Multiplication and Movement of *Xylella fastidiosa* Within Grapevine and Four Other Plants

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ABSTRACT

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Xylella fastidiosa, a xylem-limited bacterium that causes Pierce's disease of grapevine and other plant diseases, has numerous symptomless hosts. The multiplication and spread of *X. fastidiosa* within grapevine (*Vitis vinifera*) and four other reported plant hosts that were preferred plants for insect vectors (Himalayan blackberry, *Rubus discolor*; California mugwort, *Artemisia douglasiana*; watergrass, *Echinochloa crusgalli*; and Bermuda grass, *Cynodon dactylon*) were assessed after vector inoculation. The bacterium was detected by culture and enzyme-linked immunosorbent assay. The incubation times required before first detection in grape, blackberry, mugwort, and watergrass were 4, 32, 30, and 18 days, respectively. The maximum bacterial concentration (CFU per gram) and percentage of infection in these species were $>10^8$ (100%), 1×10^7 (58%), 2×10^6 (20%), and 4×10^5 (31%), respectively. Systemic movement of *X. fastidiosa* distal to the inoculation site was detected only in grapevine and blackberry. The bacterium was never detected in inoculated Bermuda grass. The wide range in the capacities of these hosts to support the bacterium's multiplication and spread suggests that the epidemiological importance of plant host species for the spread of Pierce's disease varies greatly.

Additional keywords: *Carneocephala*, *Draeculacephala*, *Graphocephala*, sharpshooter, vector transmission.

Xylella fastidiosa is a xylem-limited bacterial pathogen that causes Pierce's disease (PD) of grapevines and other diseases in alfalfa, almond, peach, citrus, forest trees, and noneconomically important plants (10,23). The bacterium is vectored by xylem sap-feeding suctorial insects such as sharpshooter leafhoppers (Homoptera, Cicadellidae) (2,6,16). In California the distribution of PD is patchy; chronically affected vineyards are located near perennial vector source habitats such as riparian vegetation (5,7, 12). Evidence indicates that primary inoculum for PD in California vineyards is from sources outside the vineyard (5,7,12). When grapevines in California become infected with *X. fastidiosa* during or after the middle of the growing season, the bacteria frequently do not survive the subsequent dormant season, and most of these vines recover during the following winter (15). In California secondary spread (vine to vine) does not begin until the mid-to-late season. Because most of the vines thus infected subsequently recover, secondary cycles of infection by *X. fastidiosa* may be of little importance (16).

In 1951 Freitag (1) reported that, based on the results of vector transmission studies, 91 of 116 plant species he tested were hosts of the causal pathogen of PD (then assumed to be viral). Most of these host plants were not symptomatic. These tests established that, under laboratory conditions, the plants were able to harbor the pathogen for acquisition by uninfected vectors. More recently, in two surveys by Raju et al. (19,20), the bacterium was detected by enzyme-linked immunosorbent assay (ELISA) or recovered by culture from 7 of 52 species of plants collected near vineyards. These included some species not previously tested by Freitag (1). Over all, these three studies indicated that 94 of 151 plant species examined in California could be hosts of *X. fastidiosa*. However, the epidemiological role and relative importance of these noncrop hosts as sources of primary inoculum that can be disseminated to vineyards are poorly understood.

The objective of this research was to determine if *X. fastidiosa* multiplied and spread within four plant species in California that might serve as inoculum reservoirs. Plant species selected for study were Himalayan blackberry (*Rubus discolor* Weihe & Nees), California mugwort (*Artemisia douglasiana* Besser), watergrass (*Echinochloa crusgalli* P. Beauv.), and Bermuda grass (*Cynodon dactylon* (L.) Pers.). These species commonly occur near vineyards and are common breeding hosts of the three most important vectors of *X. fastidiosa* in California: the blue-green sharpshooter (*Graphocephala atropunctata* (Signoret)) (7,14,21), the green sharpshooter (*Draeculacephala minerva* Ball), and the redheaded sharpshooter (*Carneocephala fulgida* Nottingham) (7,18). The rate and extent of multiplication and spread of *X. fastidiosa* within the four plants were assessed and compared to grapevine (*Vitis vinifera* L.), a very susceptible host that supports rapid and extensive bacterial multiplication.

