The Fate of *Xylella fastidiosa* in Vineyard Weeds and Other Alternate Hosts in California

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ABSTRACT

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The fate of *Xylella fastidiosa*, the bacterium that causes Pierce's disease of grape, was assessed in 29 species of plants associated with vineyards in California's San Joaquin Valley. Bacterial populations and movement in greenhouse-grown plants were measured 1, 3, and 9 weeks after mechanical and insect inoculation. *X. fastidiosa* was recovered in 27 of 29 species in greenhouse tests, with common sunflower (*Helianthus annuus*), cocklebur (*Xanthium strumarium*), annual bur-sage (*Ambrosia acanthicarpa*), morning glory (*Ipomoea purpurea*), horseweed (*Conyza canadensis*), sacred datura (*Datura wrightii*), poison hemlock (*Conium maculatum*), and fava bean (*Vicia faba* cv. Aquadulce) being infected in more than 50% of inoculation attempts. Twenty-three species supported bacterial populations in excess of 10^4 CFU/g of plant tissue. *X. fastidiosa* populations increased for 9 weeks after inoculation in six species, and were static or declined in 16 species. Although the blue-green sharpshooter (*Graphocephala atropunctata*) was more efficient than mechanical inoculation for infecting plants, median populations and systemic movement for the two methods did not differ significantly. *X. fastidiosa* colonization of greenhouse and field-grown plants was compared in five alternate hosts. *X. fastidiosa* was recovered from 23 and 34% of field-grown plants, compared with 49 and 67% of greenhouse-grown plants, in the winter and summer, respectively, with at least 10 times fewer bacteria in field plants in the winter. Although *X. fastidiosa* has a wide host plant range, its fate in most species is variable and heavily influenced by field conditions.

Additional keywords: glassy-winged sharpshooter, *Homalodisca coagulata*

Pierce's disease of grapevine (*Vitis vinifera*) is caused by *Xylella fastidiosa*, a bacterium transmitted by xylem sap-feeding sharpshooter leafhoppers and spittlebugs (21,28,30,31). In California, epidemiologically important vectors of Pierce's disease vary by region and include glassy-winged sharpshooters (*Homalodisca coagulata*) in southern California and the lower San Joaquin Valley, red-headed sharpshooters (*Xyphon* [*Carnecephala*] *fulgida*) and green sharpshooters (*Draeculacephala minerva*) throughout the San Joaquin Valley, and blue-green sharpshooters (*Graphocephala atropunctata*) along the coast (2,4). The detection of *X. fastidiosa* in *D. minerva* and *X. fulgida* from diverse habitats, including areas remote from cultivated crops, indicates that sharpshooters may acquire this pathogen from diverse feeding hosts (12). However, the fate of *X. fastidiosa* in common weedy plant species in the southern San Joaquin Valley of California has not been established.

In early insect-transmission studies with sharpshooter vectors, the Pierce's disease agent was transmitted to and recovered from 75 species of plants in greenhouse tests using insect acquisition and inoculation of the pathogen, although pathogen detection was solely based on symptom expression in alfalfa (*Medicago sativa*) and grapes (11). More accurate detection of *X. fastidiosa* in numerous species of natural vegetation became possible once serological and culture techniques were developed (6,20,27). Studies of the fate of *X. fastidiosa* in four plant species highly preferred by insect vectors revealed that the bacteria multiplied in blackberry (*Rubus discolor*), mugwort (*Artemisia douglasiana*), and watergrass (*Echinochloa crus-galli*), but not in bermudagrass (*Cynodon dactylon*). Only blackberry supported systemic bacterial movement throughout the plant (16). Further examination of the fate of *X. fastidiosa* in 33 species of riparian plants commonly found in Napa Valley confirmed that *X. fastidiosa* could multiply in most plant species, but often failed to move systemically beyond the inoculation site, suggesting infections eventually died out in nonsystemic hosts (26). Systemic movement is correlated with bacterial population (16,26), which in turn relates to vector acquisition efficiency (17).

Subfreezing temperatures influence *X. fastidiosa* overwinter survival in a given host plant (23). Moreover, temperatures regulate growth of *X. fastidiosa* in plants. For example, extremely cold temperatures can eliminate *X. fastidiosa* infections and determine the geographic distribution of *X. fastidiosa* (18). Pierce's disease-infected grapes were cured of *X. fastidiosa* infections in proportion to cold severity in laboratory and field experiments (23). Optimum temperatures for *X. fastidiosa* growth in planta were between 17 and 34°C, and bacterial populations remained static or decreased at temperatures above 34°C and below 17°C (10).

