Persistence of *Xylella fastidiosa* in Riparian Hosts Near Northern California Vineyards

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ABSTRACT

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The spread of Pierce's disease (PD) from riparian hosts to grapevines in California's north-coastal grape-growing region is a function of the proportion of *Graphocephala atropunctata* (blue-green sharpshooters [BGSSs]) that acquire *Xylella fastidiosa* from infected plant tissue. Riparian hosts that do not maintain sufficient *X. fastidiosa* populations for acquisition may not be significant inoculum reservoirs. We examined *X. fastidiosa* populations in systemically infected riparian hosts (California blackberry, California grapevine, elderberry, Himalayan blackberry, periwinkle) at two coastal locations (Mendocino and Napa) with two methods of quantitation (culturing and real-time polymerase chain reaction) from 2003 to 2004. In summer and autumn, *X. fastidiosa* populations were above the threshold for BGSS acquisition in periwinkle, Himalayan blackberry, and California grapevine at both locations. The only *X. fastidiosa*-positive plants detected in spring at both locations were periwinkle and Himalayan blackberry, suggesting that these species may contribute to long-term survival of *X. fastidiosa*. California blackberry and elderberry may not be important reservoirs of *X. fastidiosa*, given that very few plants of either species maintained infections. Higher *X. fastidiosa* populations in California grapevine, Himalayan blackberry, and periwinkle in Napa, relative to plants in Mendocino, may partially explain the higher PD incidence in Napa vineyards.

In the north-coastal grape-growing region of California, *Xylella fastidiosa* Wells et al., the bacterium that causes Pierce's disease (PD; 4), is vectored by a native sharpshooter, *Graphocephala atropunctata* (Signoret) (*Hemiptera: Cicadellidae*) (blue-green sharpshooter [BGSS]; 6,15). Purcell (14,15) demonstrated a direct relationship between incidence of PD and proximity to riparian vegetation bordering vineyards. The concentration of diseased grapevines decreases with increasing distance from riparian vegetation. BGSSs feed and breed on riparian plants (6,15). Not only do some riparian plants provide habitat for BGSSs, but they also host *X. fastidiosa* (22). *Homalodisca coagulata* (Say) (glassy-winged sharpshooter), an introduced vector of PD in southern California, does not currently occur in the north-coastal grape-growing region.

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The ability of *X. fastidiosa* to multiply and spread within a host varies among species. Hill and Purcell (7) found that *Rubus discolor* Weihe & Nees (Himalayan blackberry) supported systemic infections, whereas *Artemisia douglasiana* Besser (mugwort) and *Echinochloa crus-galli* (L.) P. Beauv. (watergrass) only supported non-systemic infections. Differences among these riparian hosts have important epidemiological consequences. In systemic hosts, *X. fastidiosa* multiplies and infects tissue beyond the inoculation site, thereby increasing the likelihood of vector acquisition and spread to other hosts. In nonsystemic hosts, *X. fastidiosa* multiplies at the inoculation site but does not spread (20). The lack of systemic movement of *X. fastidiosa* within these hosts limits their epidemiological importance as pathogen reservoirs.

Changes in temperature (3) and plant hormones (9) affect *X. fastidiosa* populations which, in turn, affect BGSS transmission (8). In general, as temperature increases, *X. fastidiosa* population densities increase in host plants (3). Seasonal fluctuations in *X. fastidiosa* populations in plants are associated with changes in rates of transmission by BGSSs. Previous research indicated that acquisition of the pathogen from an infected host by the BGSS requires at least 10^4 CFU of *X. fastidiosa* per gram of plant tissue (8). Seasonal declines to levels below this threshold reduce the spread of PD to grapevines by limiting the proportion of BGSSs that acquire *X. fastidiosa*. Conversely, perennial riparian hosts in which *X. fastidiosa* populations remain above 10^4 CFU/g of tissue may serve as important long-term inoculum sources.

The goal of our research was to further characterize the potential for common riparian species that are known reservoirs of *X. fastidiosa* to serve as inoculum sources in two important grape-growing regions of California, Mendocino and Napa Counties. To this end, we quantified temporal variation in *X. fastidiosa* populations in five systemic hosts: *R. discolor* (Himalayan blackberry), *R. ursinus* Cham. & Schldl. (California blackberry), *Sambucus mexicana* C. Presl (elderberry), *Vinca major* L. (periwinkle), and *Vitis californica* Benth. (California grapevine). All five host species are potentially important in both grape-growing regions because they also are feeding and breeding hosts of the BGSS (6,15). Our objective was to examine temporal changes in *X. fastidiosa* populations in petioles and stems of riparian hosts to determine which hosts are likely to harbor sufficient *X. fastidiosa* populations for BGSS acquisition.