MATERIALS AND METHODS

Vector, plant sources, and inoculation. All test or insect-rearing plants were propagated in protected greenhouses that were constantly pressurized with charcoal-filtered air. Alfalfa (*Medicago sativa* 'Moapa'), grapevine ('Pinot noir'), and watergrass were propagated as seedlings. Blackberry, mugwort, and Bermuda grass seedlings were propagated by rooting cuttings.

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Three vector species, the green sharpshooter (GSS), the blue-green sharpshooter (BGSS), and the redheaded sharpshooter (RHSS) were used. Colonies of GSS and RHSS were maintained for 2 and 1 years, respectively, on greenhouse-grown Bermuda grass or barley (*Hordeum vulgare*). Bermuda grass was an excellent oviposition host, and barley served mostly to rear nymphs to adulthood. The key to maximizing sharpshooter survival was to transfer the insects to new plants frequently (at least weekly) to continuously provide succulent new plant growth.

BGSS could not be maintained in laboratory colonies for multiple generations. For some experiments (from June through February), adult BGSS were collected from ornamental shrubs and trees at a site in Berkeley, CA (8). In 12 collections of 80 to 200 insects each, <1% of these BGSS were infected with *X. fastidiosa*. From March through May, BGSS nymphs from the Berkeley site were reared or gravid females were collected to oviposit, and the resultant nymphs were reared. The nymphs were reared on mugwort or mustard (*Brassica juncea*). Newly molted sharpshooters should be not be infective with *X. fastidiosa* (17), and the nymphs were transferred every 2 to 3 days to new plants to preclude transmission from the holding plants in the unlikely event that *X. fastidiosa* was present among the nymphs. All BGSS adults (whether reared from nymphs or collected as adults) were prescreened before they were used in experiments to further insure they were not infective with *X. fastidiosa*. For prescreening, groups of five insects were put on grapevine plants for 2 or more days. After 6 to 10 weeks the screening plants were tested for *X. fastidiosa* by culture and/or ELISA. Because none of the prescreened groups of BGSS transmitted PD to the screening plants, they were presumed to be free of *X. fastidiosa* when used in the experiments.

Culture and ELISA. Dilution plating of *X. fastidiosa* was used to estimate the number of viable bacterial cells per g of plant tissue. The methods for culturing *X. fastidiosa* (sample preparation, PWG growth media, and dilution plating) were previously described (8). The theoretical detection threshold for this method was calculated to be approximately 100 cultivable cells per sample or 100 cells per g of plant tissue (8).

After being processed and used for dilution plating of *X. fastidiosa*, the remaining undiluted sample solution was used in a double-antibody sandwich-ELISA for *X. fastidiosa*. The appearance, growth rate, and growth pattern of colonies of *X. fastidiosa* on PWG medium were characteristic and distinctive, but to further insure that the bacteria detected were correctly identified as *X. fastidiosa*, all samples were ELISA tested as well as dilution plated. The detection threshold for ELISA is about 10^5 cells per g of plant tissue. Thus, some plant samples yielded colonies of *X. fastidiosa* from culture, but the ELISA results were negative. In these cases, a second ELISA test was performed on the cultured cells to confirm the identity of the bacterium.

Known negative-control samples (in triplicate) of each plant species assayed were included on each 96-well ELISA plate. The assay was performed according to manufacturer's instructions (Agdia, Mishawaka, IN) with the following modifications to reduce background readings (A_{405nm}) of negative controls (readings that were prohibitively high for plants other than grapevine). (i) To each 2 ml of homogenized sample, 250 µl of sample reagent stock solution was added. Sample reagent stock solution consisted of 32 g of polyvinyl pyrolidone, 6.5 g of diethyldithio carbamic acid, 32 ml of Tween 20, 0.32 g of sodium azide, 3.2 g of egg albumin, and sufficient phosphate buffered saline (PBS) to give a final volume of 200 ml. The sample was vortexed and filtered through Miracloth (Calbiochem-Behring Corporation, La Jolla, CA). (ii) The recommended Agdia buffer for the second antibody (conjugated to alkaline phosphatase) was modified by incorporating the soluble components of healthy plants by finely chopping grape petioles and Bermuda grass stems (20 g each) in 500 ml of the recommended buffer in a kitchen blender for 5 min and then filtering through Miracloth. (iii) Sample and second antibody (alkaline phosphatase conjugated) incubations were overnight at 4°C. Before incorporating these modifications, the background readings for Bermuda grass were higher than those of the three nongrape species, but the backgrounds for all species were undesirably, if not prohibitively, high. After the modifications, the negative-control background A_{405nm} readings were reduced to 0.03 or less for all the plant species. We adopted $A_{405nm} = 0.18$ (six times maximum background) as a threshold value for a positive ELISA result. All sample results were either positive or negative (not indeterminate) using these criteria.