Our objectives were to (i) determine the fate of *X. fastidiosa* infections in previously untested plant species associated with southern San Joaquin Valley vineyards, and (ii) compare survival of *X. fastidiosa* infections in selected field and greenhouse-grown plants.

MATERIALS AND METHODS

Selection and propagation of plants to be tested. Plant species were selected for testing with input from farm advisors and the Kern-Tulare Glassy-winged Sharpshooter/Pierce's Disease Task Force. Plants were ranked by priority. Perennials and known feeding and oviposition hosts of the glassy-winged sharpshooter were tested first, then plants particularly abundant in or near vineyards (University of California Davis IPM online). Plants were grown in the greenhouse from field-collected seed, except for field bindweed (*Convolvulus arvensis*) and yellow nutsedge (*Cyperus esculentus*), which were vegetatively propagated from field-collected stems. Red gum (*Eucalyptus camaldulensis*) and blue gum (*Eucalyptus globulus*) were obtained as commercial nursery plants. Plants were grown in University of California (U.C.) Davis Mix (equal parts coarse sand, peat moss, and nitrolyzed redwood sawdust) in a greenhouse at U.C. Berkeley maintained at 25°C (+10°C/-5°C) and fertilized daily with dilute Hoagland's solution and monthly with 20-20-20 fertilizer at the label rate (8). Plants were grown with natural light conditions. Scientific and common names are listed according to the Jepson Manual (14) and the Growers Weed Identification Handbook (3). Voucher specimens were deposited in the Jepson Herbarium at U.C. Berkeley.

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Inoculation and detection of *X. fastidiosa* under greenhouse conditions. All plants were inoculated with the STL strain of *X. fastidiosa* (13), a grape strain from Napa County, CA. Plants were inoculated with *X. fastidiosa* via needle puncture or by the sharpshooters *H. coagulata*, *X. fulgida*, and *G. atropunctata*. All species inoculated with *H. coagulata* were also inoculated with *G. atropunctata*, with the exception of tree tobacco (*Nicotiana glauca*) and red gum, which were needle inoculated because *G. atropunctata* could not survive the inoculation access period. Ten to 115 plants were inoculated in at least two replications per species. Plants were maintained in a greenhouse at U.C. Berkeley as previously described.

The sharpshooter species selected depended on its preferred host plants in the field (28). Grasses were inoculated with *X. fulgida*; perennial and woody species were inoculated with *H. coagulata*; and herbaceous annuals were inoculated with *G. atropunctata*. To account for differences in inoculation efficiency between sharpshooter species, we only used data from insect groups that transmitted *X. fastidiosa* to grapes prior to exposure to alternate hosts. Insects acquired *X. fastidiosa* from infected grapevines in a 4-day acquisition access period and then were tested for presence of *X. fastidiosa* with a 4-day inoculation access period on a seedling grape (cv. Cabernet Sauvignon or Pinot Noir). Insects were then confined to plants in groups of two to four in small foam cages for 2 days (17). The cages were located on young, nonlignified, fully expanded stems, leaves, or petioles, where the plant was strong enough to support the weight of the cage. For example, prickly lettuce (*Lactuca serriola*) was inoculated on caudal leaves, poison hemlock (*Conium maculatum*) on basal petioles, and fava bean (*Vicia faba* cv. Aquadulce), red gum, cocklebur (*Xanthium strumarium*), and common sunflower (*Helianthus annuus*) on green stems.

For needle inoculation, 10 μ l of *X. fastidiosa* cells suspended in succinate-citrate-phosphate buffer (approximately 10^9 CFU/ml) were deposited on a stem, petiole, or leaf. The plant was probed three to six times through the drop with a no. 2 insect pin until the suspension was drawn into the plant (16). All inoculation suspensions were tested for viability, and inoculations were performed on sunny days so that active transpiration aided uptake of the bacterial suspension (33).

X. fastidiosa was isolated from inoculated plants by culture on periwinkle wilt-Gelrite media (7,16). Briefly, a section of plant was surface disinfested in successive rinses in 70% ethanol, 30% bleach (1.5% sodium hypochlorite), and sterile water. The plant material was chopped on sterile Whatman no. 1 filter paper and placed in 2 ml of succinate-citrate-phosphate buffer. The sample was ground by a Polytron ho-

mogenizer with a PT10/35 generator (Brinkmann Instruments, Westbury, NY) and diluted 100-fold in succinate-citrate-phosphate buffer. Twenty microliters of each dilution were plated on modified periwinkle wilt media. Plates were incubated inverted for 7 to 14 days at 28°C in the dark.