MATERIALS AND METHODS

In October 2002, 100 plants of each of the five riparian host species were established in 14-liter pots in the greenhouse. In February 2003, they were mechanically inoculated with *X. fastidiosa* strain STL (American Type Culture Collection 700963), a PD strain that was isolated originally from symptomatic grapevines in the Stag's Leap Appellation, Napa County, CA. Inoculum consisted of a turbid suspension in phosphate-buffered saline (PBS) of cells (approximately 10^8 cells/ml) collected from a 7-day-old culture on solid PD2 agar (10). Plants were inoculated in the greenhouse by depositing a 20-µl drop of inoculum onto the plant stem and piercing the stem underneath the drop with a pin (7). In July 2003, plants were confirmed to be *X. fastidiosa* positive with polymerase chain reaction (PCR) analysis, using the primers of Minsavage et al. (12) and Pooler et al. (13). Infected plants were transferred to screenhouses at two locations in the north-coastal grape-growing region of California: Oakville (Napa County) and Hopland (Mendocino County). At each location, we randomly distributed 185 infected plants, which in-

cluded 36 California blackberry, 38 California grapevine, 38 elderberry, 43 Himalayan blackberry, and 30 periwinkle. Five PBS buffer-inoculated controls of each species also were placed in each screenhouse. Vineyards throughout Mendocino and Napa Counties commonly border riparian habitats that support the five riparian hosts we examined. Average monthly temperatures were recorded by California Irrigation Management Information System (CIMIS) weather stations at both locations (Mendocino, Hopland Station #85; Napa, Oakville Station #77).

X. fastidiosa in riparian hosts at each location was quantified in October 2003, February 2004, June 2004, and August 2004. For each sampling period, a petiole located distal to and within approximately 20 cm of the stem inoculation site was collected from each plant. Additionally, we restricted petiole sampling to leaves that were fully expanded and nonsenescent. Samples were not collected from plants that did not have leaves meeting the sampling criteria. Stem tissue was collected starting in February 2004. Each sampling period required 2.5 weeks to collect and process all samples from both sites. Samples from only one plant species were collected per day, one site at a time, due to the large number of samples. On the day of collection, tissues were trimmed to a weight of approximately 0.1 g and stored at room temperature overnight. Tissue samples were processed the day following collection.

Our culturing technique was modified from that of Hill and Purcell (7) to accommodate a tissue homogenizer and a spiral plater, which allowed us to process up to 90 samples per day. Surface sterilization of each sample (1 min in 20% bleach, 10 s in 95% ethanol, and 10 s in sterile water) was followed by homogenization in 1 ml of PBS buffer in enzyme-linked immunosorbent assay mesh sample bags (Agdia Inc., Elkhart, IN) with a Homex 6 plant tissue homogenizer (Bioreba Ag, Reinach, Switzerland). From the 1-ml sap extraction, a spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) was used to deposit a 50-µl subsample on each of three plates of PD2 agar (10) amended with benomyl WP (4 mg/ml). Colonies were counted after incubation at 28ºC for 10 days. Results from plates with colonies, expressed as CFU/g of tissue, were averaged for each tissue sample and used for data analyses. On each day of culturing, we included negative controls (tissues from PBS buffer-inoculated control plants and PBS buffer) and positive controls (*X. fastidiosa* cultured from freeze-dried cells stored at –80ºC).

From the same 1-ml sap extraction used for spiral plating, a 500-µl subsample was used for DNA extraction, using the procedure of Zhang et al. (23). The real-time PCR procedure we used was modified from that of Schaad et al. (21), using DNA primers XfF1 and XfR1 to amplify the ribosomal DNA 16S-23S internal transcribed spacer (ITS). For PCR, 1 µl of DNA extract was added to 24 µl of the following mixture: Reaction Buffer (Qiagen, Valencia, CA; proprietary blend of Tris-HCL, KCL, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, pH 8.7), 3.5 mM $MgCl_2$ (Qiagen), 100 µM each dNTP (Amersham, Piscataway, NJ), 300 nM XfF1 and 300 nM XfR1 (Invitrogen, Carlsbad, CA), 100 nM 5′ 6FAM-labeled 3′ TAMRA-labeled ITS probe (MWG Biotech, High Point, NC), 0.5 units of HotStar Taq DNA polymerase (Qiagen), 500 nM ROX Reference Dye (Invitrogen), and sterile molecular biology grade water (GIBCO; Invitrogen). All reactions were performed in 200-µl, 96-well plates in an Mx3000p Real-time PCR Thermalcycler (Stratagene, La Jolla, CA). PCR cycling parameters were 95ºC for 15 min, 40 cycles at 95ºC for 15 s, and 62ºC for 1 min. Estimates of *X. fastidiosa* populations based on real-time PCR analysis represent the average number of cells per gram of tissue from three replicate PCR runs per tissue sample.

