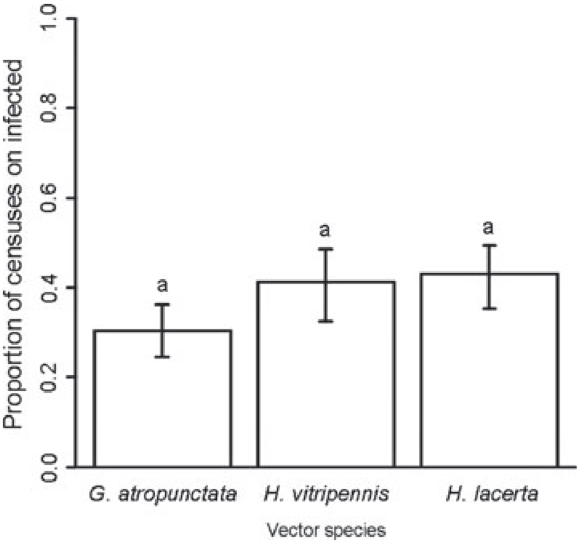

Fig. 2. Mean proportion (± SE) of censuses for which sharpshooters were on any part of the infected cane. Different letters above bars denote significant differences in proportions between (i.e. none) vector species.

There was no significant difference in first visitation rate between the two vector species ($\chi_1^2 = 0.161$, $P = 0.6883$). As in the previous trial, both sharpshooter species were less likely to alight on plants painted to look symptomatic. *Graphocephala atropunctata* first oriented to red and orange (i.e. symptomatic) plants in 13 of 40 replicates (32%), and *H. vitripennis* oriented to symptomatic plants in 9 of 32 replicates (28%). Together, the proportion of vectors that first visited symptomatic plants, 0.306, was significantly less than 0.5 ($P = 0.0006$).

Field cage trial

In the field, the mean (± SD) proportion of censuses that the three vectors spent collectively off of either the healthy or infected plant (i.e. cage wall) was 0.119 (± 0.090). Across all censuses there was not a significant difference among the vector species in the proportion of censuses spent on the infected cane ($F_{2,57} = 0.954$, $P = 0.3912$). However, the intercept (i.e. collective proportion of all three vectors) was significantly less than 0.5 (mean ± SE $= 0.381 \pm 0.041$; $t_1 = -2.927$, $P = 0.0049$). Mean values for the three vector species ranged between 0.43 for *H. lacerta* and 0.30 for *G. atropunctata* (Fig. 2). This equates to up to a 2.3-fold preference for spending time on healthy canes instead of infected canes (Fig. 2).

Sharpshooters showed a strong preference for site within the plant, which differed significantly among the vector species (Pillai's trace = 0.4974, d.f. = 2, Approx. $F_{6,112} = 6.180$, $P < 0.0001$). *Graphocephala atropunctata* was found overwhelmingly on leaf blades, and was rarely found on petioles and the stem (Fig. 3). Both *Homalodisca* spp. were found comparatively more often on petioles and stems.

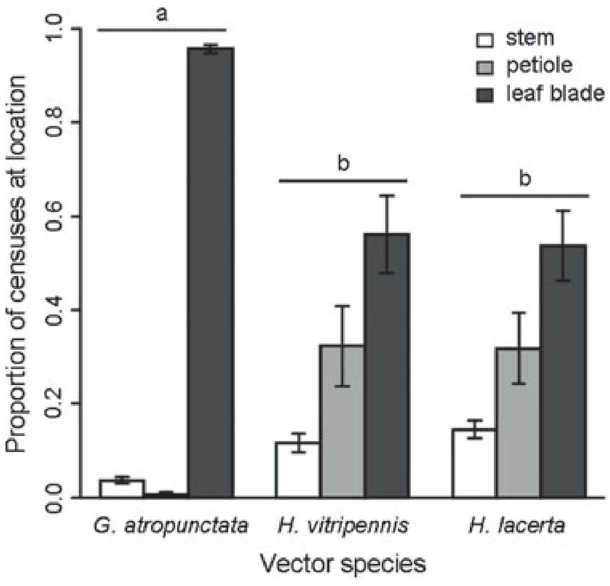

Fig. 3. Mean proportion (± SE) of censuses for which sharpshooters were on leaf stems, petioles, or leaf blades, regardless of cane infection status. Different letters denote significant differences in the collective proportions at the three locations between vector pairs.

Sharpshooter movement rates also differed significantly among vector species with respect to the number of unique movements onto infected canes ($\chi^2_2 = 13.254$, $P = 0.0013$) and the number of switches between infected and healthy canes ($\chi^2_2 = 22.252$, $P < 0.0001$). *Graphocephala atropunctata* moved more frequently based on both of these metrics than the two *Homalodisca* spp., which were similar (Fig. 4).