The inoculation site was assayed for bacteria 1, 3, and 9 weeks after inoculation, as previous studies indicated that *X. fastidiosa* died out after 3 weeks in some hosts (26). We tested a secondary stem or leaf distal to the inoculation site to detect possible systemic movement of *X. fastidiosa*, where *X. fastidiosa* recovered from distal petioles or secondary stems passed between pits to colonize the plant (33). Three uninoculated plants belonging to the same cohort were kept in the greenhouse or field and tested along with the inoculated plants to test for accidental *X. fastidiosa* presence during inoculation and culturing. The presence of plant inhibitors to *X. fastidiosa* growth in vitro was evaluated by homogenizing uninfected plants of each species, adding 20 μ l of cultured *X. fastidiosa* cells suspended in succinate-citrate-phosphate buffer, then culturing the mixture (26). It was assumed that *X. fastidiosa* population estimates obtained by culturing were reasonable indicators of a plant's ability to be a host of bacteria for insect vector acquisition. Culturing detects *X. fastidiosa* in infected grapevines below 10^4 CFU/g, the threshold population required for acquisition by *G. atropunctata*, an efficient vector (17).

Field experiments. We selected seasonally appropriate plant species previously identified as having a high rate (>50%) of systemic infection in greenhouse tests. We tested poison hemlock, a winter annual weed, and fava bean, a cover crop, in two replications from November 2001 to March 2002, and two summer annual weeds, common sunflower and cocklebur, in two replications between July and November 2002. Prickly lettuce, a long-lived winter annual, was included in both winter and summer tests. Plants were reared and inoculated in the greenhouse as previously described, except that groups of three *G. atropunctata* were allowed a 3-day inoculation access period, to allow a more reasonable planting and processing schedule with minimal overall effect on sharpshooter infectivity (24). In the second replication from November 2001 to March 2002, one-third of the plants of each species were mechanically inoculated due to lack of sharpshooters. Half the plants were kept in the greenhouse at U.C. Berkeley and the other half were planted in a screen house in Bakersfield, CA. Plants were assayed for *X. fastidiosa* by culturing at 1, 3, and 9 weeks after inoculation. Temperatures in the screen house were recorded with a portable Hobo temperature recorder (Onset Co., Pocasset, MA) and supplemented with field station temperature data

from Arvin, CA (17 km away) for one experiment where the temperature recording system failed.

Data analysis. Data analysis and statistical comparisons were made using Excel (Microsoft, Redmond, WA). Chi-square comparisons were used to compare transmission rates, with Yates' correction applied to any values less than 10.

RESULTS

Greenhouse inoculations. We detected *X. fastidiosa* infections in eight plant species more than 50% of the time in replicated greenhouse tests (Table 1). These were common sunflower, cocklebur, annual bur-sage (*Ambrosia acanthicarpa*), common morning glory (*Ipomoea purpurea*), horseweed (*Conyza canadensis*), sacred datura (*Datura wrightii*), poison hemlock, and fava bean. Of these, common sunflower, annual bur-sage, sacred datura, poison hemlock, and fava bean had median *X. fastidiosa* populations in excess 10^6 CFU/g. An additional 14 species became infected with *X. fastidiosa* 20 to 49% percent of the time (Table 1). Of these, yellow nutsedge, prickly lettuce, and tree tobacco had median populations greater than 10^6 CFU/g.

The only species that showed in vitro inhibition of *X. fastidiosa* was blue gum. In two replications, homogenized tissues of blue gum completely inhibited *X. fastidiosa* growth in one experiment and reduced viability (CFU) 100-fold in a second replication. When inoculated by *H. coagulata* and *G. atropunctata*, however, 10 of 62 blue gum stems were positive for *X. fastidiosa*, and the median bacterial population was 8×10^5 CFU/g.

White clover (*Trifolium repens*), red clover (*T. pretense* cv. Linden), alfalfa (cv. Moapa), and black nightshade (*Solanum nigrum*) had other unidentified bacteria obscure putative *X. fastidiosa* in culture. Contamination problems occurred in separate replications, and did not occur in other plant species tested on the same day.

We observed a decline in *X. fastidiosa* transmission by *G. atropunctata* one winter, from October 2002 to February 2003. Only 30% (46 of 152 groups) transmitted compared with 91% (81 of 89 groups) from April to August 2002 and 134 of 147 (91%) of sharpshooter groups used after April 2003. The previous winter (November 2001 to January 2002), 95% (122 of 128) of *G. atropunctata* groups transmitted *X. fastidiosa*.

In six species, *G. atropunctata* transmitted *X. fastidiosa* to a greater percentage of plants than did needle inoculation. For example, infective insects transmitted to 84% (36 of 43 stems) of common morning glory plants, but only 24% (12 of 51) became colonized after mechanical inoculation ($P < 0.01$, chi-square, Table 2). We also detected significantly more frequent *X. fastidiosa* recovery from *G. atropunc-*

tata—inoculated prickly lettuce, cocklebur, and quinoa (*Chenopodium quinoa*).