In each set of real-time PCR runs, we included samples of known cell concentrations to generate a standard curve, from which *X. fastidiosa* populations were estimated based on their cycle threshold (C_T) values. The standard curve was constructed by plotting the mean C_T values of the $\log_{10}$ of serial 10-fold dilutions of *X. fastidiosa* DNA, corresponding to 6×10^2 to 6×10^8 cells/ml. A sample was considered positive when its fluorescence signal was greater than five standard deviations of the background primary fluorescence. The cycle at

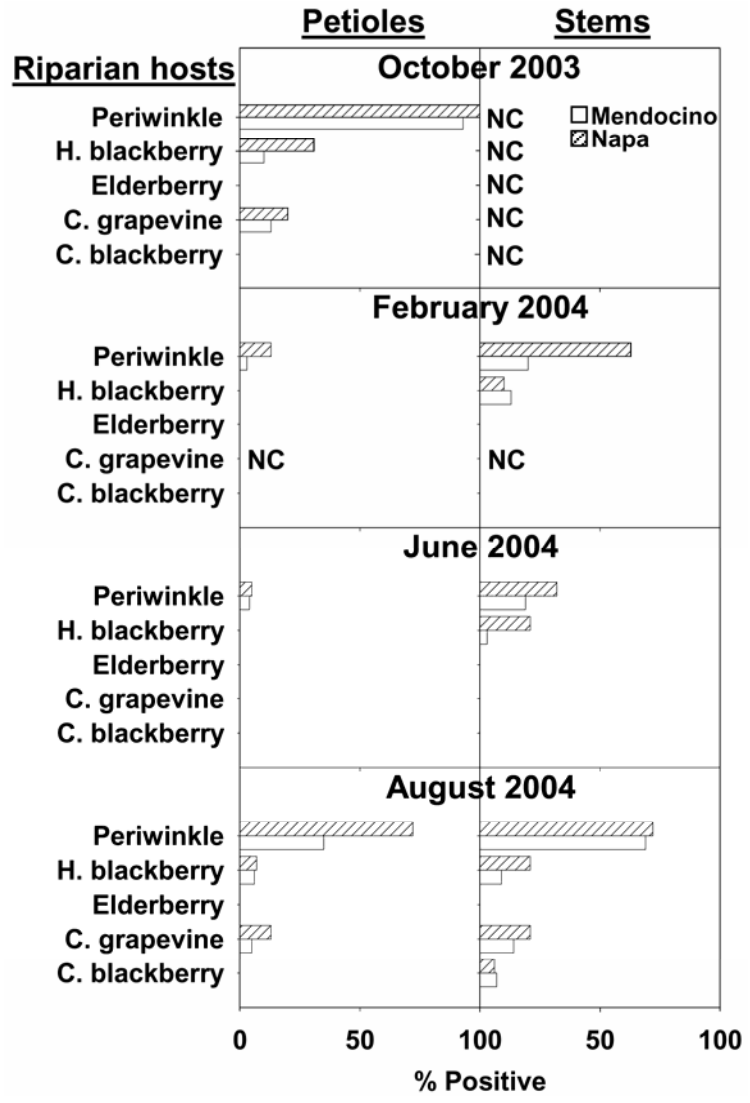

Fig. 1. Percentage of riparian hosts from which *Xylella fastidiosa* was cultured at two locations in northern California. Riparian hosts included periwinkle (*Vinca major*), Himalayan blackberry (H. blackberry, *Rubus discolor*), elderberry (*Sambucus mexicana*), California grapevine (C. grapevine, *Vitis californica*), and California blackberry (C. blackberry, *Rubus ursinus*). In July 2003, plants that were confirmed infected were placed in screenhouses at both locations. At each location, petioles were collected from between 19 and 43 plants per species for each of four sampling periods: October 2003, February 2004, June 2004, and August 2004. Stems were not collected (NC) until February 2004. Tissues were not collected from California grapevine in February 2004, when it was dormant.

which a sample's signal exceeded the background fluorescence, the C_T value, then was used to calculate the number of *X. fastidiosa* cells per milliliter in the sample with the formula obtained from the slope of the regression line from the standard curve.

Data were analyzed using the MIXED procedure in SAS (SAS System, version 8.2; SAS Institute Inc., Cary, NC) with the Kenward-Roger method of calculating degrees of freedom. Experiments were treated as completely randomized designs with separate analyses for colony counts in culture and real-time PCR data. Location, sampling period, and species were treated as fixed effects. Sampling periods were treated as repeated measures. Tukey's tests were used to compare treatment means. A $\log_{10}$ transformation was applied to all *X. fastidiosa* population data to normalize variances. Reverse-transformed means and confidence limits are presented.