Overall 46% of sharpshooters successfully acquired *X. fastidiosa* during the 3-day trials. Despite differences in movement rates and preferences for feeding site, the likelihood of acquisition did not differ significantly among vector species ($\chi^2_2 = 1.309$, $P = 0.5197$). *Graphocephala atropunctata* acquired most frequently, in 8 of 14 replicates (57%), whereas *H. vitripennis* and *H. lacerta* acquired in 8 of 18 (44%) and 3 of 9 replicates (33%), respectively. Notably, there was a trend for those individuals that spent more time on infected canes to also be more likely to acquire the bacterium ($b \pm \text{SE} = 1.926 \pm 1.122$; Fig. 5). However this relationship between the proportion of censuses that a given sharpshooter spent on the infected cane and likelihood of pathogen acquisition was not significant ($\chi^2_1 = 3.158$, $P = 0.0755$).

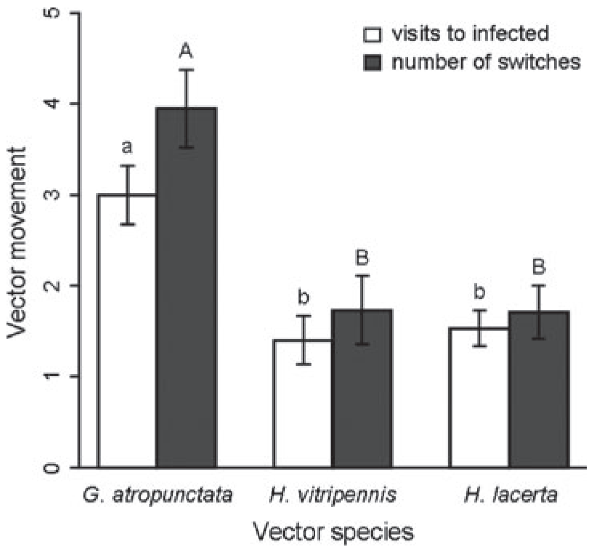

Fig. 4. Mean number of movements (± SE) made by sharpshooters among censuses. Different lower-case letters denote significant differences among vector species in the minimum number of times sharpshooters moved onto infected canes. Different upper-case letters denote significant differences among vector species in the minimum number of times vectors moved between infected and healthy.

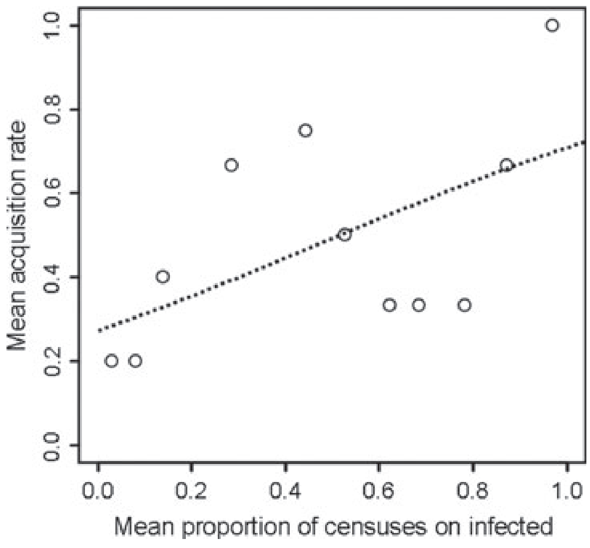

Fig. 5. Likelihood of vectors acquiring *Xylella fastidiosa* as a function of the proportion of censuses on the infected cane. Because the response variable is binary, the figure was created by ranking according to the proportion of censuses individual sharpshooters spent on the infected cane, then calculating a mean acquisition rate for groups of three to four vectors, based on natural breaks in the proportion of censuses on infected. The dotted line denotes the fit of the GLM.

Discussion

For vector-borne pathogens, it is becoming increasingly apparent that disease dynamics are contingent on behavioural decisions made by vectors at the scale of the individual host (Daugherty *et al*., 2010a), host population (McElhany *et al*., 1995), and host community (Kilpatrick *et al*., 2006). In this study we focused on vector behaviour at the population scale, evaluating the preference of leafhopper vectors based on host infection status. The results indicate that this important group of vectors, including the invasive *H. vitripennis*, consistently avoids diseased hosts during both orientation and feeding. Ultimately, this discrimination against infected hosts may affect transmission by reducing vector exposure to the pathogen.