Needle inoculation was more efficient than *H. coagulata* inoculation in four other plants. In cheeseweed (*Malva parviflora*), *X. fastidiosa* infections developed at 51% (29 of 57) of needle-inoculated sites but only at 8% of *H. coagulata*—inoculated sites (1 of 12, $P < 0.01$, chi-square with Yates' correction). Similar results occurred for sacred datura (needle inoculation: 43 of 56; *H. coagulata*: 1 of 7, $P < 0.01$) and red

gum (needle inoculation: 12 of 33; *H. coagulata*: 0 of 5, $P > 0.05$, not significant). When sharpshooter groups inoculated seedling grapes during a 4-day inoculation access period, 30% of *H. coagulata* groups transmitted *X. fastidiosa*, compared with 96% of *G. atropunctata* groups. *H. coagulata* were even less efficient at transmitting *X. fastidiosa* to hosts other than grapes.

Bacterial spread and multiplication over time. Results for nine representative high population, systemic hosts, and poor

and moderate hosts are shown in Figure 1. Data are not shown for an additional 13 species. Among these 22 species, *X. fastidiosa* populations in infected plants peaked at 3 weeks at a median population of 2×10^6 CFU/g, and were 9×10^4 CFU/g and 6×10^5 CFU/g at 1 and 9 weeks, respectively.

Populations increased over time in six species: field bindweed, yellow nutsedge, common sunflower, prickly lettuce, fava bean, and sacred datura. Populations were

Table 1. *Xylella fastidiosa* infections in greenhouse-grown plants following insect vector and needle inoculation

Species	No. tested	No. infected ^a	CFU/g ^b	No. systemic ^c	Inoculation ^d
Annual bur-sage – <i>Ambrosia acanthicarpa</i>	47	31	6.2	25/31	GW, BG, NI
Annual sowthistle – <i>Sonchus oleraceus</i>	31	6	4.1	1/6	GW, BG
Blue gum – <i>Eucalyptus globulus</i>	62	10	5.9	3/10	GW, BG
Cheeseweed – <i>Malva parviflora</i>	69	21	3.8	9/14	GW, BG, NI
Cocklebur – <i>Xanthium strumarium</i>	111	65	5.5	40/65	GW, BG, NI
Common morning glory – <i>Ipomoea purpurea</i>	94	48	5.2	26/53	BG, NI
Common purslane – <i>Portulaca oleracea</i>	39	9	5.7	3/9	BG
Common sunflower – <i>Helianthus annuus</i>	76	63	6.3	48/60	GW, BG, NI
Curly dock – <i>Rumex crispus</i>	25	11	5.7	1/9	BG
Eggplant – <i>Solanum melongena</i> cv. Violeta lunga	54	22	4.0	16/23	NI
Fava bean – <i>Vicia fava</i> cv. Aquadulce	67	46	6.7	26/45	BG
Field bindweed – <i>Convolvulus arvensis</i>	39	11	5.9	5/6	BG
Horsetweed – <i>Conyza canadensis</i>	15	13	5.1	7/13	GW, BG
Johnsongrass – <i>Sorghum halepense</i>	62	14	4.7	2/14	RH, BG, GW
Joboba – <i>Simmondsia chinensis</i>	115	8	4.2	3/8	BG, NI
Poison hemlock – <i>Conium maculatum</i>	37	24	6.4	16/24	BG, NI
Prickly lettuce – <i>Lactuca serriola</i>	110	48	6.4	15/46	GW, BG, NI
Prostrate pigweed – <i>Amaranthus blitoides</i>	13	1	-	1/1	GW, BG
Quinoa – <i>Chenopodium quinoa</i>	79	25	3.6	9/25	GW, BG, NI
Red gum – <i>Eucalyptus camaldulensis</i>	37	12	4.8	5/12	GW, NI
Sacred datura – <i>Datura wrightii</i>	56	43	6.8	14/45	NI
Southwestern cupgrass – <i>Eriochloa gracilis</i>	22	5	2.8	3/5	BG
Tomato – <i>Lycopersicon esculentum</i> cv. Ace	82	35	5.1	20/27	NI
Tree tobacco – <i>Nicotiana glauca</i>	44	14	6.5	7/15	GW, NI
Watergrass – <i>Echinochloa crus-galli</i>	10	1	5.4	1/1	RH
Whitestem filaree – <i>Erodium moschatum</i>	42	9	4.7	4/9	GW, BG, NI
Yellow nutsedge – <i>Cyperus esculentus</i>	76	34	6.2	2/20	RH, BG

^a Results combined from two to six replications cultured 1, 3, and 9 weeks after inoculation.