With only one *X. fastidiosa*-positive plant for some location by sampling period by species combinations, it was not possible to perform a four-way analysis of variance (ANOVA) for results from each quantitation method. Instead, a series of seven ANOVAs that best utilized the most complete data sets was performed. A two-way ANOVA was used to determine main and interactive effects of location (Mendocino or Napa) and sampling period (October 2003, February 2004, June 2004, or August 2004) on *X. fastidiosa* populations in periwinkle petioles, as quantified by real-time PCR, and stems, as quantified by both colony counts in culture and by real-time PCR. A two-way ANOVA was used to determine main and interactive effects of location and sampling period on *X. fastidiosa* populations in Himalayan blackberry stems, as quantified by real-time PCR. Between-period comparisons involving stem tissue were restricted to February 2004, June 2004, or August 2004 (stems were not collected in October 2003).

Among-species comparisons were restricted to October 2003 and August 2004, sampling periods in which there were at least two *X. fastidiosa*-positive plants of several species at both locations. A two-way ANOVA was used to determine main and interactive effects of location and species (California grapevine, Himalayan blackberry, or periwinkle) on *X. fastidiosa* populations in petioles collected in October 2003, as quantified by both culture and real-time PCR. A three-way ANOVA was used to determine main and interactive effects of location, species, and tissue on *X. fastidiosa* populations in petioles and stems from California grapevine, Himalayan blackberry, and periwinkle collected in August 2004, as quantified by culturing.

RESULTS

Periwinkle had the highest percentage of *X. fastidiosa*-positive plants at all sampling periods, at both locations, with both quantitation methods, and in both tissue types (Figs. 1 and 2). Periwinkle had the highest populations of *X. fastidiosa* compared with Himalayan blackberry and California grapevine in October 2003, as quantified by both culturing (Table 1; Fig. 3) and real-time PCR (9.07×10^7 cells/g; $n = 60$; Table 1). Similar results were found in August 2004, based on culturing from both petioles and stems (Table 2); periwinkle had the highest populations at both locations (2.39×10^5 CFU/g; $n = 69$).

In periwinkle, we detected similar temporal changes in *X. fastidiosa* populations with both methods and in both tissue types (Table 3). Based on real-time PCR, populations in periwinkle tissues declined significantly to their lowest point in June 2004, followed by a significant increase in August 2004 (Fig. 4A and B). Based on colony counts, *X. fastidiosa* populations in periwinkle stems were lowest in June 2004 (6.39×10^3 CFU/g; $n = 11$) and highest in August 2004 (2.41×10^5 CFU/g; $n = 39$), with February 2004 being intermediate (6.00×10^4 CFU/g; $n = 25$).

We found significant differences in *X. fastidiosa* populations in petioles and stems of periwinkle ($P < 0.0001$). Petioles had significantly lower populations (8.97×10^6 cells/g; $n = 126$) than stems (5.74×10^7 cells/g; $n = 135$) at all sampling periods, based on real-time PCR results. We also found significant differences in *X. fastidiosa* populations in periwinkle at the two locations (Table 3). Based on real-time PCR results for all four sampling periods,