Studies of vector preference often show initial attraction to infected hosts, which stem from pathogen-induced changes in host phenotype (Baker, 1960; Eigenbrode *et al*., 2002; Medina-Ortega *et al*., 2009; Mauck *et al*., 2010). Yet other studies have demonstrated avoidance of infected hosts (Jennersten, 1988; Blua & Perring, 1992; Altizer *et al*., 1998). In the orientation preference trials we conducted, the two sharpshooter species tested, *G. atropunctata* and *H. vitripennis*, behaved similarly. Neither vector appeared to distinguish between healthy and infected-but-asymptomatic plants. Thus, *X. fastidiosa* infection, alone, does not appear to be sufficient to elicit an orientation preference from the vectors. However, the sharpshooters were up to three times more likely to first choose asymptomatic versus Pierce's disease symptomatic cuttings collected from the field, and were similarly more likely to first choose grapevines painted to look healthy over those with symptomatic coloration. Collectively, these results suggest that sharpshooters discriminate against grapevines exhibiting Pierce's disease symptoms, in a manner similar to pollinator avoidance of flowers infested with anther smut fungi (Jennersten, 1988; Altizer *et al*., 1998).

Many insects use visual and/or olfactory cues for orientation and host selection (Prokopy & Owens, 1983; Hardie, 1989; Fereres & Moreno, 2009). Understanding the role of pathogen-mediated effects on host-plant morphology and physiology is important in vector-borne pathogen systems as these changes can manipulate host-plant palatability and profitability to vectors (Hodge & Powell, 2008), and subsequently affect pathogen distribution (Fiebig *et al*., 2004). Leafhoppers are known to use visual cues for orientation purposes and previous studies indicated their attraction to bright yellow wavelengths (Patt & Setamou, 2007; Fereres & Moreno, 2009). The results of the orientation preference trials suggest that sharpshooters avoid leaf colours associated with Pierce's disease symptoms. However, we did not evaluate explicitly the role of chemosensory cues in vector host preference (Eigenbrode *et al*., 2002). Therefore, the possibility exists for these vectors that olfaction may function as a synergistic stimulator in conjunction with visual cues (Patt & Setamou, 2007).

Studies of aphid-borne plant viruses suggest that feeding preference based on infection status may be an inherent feature of the transmission mode. The spread of non-persistent viruses, whose transmission requires relatively short feeding periods, should be favoured by increasing the attractiveness of infected hosts to vectors then encouraging them to disperse by reducing the nutritional quality of infected hosts (Mauck *et al*., 2010). Conversely, because persistent viruses require extended feeding periods for transmission, spread is favoured by arresting vectors through increased nutritional quality of infected hosts (Eigenbrode *et al*., 2002; Medina-Ortega *et al*., 2009). *Xylella fastidiosa* transmission biology is analogous to persistent viruses in that once the pathogen is acquired by adult sharpshooters it is maintained for the duration of the insect's life, albeit in the foregut (Severin, 1949; Purcell & Finlay, 1979). In addition, like persistent viruses, feeding periods on the order of several hours to days are required for efficient sharpshooter transmission of *X. fastidiosa* (Daugherty & Almeida, 2009). *Graphocephala atropunctata* efficiency after 1 h acquisition access period resulted in only 30% transmission success, whereas transmission rates given periods of 24 h or more were approximately 90% (Purcell & Finlay, 1979). However, there is no evidence that this pathogen manipulates positively either the attractiveness or nutritional quality of infected plants for sharpshooters. Instead, in our field experiment we found that all three vector species tested showed similar patterns of feeding preference, with all three spending more time on healthy grapevine canes. Sharpshooter discrimination against symptomatic grapevines appears to be a general behaviour of this group of vectors, one which is consistent with other pathogens that reduce host quality (Jennersten, 1988; Blua & Perring, 1992). As xylem sap-feeding insects, sharpshooters must process many times their body weight (up to 1000 times – Mittler, 1967) in plant fluid each day. More severe xylem tension associated with water stress impinges on their ability to do so (Andersen *et al*., 1992). Thus, it is plausible that plants infected with *X. fastidiosa* are perceived by sharpshooters as poor quality hosts because they exhibit physiological traits that are consistent with increased water stress (Daugherty *et al*., 2010b). It is worth considering, however, whether such discrimination against infected hosts varies among host species. Sharpshooters, particularly *H. vitripennis* and *X. fastidiosa*, are notable for having broad host ranges; upwards of 300 and 100 species, respectively (CDFA, 2011; Purcell, 2011). Pathogen infection level and symptom severity differ among these host species (Hill & Purcell, 1995; Purcell, 1997). Therefore, attempts to understand whether these alternate hosts act as pathogen reservoirs should consider how sharpshooters respond to infection in these host species.