^b Median *X. fastidiosa* populations at inoculation site, log₁₀ CFU per gram plant tissue, combined 1, 3, and 9 weeks.

^c Systemic infections are *X. fastidiosa* recovery by culture from an adjacent stem or petiole distal to the inoculation site. In common morning glory and eggplant, more *X. fastidiosa* infections were detected systemically than at the inoculation site because *X. fastidiosa* was recovered from distal stems after the inoculated leaf senesced.

^d Inoculation by *Homalodisca coagulata* (glassy-winged sharpshooter: GW), *Graphocephala atropunctata* (blue-green sharpshooter: BG), *Xyphon* [*Carneiocephala*] *fulgida* (red-headed sharpshooter: RH), or needle inoculation (NI).

Table 2. Comparison of *Xylella fastidiosa* infection in *Graphocephala atropunctata* (blue-green sharpshooter: BG) and mechanically (NI: needle) inoculated greenhouse-grown plants

Plant	Inoc.	No. tested	No. infected (%)	CFU/g ^a (range)	No. systemic ^b (%)
Joboba	BG	41	5 (12)	4.1 (3.1 – 4.3)	1/5 (20)
Joboba	NI	74	3 (4)	5.3 (4.1 – 6.4)	2/3 (67)
Prickly lettuce	BG	56	*35 (63) ^c	6.4 (2.9 – 8.0)	8/33 (24)
Prickly lettuce	NI	54	*13 (24)	6.3 (3.7 – 8.2)	7/19 (37)
Cocklebur	BG	71	*54 (76)	5.5 (2.5 – 7.1)	33/54 (61)
Cocklebur	NI	32	*11 (34)	4.3 (2.5 – 5.8)	7/11 (64)
Ambrosia	BG	27	21 (78)	6.3 (3.4 – 7.3)	16/21 (76)
Ambrosia	NI	19	10 (53)	6.2 (2.6 – 7.2)	9/10 (90)
Common morning glory	BG	43	*36 (84)	5.4 (3.5 – 7.4)	19/36 (53)
Common morning glory	NI	51	*12 (24)	4.8 (3.4 – 6.6)	7/17 (41)
Poison hemlock	BG	22	14 (64)	6.7 (2.8 – 7.7)	11/14 (79)
Poison hemlock	NI	15	10 (67)	6.3 (3.9 – 7.1)	5/10 (50)
Quinoa	BG	24	*12 (50)	3.8 (2.6 – 5.6)	2/12 (17)
Quinoa	NI	49	*13 (27)	3.5 (2.8 – 4.8)	7/13 (54)

^a *X. fastidiosa* populations in log₁₀ CFU per gram of plant tissue at inoculation site.

^b Systemic infections are *X. fastidiosa* recovery by culture from an adjacent stem or petiole distal to the inoculation site. In common morning glory and prickly lettuce, more *X. fastidiosa* infections were detected systemically than at the inoculation site because *X. fastidiosa* was recovered from distal stems after the inoculated leaf senesced.

^c * = Numbers infected are significantly different between BG and NI inoculation ($P < 0.05$, chi-square with Yates' correction).

static in another six species: cheeseweed, cocklebur, annual bur-sage, common morning glory, quinoa, and tomato (*Lycopersicon esculentum* cv. Ace). Bacterial populations decreased or were undetectable in johnsongrass (*Sorghum halepense*), jojoba (*Simmondsia chinensis*), annual sowthistle (*Sonchus oleraceus*), southwestern cupgrass (*Eriochloa gracilis*), whitestem filaree (*Erodium moschatum*), curly dock (*Rumex crispus*), poison hemlock, blue gum, tree tobacco, and eggplant (*Solanum melongea* cv. Violeta lunga). Few *X. fastidiosa* infections moved into the plant beyond the inoculation site in plants with typical basal rosette morphology (curly dock, whitestem filaree, annual sowthistle, and poison hemlock), where basal leaves inoculated with *X. fastidiosa* senesced as the plant matured.

Comparison of *X. fastidiosa* infection in field versus greenhouse. There were fewer *X. fastidiosa* infections in field-grown plants compared with greenhouse-grown plants for the 9-week sampling period in both summer and winter experiments (Fig. 2). Bacterial populations in field-grown plants were lower, and

these plants had fewer systemic infections detected beyond the inoculation site (Table 3).