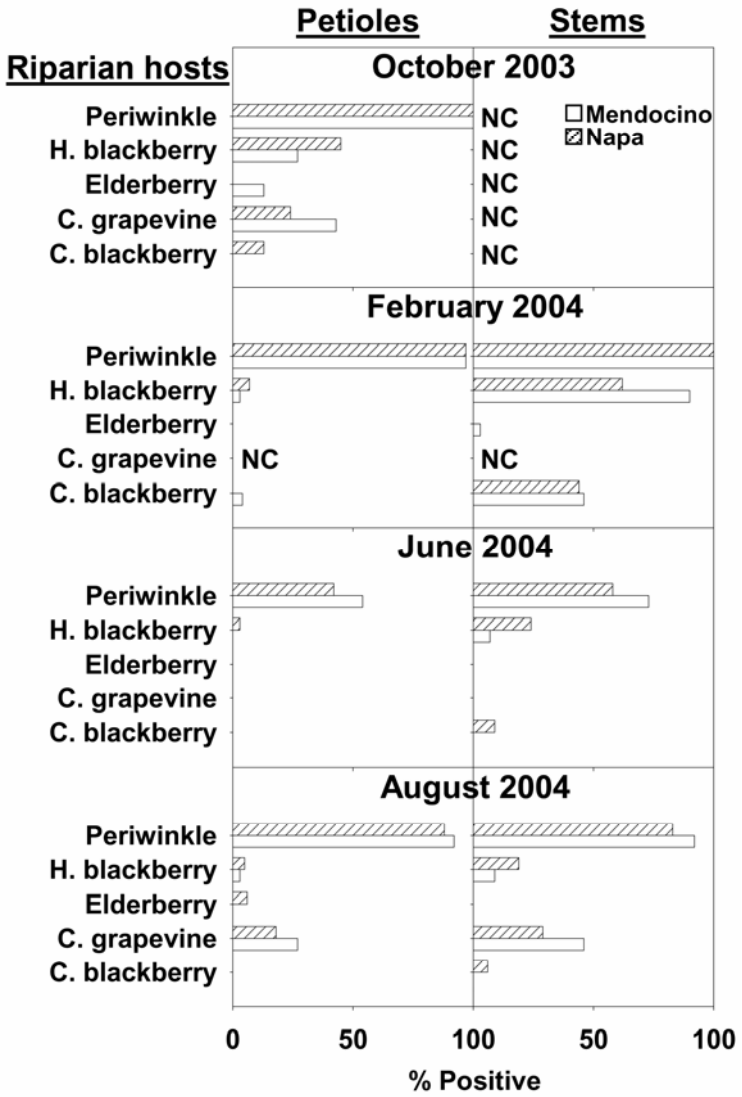

Fig. 2. Percentage of riparian hosts from which *Xylella fastidiosa* was detected by real-time polymerase chain reaction at two locations in northern California. Riparian hosts included periwinkle (*Vinca major*), Himalayan blackberry (H. blackberry, *Rubus discolor*), elderberry (*Sambucus mexicana*), California grapevine (C. grapevine, *Vitis californica*), and California blackberry (C. blackberry, *Rubus ursinus*). In July 2003, plants that were confirmed infected were placed in screenhouses at both locations. At each location, petioles were collected from between 19 and 43 plants per species for each of four sampling periods: October 2003, February 2004, June 2004, and August 2004. Stems were not collected (NC) until February 2004. Tissues were not collected from California grapevine in February 2004, when it was dormant.

periwinkle petioles in Napa had significantly higher populations (2.41×10^7 cells/g; $n = 90$) than those in Mendocino (9.78×10^6 cells/g; $n = 97$).

X. fastidiosa was detected consistently in Himalayan blackberry at all sampling periods, at both locations, and with both quantitation methods (Figs. 1 and 2). In August 2004, *X. fastidiosa* populations in Himalayan blackberry (5.71×10^4 CFU/g; $n = 17$) were intermediate between those of periwinkle (2.39×10^5 CFU/g; $n = 69$) and California grapevine (1.34×10^4 CFU/g; $n = 20$), based on colony counts in culture (Table 2). Temporal changes in *X. fastidiosa* populations in Himalayan blackberry stems did not follow the same trend as those of periwinkle, based on real-time PCR. Although populations in periwinkle were lowest in June 2004 (Fig. 4A and B), they were lowest in August 2004 for Himalayan blackberry (Fig. 5). Populations in Himalayan blackberry stems declined from February to August 2004 in Mendocino, but remained high in Napa through all three sampling periods (Fig. 5); hence the significant location–sampling period interaction ($P = 0.0015$).

California grapevine tested *X. fastidiosa* positive at both locations and with both quantitation methods in October 2003 and August 2004 (Figs. 1 and 2). In October 2003, California grapevine petioles had the lowest populations in Mendocino compared with those of Himalayan blackberry and periwinkle; however, in Napa, they were almost as high as that of periwinkle (Table 1; Fig. 3). Based on real-time PCR results for the same sampling period (October 2003), *X. fastidiosa* populations in California grapevine petioles were second to those of periwinkle at both locations (1.54×10^6 cells/g; $n = 19$) (Table 1). In August 2004, California grapevine had the lowest populations in petioles and stems at both locations (1.34×10^4 CFU/g; $n = 20$), based on culturing (Table 2).

In August 2004, when the number of *X. fastidiosa*-positive plants was high, based on colony counts in culture (Fig. 1), meaningful among-species comparisons between the two locations were possible. In this case, we found similar location effects for petioles and stems of California grapevine, Himalayan blackberry, and periwinkle (Table 2) and significantly higher *X. fastidiosa* populations in Napa (2.53×10^5 CFU/g; $n = 67$) than in Mendocino (1.27×10^4 CFU/g; $n = 39$). In October 2003, all three species, again, had significantly higher *X. fastidiosa* populations in Napa (5.87×10^6 cells/g; $n = 53$) than in Mendocino (1.76×10^6 cells/g; $n = 55$), as quantified in petioles by real-time PCR (Table 1). Among-species comparisons based on colony counts in October 2003 were slightly different, due to a significant location–species interaction (Table 1); populations in California grapevine and periwinkle were significantly higher in Napa, but there were no significant location differences in *X. fastidiosa* populations in Himalayan blackberry (Fig. 3).

Few *X. fastidiosa*-positive California blackberry and elderberry plants were detected, regardless of method (Figs. 1 and 2). Culturing revealed no *X. fastidiosa*-positive petioles from California blackberry or elderberry in any sampling period and only four positive stems from California blackberry (all collected in August 2004). All other *X. fastidiosa*-positive samples from California blackberry and elderberry were detected by real-time PCR. For California blackberry, only 5 of 37 *X. fastidiosa*-positive samples were from petioles, whereas 6 of 7 *X. fastidiosa*-positive elderberry samples were from petioles.