It is important to note that the effects of pathogen infection on host phenotype may not be consistent over time. For example, shortly after inoculation *Zucchini yellow mosaic virus* infected plants are higher-quality hosts for aphids than are healthy hosts, but weeks later they are of significantly lower quality (Blua & Perring, 1992). Such trends in quality may drive changes in vector preference over time. For example, aphid emigration from *Potato leafroll virus* infected plants at different times post inoculation was similar to that from healthy plants at early and late timescales, but was significantly higher at intermediate timescales (Werner *et al*., 2009). Our experiments evaluated sharpshooter preference at a single time point – generally at low and high extremes of the disease state. Therefore it is not possible to determine from this study whether sharpshooter preference switches as disease progresses. However, Pierce's disease is a chronic, degenerative disease that disrupts xylem sap transport, plant growth, and fruit production (Purcell, 1997; Krivanek *et al*., 2005). At later disease stages vector discrimination against symptoms is likely to be more pronounced, which should reduce pathogen spread (M.P. Daugherty, unpublished). But we don't expect to see qualitative shifts in the trajectory of sharpshooter preference for host infection status over a gradient of disease severity.

We found substantial individual variation in the amount of time sharpshooters spent on infected grapevine canes in the field trial, ranging from 0 to nearly 97% of censuses. This is notable because those individuals that spent less time on infected plants tended to acquire the pathogen at a lower frequency. This result needs further testing, but it is interesting because it provides the link between vector behaviour and disease epidemiology that is postulated by various models (Kingsolver, 1987; McElhany *et al*., 1995; Sisterson, 2008), yet which has been largely untested in studies of vector behaviour. Although it may be safe to assume that a vector-host contact equates to a transmission event in the case of highly efficient vectors, for less efficient vectors this is not necessarily the case. For relatively less efficient vectors the 'quality' of a contact moderates acquisition or inoculation success. Sharpshooter transmission of *X. fastidiosa*, for example, depends strongly on the duration of feeding and frequency of unique probes (Daugherty *et al*., 2009). Therefore simply noting vector preference in the absence of transmission provides an incomplete metric of the epidemiological significance of vector behaviour in certain pathosystems. This link between individual preference and transmission has been documented for pollinator-associated fungal pathogens. In those systems bees tend to avoid infected flowers, but individuals that visited infected flowers more often were more likely to pick up fungal spores (Altizer *et al*., 1998). Thus these results support the prediction that greater vector residency on infected hosts promotes greater pathogen acquisition. This is likely to be a general phenomenon for pathosystems in which transmission efficiency requires protracted feeding. Other studies of vector behaviour need to consider transmission, because differences in behaviour may explain differences in transmission efficiency among vector species.

Sharpshooter transmission of *X. fastidiosa* is highly context dependent, with several ecological factors underpinning variation in transmission efficiency (Almeida & Purcell, 2003; Daugherty & Almeida, 2009; Lopes *et al*., 2009). Species-dependent transmission rates may stem from innate differences in their perception of the quality of host species or differences in preferred feeding sites within a host (Daugherty *et al*., 2010a). Thus, we expected the differences in sharpshooter movement rates and feeding site preferences in the current study to drive differences in acquisition rates among the vectors. Although *G. atropunctata*, the vector that moved with the greatest frequency and had the strongest preference for leaf tissue, was marginally more efficient at acquiring *X. fastidiosa* than were the two *Homalodisca* spp., the difference was not significant. This result contradicts the general view of *G. atropunctata* being the most efficient vector of *X. fastidiosa* to grapevines in the Western U.S.A. (Almeida & Purcell, 2003) – two- to four-fold more efficient over a range of conditions than is the invasive *H. vitripennis* (Daugherty & Almeida, 2009). However these previous efficiency tests were conducted under no-choice conditions. Under conditions where choice is possible, which is arguably more representative of field conditions than are no-choice bioassays, such differences among species appear to be depressed.

Epidemiological models predict that the extent to which vectors are attracted to and retained on infected hosts determines the likelihood or severity of disease outbreaks (Kingsolver, 1987; McElhany *et al*., 1995; Sisterson, 2008). Unlike vector-borne plant viruses that facilitate attraction to infected hosts (Medina-Ortega *et al*., 2009; Mauck *et al*., 2010), and increase retention on infected hosts (Eigenbrode *et al*., 2002), *X. fastidiosa* reduced vector preference during both orientation and feeding phases. Under most conditions, particularly in the early phases of an outbreak (McElhany *et al*., 1995), this consistency in response should constrain pathogen transmission and spread (Kingsolver, 1987). The invasion of *H. vitripennis* into Southern California precipitated Pierce's disease outbreaks on a scale much greater than native sharpshooters appear to have caused (Almeida *et al*., 2005). Yet, given the similarity in preference we observed among sharpshooter species, vector preference for infection status is not likely to contribute appreciably to differential Pierce's disease incidence caused by native versus invasive sharpshooters. Instead, the impact of the invasive *H. vitripennis*, which is not a more efficient vector (Almeida & Purcell, 2003; Daugherty & Almeida, 2009), is likely to be tied to some other aspect of its ecology.

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