In winter annuals grown from November 2001 to March 2002, *X. fastidiosa* was recovered from 23% (26 of 114) of field-grown plants and 49% (51 of 105) of greenhouse-grown plants. Median bacterial populations in field-grown plants were approximately 100-fold lower (2×10^6 CFU/g in the greenhouse and 3×10^4 CFU/g in field-grown plants). We detected systemic movement of *X. fastidiosa* in 49% of infected plants in the greenhouse, but only in 12% of infected field-grown plants. Median *X. fastidiosa* populations increased in greenhouse-grown plants over the 9-week sampling period (2×10^5 CFU/g at 1 week, 6×10^6 CFU/g at 3 weeks, and 1×10^8 CFU/g at 9 weeks), but decreased in field-grown plants (8×10^3 CFU/g at 1 week, 1×10^5 CFU/g at 3 weeks, and 4×10^4 CFU/g at 9 weeks). During winter-spring field tests, most temperatures (2,229 h of 2,789 total h) in the screen cage at Bakersfield were below the growth threshold for *X. fastidiosa*,

while greenhouse temperatures were set at 25°C, near the optimum temperature for *X. fastidiosa* growth.

Results were similar from July to November 2002: field-grown plants were less-frequently infected than greenhouse-grown plants. Over 9 weeks, *X. fastidiosa* was detected in 67% (75 of 112) of greenhouse-grown plants compared with 34% (28 of 83) of field-grown plants ($P < 0.001$, chi-square comparison). However, median *X. fastidiosa* populations in infected plants were not significantly different: 2×10^6 CFU/g in greenhouse plants compared with 6×10^5 CFU/g in field-grown plants (two-sample *t* test, $n = 90$, $P = 0.16$). Systemic movement was detected in 51% (36 of 71) of infected greenhouse plants, but only in 26% (5 of 27, $P < 0.05$, chi-square with Yates correction) of infected field-grown plants. Greenhouse temperatures were more moderate than field temperatures in the summer-fall experiments (2,501 of 2,606 h between 17 and 34°C in greenhouse; 2,042 of 3,054 h were between 17 and 34°C in the field). Summer field site temperature data were

Fig. 1. *Xylella fastidiosa* infection in nine representative greenhouse-grown alternate hosts at 1, 3, and 9 weeks after inoculation. Black areas represent the number of sites with *Xf* recovery, and striped areas represent sites without *X. fastidiosa*. Populations (line, on the secondary y-axis) are in $\log_{10}$ CFU per gram of plant tissue. Sacred datura, common sunflower, cocklebur, prickly lettuce, and fava bean are examples of high population, frequently infected hosts. Quinoa and johnsongrass were infrequently infected and reached lower bacterial populations. Southwestern cupgrass was not a host after 1 week.

*All inoculation sites senesced from poison hemlock by 9 weeks.

from Arvin station. Although 17 km from the field site, the two locations were geographically similar and had only a 1.1°C difference in comparisons of concurrent temperature data from both sites.

DISCUSSION

Our study confirmed that plant species cannot be simply classified as either “hosts” or “nonhosts” of *X. fastidiosa* but vary considerably among plant species in supporting growth and movement of the bacterium. In addition, environmental conditions have a major impact on bacterial growth in host plants. We propose that a good source plant for vector acquisition of *X. fastidiosa* has four characteristics: it must (i) be a feeding source for sharpshooters, (ii) frequently develop infections when inoculated by infective insects, (iii) allow systemic bacterial movement throughout the plant, and (iv) support high *X. fastidiosa* populations. The majority of the plants we tested met the first criterion. Both *G. atropunctata* and *H. coagulata*, typical of many xylem feeders, feed on an extremely wide range of plant species (22,32) and can acquire *X. fastidiosa* after only a few hours of feeding on Pierce’s diseased grapevines (2,24). In our inoculation attempts, an average 77% of sharpshooters survived at least 2 days on the plants we tested, and even feeding access periods of a few hours are sufficient for vector acquisition of *X. fastidiosa* (2,24). Both *G. atropunctata* and *H. coagulata* have adult life spans of many months (29,32), which increases opportunities for the acquisition of *X. fastidiosa* from the variety of plants on which they feed.

Considerable interspecific variation was found for plants for the last three host criteria listed above. Our findings are similar

to those of Freitag (11), Raju et al. (27), Hill and Purcell (16), and Purcell and Saunders (26), where most plants tested supported *X. fastidiosa* populations to some degree. However, our study also assessed *X. fastidiosa* populations at regular intervals after inoculation, and found that the plants tested supported three possible fates of *X. fastidiosa* infections: they increased over time (e.g., sacred datura), peaked at 6 weeks (johnsongrass), or remained static over the testing interval (cocklebur).