A set of standards and positive controls was included on each ELISA plate. A suspension of *X. fastidiosa* cells was made in PBS with 0.05% sodium azide (approximately 10^7 cells per ml), and 0.5-ml aliquots of both the undiluted and 1:10 dilution of the suspension were stored frozen (–20°C) until used. For each ELISA plate, a pair of the aliquots was thawed, and the two cell suspensions (two wells each) were included on the plate as a standard. Several readings of each ELISA plate were obtained as the color developed, and the readings for which the A_{405nm} of the undiluted and the 1:10 dilution standards were close to 1.3 and 0.5, respectively, were selected in all cases for the results of the test. Use of these standards provided a basis for comparing the readings of samples in ELISA tests done on separate occasions. In addition to the two standard cell suspensions, two or more plant tissue samples from known PD-infected grapevines were used on each ELISA plate as positive controls.

Insect inoculation protocols. Infective BGSS were obtained by caging groups of up to 100 to feed for 2 to 6 days on each of two grapevines that had noticeable disease symptoms and that were positive for *X. fastidiosa* by culture and ELISA. Alfalfa plants did not exhibit noticeable disease symptoms, so alfalfa plants with positive culture and ELISA results were used as acquisition sources for GSS and RHSS. The acquisition plants had been inoculated with a strain of *X. fastidiosa*, designated YVPD (8), we originally isolated from plants in a vineyard with PD in Yountville, Napa County, CA. A suspension in PBS of the YVPD strain was stored frozen at –70°C. The acquisition plants were inoculated either by needle (9), using a thawed aliquot of the stored cell suspension, or by insect vectors that had acquired the bacterium from needle-inoculated plants.

There were three repetitions of the experiments for each of the five plant species. Each plant in an experiment was inoculated by a group of four infective insects caged for 24 to 48 h on a marked inoculation site limited to 2.5 cm of stem or petiole. Light-weight clamshell-type insect cages were made from 6-cm-long segments of foam-rubber pipe insulation with an inside diameter of 2.5 cm. The open ends of the segment were covered by nylon mesh glued over the opening. A transverse cut deeper than halfway through the foam-rubber pipe was made in the middle of the segment. The cages were pulled open at the cut, clamped around the plant stem, and taped closed so the plant stem traversed the 2.5 cm inside diameter of the cage.

After feeding on the experimental plant, each insect group's inoculativity was confirmed by caging it for 2 days on an uninfected grapevine or alfalfa plant. This "confirmation plant" was tested after 6 to 12 weeks for *X. fastidiosa* by culture and ELISA. If an insect group was not confirmed as inoculative (because it did not infect the confirmation plant), the associated experimental plant was omitted from the experiment. In each experiment, 15 to 30 plants were inoculated, depending on the number of inoculative vectors available. At various time intervals after inoculation, three to six of the experimental plants were sacrificed, and the marked inoculation site on the plant was tested for *X. fastidiosa* by culture and ELISA. Plants from two to five experiments were sacrificed and sampled per day. Because of the logistics of insect inoculation and sample processing, the postinoculation time intervals for sampling were not uniform. In a typical ex-

ample, an experiment with 28 inoculated plants was sampled on days 6, 14, 24, 28, and 36. From many of the plants, additional samples of stem or petiole of comparable sizes that were 5 cm or more distal to the inoculation site also were tested by culture and ELISA.