The average temperatures for 30 days prior to each sampling period in Mendocino were 21ºC, September 2003; 7ºC, January 2004; 15ºC, May 2004; and 22ºC, July 2004. For Napa, the average temperatures were 19ºC, September 2003; 9ºC, January 2004; 17ºC, May 2004; and 19ºC, July 2004. At both locations, the average temperatures for three consecutive months prior to the February 2004 sampling period were ≤10ºC.

DISCUSSION

Using repeated tissue sampling and two methods of quantitation, we examined the potential of five common riparian hosts (California blackberry, California grapevine, elderberry, Himalayan blackberry, and periwinkle) to serve as important inoculum sources of *X. fastidiosa* to vineyards in Mendocino and Napa Counties. Our findings that *X. fastidiosa* achieved sufficient *X. fastidiosa* populations for acquisition, $\geq 10^4$ CFU/g tissue (8), in periwinkle for three of four sampling periods (August, October, and February), and in both Himalayan blackberry and California grapevine for two of four sampling periods (August and October) indicate that the presence of these species in proximity to vineyards is likely to increase the risk of PD. In contrast, the extremely low detection frequency of *X. fastidiosa* in California blackberry and elderberry indicates that these hosts may add little risk of PD in adjacent vineyards. Although it has been shown that grapevines infected with *X. fastidiosa* before June are more likely than those infected after June to develop permanent PD (2,19), our results suggest that the spring reservoir of *X. fastidiosa* in riparian areas where these five riparian hosts occur may be limited.

Despite the fact that none of the riparian hosts we examined had sufficient *X. fastidiosa* populations for acquisition in spring, this does not diminish their significance as inoculum reservoirs for acquisition in summer and autumn. The BGSSs that feed on grapevines in spring are the overwintering adults, the spring distribution of which is reflected in the distribution of diseased grapevines (15). Most overwintering adults that are infective in spring likely acquire *X. fastidiosa* prior to winter, as evidenced by the increasing proportion of infective BGSSs in Napa vineyards in July to September (15). Additional support for this hypothesis is the higher percentage of *X. fastidiosa*-positive riparian hosts we detected in August and October. Our findings suggest that the long-term survival of *X. fastidiosa* in areas where no or few infective BGSSs overwin-

Table 1. Analyses of variance to test for effects of location and species on October 2003 *Xylella fastidiosa* populations in California grapevine, Himalayan blackberry, and periwinkle at two locations in northern California[a]

		Colony counts in culture (CFU/g of petiole)		Real-time PCR (cells/g of petiole)	
Source[b]	Num df	Den df	*F* value	Den df	*F* value
Location	1	73	6.29**	94	7.95**
Location × species	2	73	3.25*	94	0.15
Species	2	73	9.60**	94	96.55***

[a] For plants with petioles confirmed as *X. fastidiosa* positive in October 2003. PCR = polymerase chain reaction; Num = numerator and Den = denominator; *, **, and *** indicate significance at 0.05, 0.01, and <0.0001 probability levels, respectively.

[b] Source of variation: Mendocino or Napa (location); California grapevine, Himalayan blackberry, or periwinkle (species).

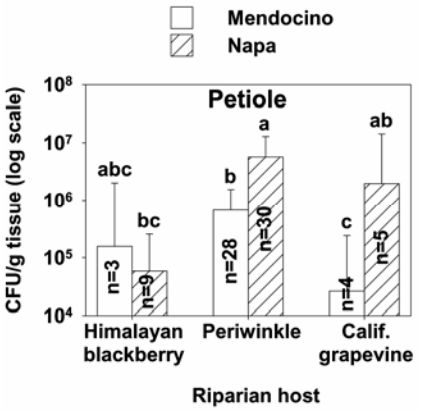

Fig. 3. October 2003 *Xylella fastidiosa* populations in Himalayan blackberry, periwinkle, and California grapevine petioles at two locations in northern California, as determined by colony counts in culture. Bars with different letters are significantly different at $P \leq 0.05$ (Tukey's test). Error bars represent 95% confidence limits; n = number of *X. fastidiosa*-positive plants.

ter may depend on the presence of periwinkle. Purcell and Saunders (20) came to similar conclusions, based on overwinter survival of *X. fastidiosa* in 100% of their inoculated periwinkle plants. Purcell and Saunders (20) came to different conclusions than we did for California blackberry, based on overwinter survival of *X. fastidiosa* in 29% of their inoculated California blackberry plants. Differences in our results may be due to differences in year, method of inoculation, or time of culturing.