Inoculation efficiency varies between sharpshooter species (2,28). In this study, sharpshooter inoculation with *G. atropunctata* was usually more efficient than needle inoculation for establishing *X. fastidiosa*

infections in the plants we tested, while inoculation with *H. coagulata* was least efficient. However, the incidence of systemic infections detected in needle-inoculated plants was often higher than for sharpshooter-inoculated plants. This should be considered when interpreting studies of systemic *X. fastidiosa* movement in plants.

Other factors that have been shown to influence the establishment and persistence of *X. fastidiosa* infection in almonds and grapes include bacterial strain, plant growth hormones, and pruning (1,9,19), the sharpshooter species inoculating the plant, and field conditions and other environmental factors. Initial *X. fastidiosa* population may not affect the probability of colonization. Although *X. fastidiosa*

Table 3. Systemic movement of *Xylella fastidiosa* beyond the insect vector feeding site in hosts in the greenhouse and field in winter (November 2001 to March 2002) and summer (July to November 2002)^a

Plant	Season	Location	No. systemic ^b / no. infected	CFU/g ^c (median)
Poison hemlock	Winter	GH	16/24	6.5
		Field	0/12	...
Fava bean	Winter	GH	5/15	4.7
		Field	0/4	...
Prickly lettuce	Winter	GH	3/10	2.5
		Field	3/9	3.2
	Summer	GH	5/24	3.1
		Field	0/10	...
Common sunflower	Summer	GH	17/23	5.5
		Field	2/5	4.5
Cocklebur	Summer	GH	14/24	6.0
		Field	5/12	4.7

^a Alternate hosts were inoculated with *X. fastidiosa* by blue-green sharpshooters during 3 days in the greenhouse, then kept in the greenhouse (GH) or planted outside (field) 1 day later. Populations are in log₁₀ CFU per gram of plant material.

^b Systemic infections are *X. fastidiosa* recovery by culture from an adjacent stem or petiole distal to the inoculation site.

^c Median *X. fastidiosa* populations in log₁₀ CFU per gram of plant tissue in an adjacent stem or petiole distal to the inoculation site.

Fig. 2. *Xylella fastidiosa* infection at the caged insect feeding site in five alternate host species grown in the greenhouse (GH) and field in winter (November 2001 to March 2002) and summer (July to November 2002). Black area represents the number of inoculation sites with *X. fastidiosa* recovery; striped area is the number of bacteria-free inoculation sites. Diamonds (secondary y-axis) represent median *X. fastidiosa* populations in log₁₀ CFU/g.

populations were not measured immediately after inoculation in this study, an estimated 10^6 CFU of *X. fastidiosa* were introduced by each mechanical inoculation, which had lower infection rates than *G. atropunctata* inoculation. In contrast, few bacteria are required for infection by sharpshooters, since *G. atropunctata* can infect plants even when the population of bacteria in their mouthparts is below detectable levels (15).

This study showed that *X. fastidiosa* growth was markedly affected by environmental conditions. Although it was impossible to account for the impact of temperature alone in field experiments, temperature is likely a major driver or limiter of bacterial growth. For a subtropical and tropical pathogen, *X. fastidiosa* has an unexpectedly low maximum temperature for indefinite survival, 34°C (10). In this study, summer field temperatures were generally higher than the optimal growth temperature for *X. fastidiosa* and sometimes higher than the growth limiting temperature of 34°C. This may explain the lower populations of *X. fastidiosa* that we detected in summer field plants compared with plants maintained in the greenhouse. Cool but above-freezing (5°C) temperatures also decreased viable populations of *X. fastidiosa* (10), consistent with our findings that *X. fastidiosa*-inoculated plants in the same cohort reached much lower populations in winter field conditions than did plants held in greenhouse conditions optimal for *X. fastidiosa* growth.

Alternate host studies utilizing enzyme-linked immunosorbent assays (27) or polymerase chain reaction (5) have identified plants harboring *X. fastidiosa* in the field, but population estimates in field-grown plants are needed to estimate their importance as sources of *X. fastidiosa* for vectors. In our summer field study, all species grown in the greenhouse developed *X. fastidiosa* populations greater than 10^6 CFU/g, but similar populations were reached only in prickly lettuce in the field-grown plants. Field-grown common sunflower and cocklebur also supported *X. fastidiosa* populations around 10^5 CFU/g, 10 times greater than the threshold for sharpshooter acquisition of *X. fastidiosa* from grape (17). In winter tests, *X. fastidiosa* populations in field-grown fava bean and poison hemlock were also between 10^4 and 10^5 CFU/g, while prickly lettuce did not support populations sufficient for sharpshooter acquisition. Further study is needed to determine factors governing systemic movement, even in grape, where it may vary with time of year (9) and variety (25).