We used BGSS in all experiments with grapevine, blackberry, and mugwort. For both Bermuda grass and watergrass, one of the three replicates used GSS that had fed on infected alfalfa as the infective vector. The other two replicates used BGSS as the vector. Four other replicates of experiments that attempted to use GSS that had fed on infected grapevine were not included because the vector groups did not transmit *X. fastidiosa* to the confirmation plants.

The experimental method of Freitag (1) was used to test vector acquisition from three Bermuda grass plants. Three large groups of adult RHSS (50, 50, and 80 insects) were fed on PD-symptomatic grapevines to acquire *X. fastidiosa*. The infective insect groups were put onto three caged Bermuda grass plants, on which they fed and oviposited for 7, 16, and 18 days, respectively. The infectivity of the adult RHSS was confirmed by caging them in groups of five on uninfected grapevine seedling confirmation plants. These confirmation plants were tested after 6 to 12 weeks for *X. fastidiosa* by culture and ELISA. The nymphs that emerged from eggs laid on Bermuda grass by the infective RHSS were reared on these three Bermuda grass experimental plants for 4 weeks. These nymphs were tested to detect whether they acquired *X. fastidiosa* from the inoculated Bermuda grass plants; they were removed and divided into two groups of 15 nymphs and put onto two test plants, a grapevine seedling and an alfalfa plant, for 9 days. The resulting six test plants were tested after 6 to 12 weeks for *X. fastidiosa* by culture and ELISA. Two samples each also were taken from the three Bermuda grass plants 60 days or more after removal of the infectious RHSS adults. These six samples were tested for *X. fastidiosa* by culture and ELISA.

Mugwort needle-inoculation experiment. Eleven mugwort plants were inoculated using a needle and a suspension of bacterial cells. A 5-µl droplet of saline containing 10^9 cells per ml of *X. fastidiosa* was pipetted onto the plant stem, and a fine needle was passed through the droplet and into the xylem zone of the stem until the droplet was drawn into the stem. These mugwort plants were tested for *X. fastidiosa* by culture 6 weeks or more after inoculation by sampling the needle puncture wound site or the stem adjacent and distal to the wound. Additional samples were taken 1.5 and 4 cm distal to the puncture wound.

TABLE 1. Number of plants tested for *Xylella fastidiosa* and number of infections verified by culture and enzyme-linked immunosorbent assay during 10-day intervals after vector inoculation

Host plant	Days after inoculation			
	1–10	11–20	21–30	>30[x]
Grape	15/55[y]	32/36	9/9	16/16 a
Blackberry	...[z]	0/16	0/7	25/43 b
Mugwort	...	0/19	0/7	11/42 c
Watergrass	...	1/8	...	10/32 c
Bermuda grass	...	0/22	0/10	0/27 d

[x] All possible paired comparisons were made using Chi-square 2 × 2 contingency with Yates' correction. Results followed by different letters differ significantly at $P < 0.05$.

[y] Number of verified *X. fastidiosa* infections/number of inoculated plants tested in the postinoculation interval.

[z] Ellipses indicate tests not available for the interval.

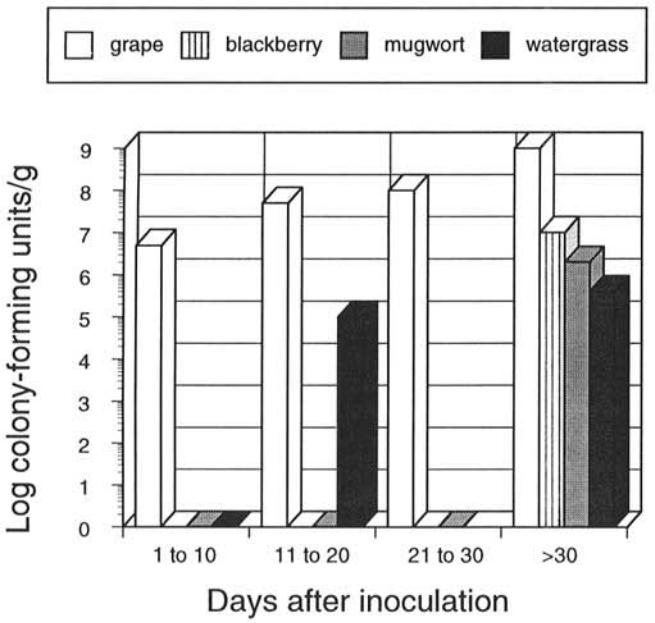

Fig. 1. The maximum population size of *Xylella fastidiosa* (CFU/g of plant tissue) for each plant species tested during each 10-day postinoculation time interval. Detection threshold was 10^2 CFU/g.