Our results are relevant to riparian revegetation, an approach to PD management that involves replacement of hosts of both *X. fastidiosa* and the BGSS with nonhosts. Revegetation offers the potential to reduce insecticide applications, but it is a labor-intensive tactic. Our findings suggest that California blackberry and elderberry may not be important reservoirs of *X. fastidiosa* and, therefore, efforts expended in removing them may not be repaid with a reduction in disease incidence. Grape growers instead should focus on removing Himalayan blackberry, California grapevine, and, especially, periwinkle, which appears to be a year-round host of *X. fastidiosa*. Periwinkle retains its leaves in winter, making it an appealing host when others are dormant and, with herbaceous petioles and stems, periwinkle has more suitable feeding sites than woody-stemmed riparian hosts. Indeed, BGSSs have been observed on periwinkle in winter and spring, although periwinkle is not visited as frequently as other hosts (20). In Himalayan blackberry, we detected similar *X. fastidiosa* populations in both petioles and stems in August, based on colony counts in culture. This finding is relevant to potential infestation of the north-coastal grape-growing region by the glassy-winged sharpshooter, an introduced PD vector that is capable of acquiring *X. fastidiosa* by feeding on woody stems (1).

Low temperatures inhibit *X. fastidiosa*. In a controlled environment study, *X. fastidiosa* populations declined in Cabernet Sauvignon seedlings grown at temperatures <17ºC (3). A significant proportion of grapevines with PD recovered after incubation at low temperatures (–12 to –8ºC) (17) and after overwinter exposure in locations in the western United States with cold winters (18). If low temperature was the sole factor affecting seasonal changes in *X. fastidiosa* populations in riparian hosts, we might expect to find lower detection frequencies and lower populations among samples collected in February 2004, after plants were exposed to three consecutive months of average temperatures ≤10ºC at both locations. The number of *X. fastidiosa*-positive plants declined from October 2003 to February 2004, but, surprisingly, there were even fewer positive plants in June 2004. Furthermore, the lowest *X. fastidiosa* populations in periwinkle were measured in June 2004. Low detection frequency in June 2004 may be a function of rapid spring shoot growth, which may have exceeded the rate of colonization of new shoot tissue by *X. fastidiosa*. Hopkins and Thompson (11) and Henneberger et al. (5) came to similar conclusions regarding low *X. fastidiosa* populations in spring in grapevines and sycamore, respectively.

We detected significantly higher *X. fastidiosa* populations in Napa plants than in Mendocino plants for three host species (California grapevine, Himalayan blackberry, and periwinkle), at multiple sampling periods, and with both quantitation methods. Although differences in winter temperatures between our two locations were small, they did exist. Lower winter and spring temperatures may have inhibited *X. fastidiosa* growth in plants maintained in Mendocino compared with those in Napa. This hypothesis is supported by research on the persistence of *X. fastidiosa* in grapevines inoculated in different locations in California (2), two of which included the same locations we examined, Hopland and Oakville (referred to as 'Mendocino' and 'Napa' in our study). None of the grapevines in Hopland were found to be *X. fastidiosa* positive the year following inoculation; whereas, in Oakville, there were grapevines that were found to be *X. fastidiosa* positive (2). Feil et al. (2) demonstrated that location, specifically the same two locations we examined, affects *X. fastidiosa* persistence and that *X. fastidiosa* persisted in more plants in Oakville than in Hopland. Higher *X. fastidiosa* populations among riparian hosts in Napa may partially explain the higher incidence of PD in this grape-growing region compared with that of Mendocino.

Both methods of quantitation provided the same general results. For example, the most *X. fastidiosa*-positive plants were detected in autumn and summer with both methods. However, there were differences between the methods at finer levels of comparison. For example, in periwinkle in

Table 2. Analysis of variance to test for effects of location, species, and tissue on August 2004 *Xylella fastidiosa* populations in California grapevine, Himalayan blackberry, and periwinkle at two locations in northern California[a]

	Colony counts in culture (CFU/g of tissue)		
Source[b]	**Num df**	**Den df**	***F* value**
Location	1	94	13.85**
Location × species	2	94	2.42
Location × species × tissue	2	94	0.55
Location × tissue	1	94	0.89
Species	2	94	6.12**
Species × tissue	2	94	0.13
Tissue	1	94	0.06

[a] For plants with petioles and stems confirmed as *X. fastidiosa* positive in August 2004. Num = numerator and Den = denominator; *, **, and *** indicate significance at 0.05, 0.01, and <0.0001 probability levels, respectively.

[b] Source of variation: Mendocino or Napa (location); California grapevine, Himalayan blackberry, or periwinkle (species), petiole or stem (tissue).