RESULTS

Insect inoculation experiments. *X. fastidiosa* was first isolated from grapevine after an incubation period of 4 days (but not at 2 or 3 days) following inoculation by BGSS. The bacterium was recovered from all of the grapevines by 20 days after inoculation. The bacterium was first isolated after 32, 30, and 18 days from blackberry, mugwort, and watergrass, respectively. Pooled results have been grouped by 10-day intervals (Table 1; Fig. 1) to facilitate comparisons among host species and to clarify changes in *X. fastidiosa* population sizes over time. The percentage of inoculations that resulted in detectable bacterial infections varied significantly among the five host plants, from 100% in grapevine to 0% in Bermuda grass.

X. fastidiosa was not recovered from 56 inoculated Bermuda grass plants at the inoculation site. In addition, *X. fastidiosa* was not recovered from the three Bermuda grass plants inoculated by Freitag's method (1) of caging 50 infective RHSS on the entire plant. There also was no transmission of *X. fastidiosa* to the three grapevine and three alfalfa plants used to test for acquisition of *X. fastidiosa* by the nymphs reared on the inoculated Bermuda grass.

The highest number of *X. fastidiosa* CFU/g of plant tissue for each species (Fig. 1) was not attained until the 30- to 90-day postinoculation interval. The range of bacterial populations (per gram) during this interval for grapevine, blackberry, mugwort, and watergrass was 10^8 to $>10^9$, 0.2 to 1×10^7, 0.02 to 2×10^6, and 2 to 4×10^5, respectively. The dilutions used in culturing did not allow for accurate estimates of population sizes greater than 10^9.

GSS were not efficient at acquiring the bacterium from or transmitting it to grapevine. In experiments in which GSS fed on PD-symptomatic grapevines to acquire *X. fastidiosa,* only 2 of 46 groups of 5 GSS were confirmed as inoculative (evidenced by infecting a grapevine seedling). When GSS acquired *X. fastidiosa* from alfalfa, all of 23 groups were inoculative (evidenced by infecting another alfalfa seedling).

We detected spread of the bacterium in plants beyond the inoculation site by culture and ELISA only in grapevine and blackberry. *X. fastidiosa* was found in all of more than 30 samples taken from 5 cm or more distal to the inoculation site in grapevines. Within a few days of detecting the bacterium at the inoculation site, the bacterium was detected in grapevine seedlings throughout the stems (15 to 40 cm in stem length) up to the two or three small developing leaves behind the growing tip. In blackberry, *X. fastidiosa* was recovered from all 15 petiole and stem samples as far as 40 cm distal to the inoculation site. Fourteen blackberry plants with no detectable bacterial populations at the inoculation site also had no detectable populations beyond the inoculation site. In nine mugwort plants from which the bacteria were recovered

from the inoculation site, *X. fastidiosa* was not recovered from samples 5 and 15 cm distal to that site. We did not recover *X. fastidiosa* from any of the 11 mugwort plants inoculated by needle puncture. Isolations were attempted from five of these plants from the needle puncture and yielded unidentified bacteria and fungi that precluded detection of *X. fastidiosa*. From the remaining six mugwort plants, stem tissue immediately distal to the puncture wound was cultured, and one sample yielded other, unidentified bacteria. We did not recover *X. fastidiosa* or other bacteria from samples 1.5 to 4 cm distal to the needle puncture. Similarly, *X. fastidiosa* was not recovered from four infected watergrass plants by sampling one or more internodes above an infected inoculation site. The rapid leaf senescence and growth pattern of watergrass made culture of tissue distal to the inoculation site difficult in many of the infected plants.

Infected grapevine and blackberry plants developed PD symptoms, but the other three plant species exhibited no disease symptoms. In blackberry, the symptoms occasionally included slight (1 to 5 mm) marginal leaf necrosis (scorch) with concentric zones of reddening interior to the necrotic margins but did not include additional symptoms typical of PD-symptomatic grapevines, such as dieback, patches of mature and green bark, and dead leaves dropping from the tip of the still green petiole rather than from an abscission layer at the petiole base. Symptoms appeared in infected blackberry plants only after more than 3 months, whereas symptoms appeared in grapevine after 4 to 8 weeks.

DISCUSSION

Three separate studies in California (1,19,20) identified 94 plant species in more than 28 plant families as sources of inoculum of *X. fastidiosa* for acquisition by vector insects under laboratory conditions. Because the capacity to harbor *X. fastidiosa* was established, presumably these 94 species also could serve as sources of *X. fastidiosa* inoculum for transmission to vineyards. No differences have been proposed among the various plant hosts of *X. fastidiosa* regarding their relative importance as sources for transmission to grapevine. The conclusions from previous studies (1, 19,20) for the five plant species used in this study are summarized in Table 2. All five of these species also are breeding hosts for one or more of the most common sharpshooter vectors of *X. fastidiosa*. Grapevine, blackberry, and mugwort are common breeding hosts of BGSS (6,13,14), and watergrass and Bermuda grass are breeding hosts of GSS and RHSS (17,18). On the basis of previous work, it is reasonable to conclude that all four nongrape hosts are important sources of *X. fastidiosa* for spread to grapevine. However, this study demonstrated substantial differences among these four plant species in their ability to support multiplication and systemic movement of *X. fastidiosa*.

Our studies identified five differences in plant-bacteria interactions among plant hosts relative to their capacities as hosts of *X. fastidiosa*. (i) Differences in the length of the incubation periods after inoculation before the first detection of *X. fastidiosa* suggested that the multiplication rate of *X. fastidiosa* differed within these hosts. (ii) The wide ranges among plant species in the number of cultivable bacteria per g of plant tissue attained during the 30- to 90-day postinoculation interval indicated that the plant species vary in the maximum concentration of *X. fastidiosa* supported. (iii) The extent of within-plant spread of *X. fastidiosa* from the site of inoculation differed among the plant species. Spread was not detected in mugwort, watergrass, and Bermuda grass. (iv) There were significant differences in the percentage of plants infected by vector inoculation in the 30- to 90-day postinoculation category. This indicates that under inoculation pressure sufficient to infect all of the grapevines, many fewer blackberry, watergrass, and mugwort and no Bermuda grass plants were infected. (v) Only two of the five species had symptoms, and symptoms were much milder in blackberry than in grapevine.

Mugwort and watergrass, the two species that had lower proportions of inoculations that resulted in infection and lower maximum concentrations of *X. fastidiosa* in tissues, also showed no evidence of systemic bacterial movement. The conclusion that watergrass supports no systemic movement is tentative in that cultures were attempted above the inoculation site in only four infected plants. The relationship between bacterial concentration and systemic movement suggests that bacterial multiplication to high concentrations (10^7 to $>10^9$ CFU/g) is needed for systemic movement.

The probability of a plant serving as a source for vector acquisition of *X. fastidiosa* is contingent on the extent to which the first four of the five plant-dependent characteristics described above will be expressed plus the attractiveness and acceptability of the plant for feeding by a vector. On the basis of our findings for the YVPD strain of *X. fastidiosa*, a mugwort plant would be a significant source for vector acquisition only if it were inoculated at numerous points early enough in the growing season to permit adequate bacterial multiplication. Because xylem sap-feeders generally feed on succulent primary growth, as is the case for BGSS (13,14) on mugwort (B. L. Hill and A. H. Purcell, *unpublished data*), adults would most likely feed on the newest growth. The newest primary growth on central and axillary shoots would be the least likely to harbor bacteria because of the incubation period required for the bacteria to multiply at the site of inoculation. Moreover, mugwort foliage dies down to roots each winter, so *X. fastidiosa* should not survive the winter in mugwort. In contrast, *X. fastidiosa* multiplies and spreads within grapevine or blackberry, so a single infection event should expand the availability of the *X. fastidiosa* for acquisition by vectors in both space and time on these plants.