Table 3. Analyses of variance to test for effects of location and sampling period on *Xylella fastidiosa* populations in periwinkle at two locations in northern California[a]

	Colony counts in culture			Real-time PCR					
	CFU/g of stem			Cells/g of petiole			Cells/g of stem		
Source[b]	**Num df**	**Den df**	***F* value**	**Num df**	**Den df**	***F* value**	**Num df**	**Den df**	***F* value**
Location	1	69.0	2.23	1	38.0	6.81**	1	84.6	0.51
Location × sampling period	2	69.0	2.42	3	65.1	0.08	2	67.9	0.87
Sampling period	2	69.0	5.26**	3	65.1	27.22***	2	67.9	25.76***

[a] For plants with petioles confirmed as *X. fastidiosa* positive in October 2003 and in February, June, and August 2004; for plants with stems confirmed as *X. fastidiosa* positive in February, June, and August 2004. Statistical analyses of corresponding CFU/g of petiole were not done, due to insufficient numbers of *X. fastidiosa*-positive periwinkle plants at both locations in February and June 2004. PCR = polymerase chain reaction; Num = numerator and Den = denominator; *, **, and *** indicate significance at 0.05, 0.01, and <0.0001 probability levels, respectively.

[b] Source of variation: Mendocino or Napa (location); October 2003, February 2004, June 2004, or August 2004 (sampling period). Stems were not collected in October 2003.

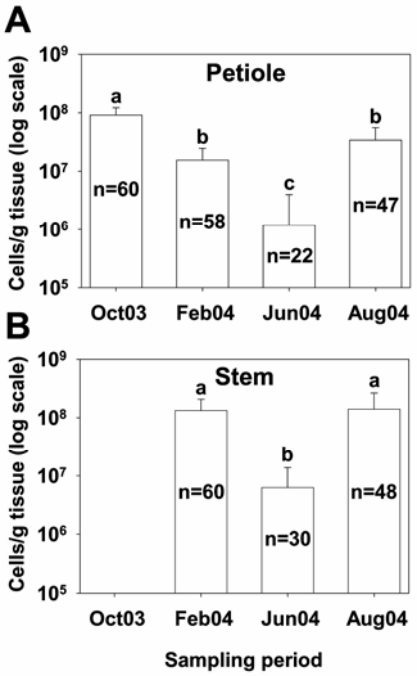

Fig. 4. Temporal changes in *Xylella fastidiosa* populations in periwinkle, as determined by real-time polymerase chain reaction, from **A,** petioles and **B,** stems. Petioles were sampled at four consecutive periods, starting in October 2003. Stems were sampled at three consecutive periods, starting in February 2004. Bars with different letters are significantly different at $P \leq 0.05$ (Tukey's test). Error bars represent 95% confidence limits; n = number of *X. fastidiosa*-positive plants summed over both locations.

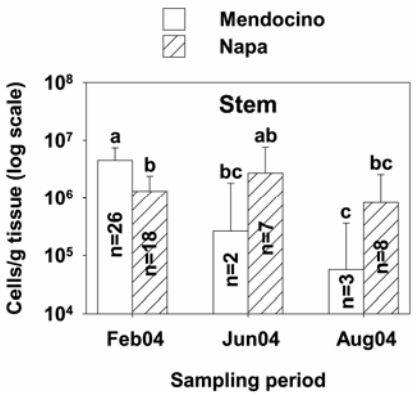

Fig. 5. Temporal changes in *Xylella fastidiosa* populations in Himalayan blackberry stems at two locations in northern California, as determined by real-time polymerase chain reaction. Bars with different letters are significantly different at $P \leq 0.05$ (Tukey's test). Error bars represent 95% confidence limits; n = number of *X. fastidiosa*-positive plants.

winter, more *X. fastidiosa*-positive plants were detected by real-time PCR than by culturing, indicating either that DNA was amplified from dead cells or that *X. fastidiosa* was viable but nonculturable in winter. The original estimate of 10^4 CFU/g of tissue as the threshold population density for acquisition (8), which was determined from colony counts in culture, may be an underestimate, given that this method often underestimated the number of cells in our study. Nonetheless, culture provides a measure of viability, whereas real-time PCR quantifies DNA, sources of which include viable and nonviable cells. Another advantage of culturing *X. fastidiosa* is that inferences can be made with respect to pathogen populations and sharpshooter transmission. A direct relationship between *X. fastidiosa* populations as quantified by culturing and BGSS transmission has been established (8). No such evidence exists for real-time PCR.

The importance of a plant species as a pathogen reservoir is determined by the frequency of occurrence of the species, the size of the *X. fastidiosa* populations it supports, and the frequency of visitation by insect vectors. In this regard, it is noteworthy that BGSSs are found more frequently on some riparian hosts than others, suggesting that feeding preferences exist (16). A common riparian host that is fed upon frequently likely will contribute more to the spread of PD. Future work is needed to evaluate the significance of riparian hosts based on *X. fastidiosa* populations and BGSS feeding preferences, in order to provide a more complete understanding of how riparian hosts impact the spread of PD.

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