X. fastidiosa multiplies and spreads faster within the more susceptible cultivars and species of grapevine (3,4,9,15). Some of the relationships between strains of *X. fastidiosa* and various hosts have been examined for host species occurring in the southeastern United States (10,11), but strain differences have not been reported in California. Strains of *X. fastidiosa* may vary in host specificity. For example, two strains of *X. fastidiosa* that were isolated from naturally infected American elm (*Ulmus americana*) and American sycamore (*Platanus occidentalis*) were pathogenic when inoculated into seedlings of elm and sycamore, respectively, but did not cause symptoms and could not be recovered by culture when inoculated into seedlings of the reciprocal hosts (22). Thus, it is not surprising that the 94 plant species previously identified in California as hosts of *X. fastidiosa* would not be equally susceptible to naturally occurring strains virulent in grapevine. The host studies based on ELISA and culture in California (19,20) and Florida (11) detected unknown strains of *X. fastidiosa*, some of which might not cause PD in vineyards. Addi-

TABLE 2. Host study results for *Xylella fastidiosa* previously reported on the plant species used in this study

	Criteria for host status			
Plant	Laboratory vector transmission[w]	Field vector recovery[x]	Field recovery by ELISA or culture[y]	Symptoms[z]
Grape	Yes	Yes	Yes	Severe
Blackberry	Yes	Yes	Yes	Mild
Mugwort	Yes	Not done	Not done	None
Watergrass	Yes	Yes	Not done	None
Bermuda grass	Yes	Yes	No	None

[w] Infective vectors caged on plants, removed, and replaced by noninfective vectors that acquired *X. fastidiosa* (Freitag [1]).

[x] Noninfective vectors acquired *X. fastidiosa* while caged on plants in the field (Freitag [1]).

[y] Surveyed plants near Pierce's disease sites using culture and enzyme-linked immunosorbent assay (ELISA) (Raju et al. [19,20]).

[z] Raju et al. (19,20).

tional information may be required to determine which of the 94 previously identified plant hosts can actually serve as reservoirs for the transmission of the PD-causing bacterium to grapevine.

X. fastidiosa was not recovered from Bermuda grass inoculated with the grapevine-virulent strain used in this study. The lack of recovery of *X. fastidiosa* from Bermuda grass is surprising in light of the fact that in the 1940s Freitag (1) recovered *X. fastidiosa* from 14 of 29 Bermuda grass plants experimentally infected by vectors using a technique that was similar to that used in this study. He also was able to recover *X. fastidiosa* from 6 of 38 Bermuda grass plants in the field by caging noninfective vectors on them. Raju et al. (19,20) did not detect *X. fastidiosa* in any of 50 Bermuda grass plants tested during the 1970s from areas adjacent to PD-infected vineyards. Could predominant strains of *X. fastidiosa* have had different host ranges than now? Hopkins (9) showed that the virulence of *X. fastidiosa* strains changed over 18 months of weekly serial transfers in vitro. In this case, only strains capable of systemic movement in grape were virulent to grape.

Efforts to control PD by management of outlying vegetation should take into account the differences among plants that serve as acquisition hosts. In mugwort and watergrass, for example, because the bacterium does not move systemically and the plant dies or dies back to ground level in the fall, *X. fastidiosa* is not likely to survive over winter. Further research is needed to determine the over-wintering capacities of plant species such as blackberry in which *X. fastidiosa* spreads systemically. Our studies were conducted in a heated greenhouse where bacterial growth in grapevines was easily detected within a few days.

We have not been able to culture *X. fastidiosa* from new shoots of chronically infected grapevines in the field before 8 weeks after bud break (B. L. Hill and A. H. Purcell, *unpublished data*). Growth of *X. fastidiosa* in field conditions, especially during the cooler spring months might be much slower than in the greenhouse. Studies relating the concentration of *X. fastidiosa* in plants to vector acquisition efficiency also are needed.

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