Our study reinforces the need for weed control in irrigation ditches and roadsides adjacent to vineyards, in regions with

chronic Pierce's disease and established populations of sharpshooters, especially in warm weather. Five species tested in this study have characteristics that could make them important in the spread of *X. fastidiosa* under field conditions, depending on vector populations. Common sunflower, annual bur-sage, common morning glory, poison hemlock, and fava bean (a cover crop plant) had at least 60% of insects survive the testing interval, supported bacterial populations of 10^6 CFU/g or greater, and had systemic movement beyond inoculation sites. In contrast, cheeseweed and sowthistle have been observed as feeding hosts of *H. coagulata* (A. Purcell, unpublished data), but our studies indicate that these two plant species are unlikely to support adequate populations of the bacterium for frequent vector acquisition.

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LITERATURE CITED

- Almeida, R. P. P., and Purcell, A. H. 2003. Biological traits of *Xylella fastidiosa* strains from grapes and almonds. *Appl. Environ. Microbiol.* 69:7447-7452.
- Almeida, R. P. P., and Purcell, A. H. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata*. *J. Econ. Entomol.* 96:264-271.
- Anonymous. 1999. The Growers Weed Identification Handbook. Univ. Calif. Div. Agric. Natl. Resources Publ. no. 4030.
- Blua, M. J., Phillips, P. A., and Redak, R. A. 1999. A new sharpshooter threatens both crops and ornamentals. *Calif. Agric.* 53:22-25.
- Costa, H. S., Raetz, E., Pinckard, T. R., Gispert, C., Hernandez-Martinez, R., Dumenyo, C. K., and Cooksey, D. A. 2004. Plant hosts of *Xylella fastidiosa* in and near southern California vineyards. *Plant Dis.* 88:1255-1261.
- Davis, M. J., Purcell, A. H., and Thomson, S. V. 1980. Isolation media for the Pierce's disease bacterium. *Phytopathology* 70:425-429.
- Davis, M. J., Raju, B. C., Brlansky, R. H., Lee, R. F., Timmer, L. W., Norris, R. C., and McCoy, R. E. 1983. Periwinkle wilt bacterium: Axenic culture, pathogenicity, and relationships to other gram-negative, xylem-inhabiting bacteria. *Phytopathology* 73:1510-1515.
- Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley & Sons, New York.
- Feil, H., Feil, W. S., and Purcell, A. H. 2003. Effects of date of inoculation on the within-plant movement of *Xylella fastidiosa* and persistence of Pierce's disease within field grapevines. *Phytopathology* 93:244-251.
- Feil, H., and Purcell, A. H. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. *Plant Dis.* 85:1230-1234.
- Freitag, J. H. 1951. Host range of the Pierce's disease virus of grapes as determined by insect transmission. *Phytopathology* 41:920-931.

- Freitag, J. H., and Frazier, N. W. 1954. Natural infectivity of leafhopper vectors of Pierce's disease virus of grape in California. *Phytopathology* 44:7-11.
- Hendson, M., Purcell, A. H., Chen, D., Smart, C., Guilhabert, M., and Kirkpatrick, B. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. *Appl. Environ. Microbiol.* 67:895-903.
- Hickman, J. C., ed. 1993. The Jepson Manual: Higher Plants of California, 2nd ed. University of California Press, Berkeley.
- Hill, B. L., and Purcell, A. H. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. *Phytopathology* 85:209-212.
- Hill, B. L., and Purcell, A. H. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. *Phytopathology* 85:1368-1372.
- Hill, B. L., and Purcell, A. H. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. *Phytopathology* 87:1197-1201.
- Hopkins, D. L. 1976. Pierce's disease of grapevines. *Am. Wine Soc.* 8:26-27.
- Hopkins, D. L. 1985. Effects of plant growth regulators on development of Pierce's disease symptoms in grape. *Plant Dis.* 69:944-946.
- Hopkins, D. L., and Adlerz, W. C. 1988. Natural hosts of *Xylella fastidiosa* in Florida. *Plant Dis.* 72:429-431.
- Houston, B. R., Esau, K., and Hewitt, W. B. 1947. The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. *Phytopathology* 37:247-253.
- Purcell, A. H. 1976. Seasonal changes in host plant preference by the blue-green sharpshooter (*Hordnia circellata*). *Pan-Pac. Entomol.* 52:33-37.
- Purcell, A. H. 1980. Environmental therapy for Pierce's disease of grapevines. *Plant Dis.* 64:388-390.
- Purcell, A. H., and Finlay, A. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. *Phytopathology* 69:393-395.
- Purcell, A. H. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. *Phytopathology* 71:429-435.
- Purcell, A. H., and Saunders, S. R. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. *Plant Dis.* 83:825-830.
- Raju, B. C., Goheen, A. C., and Frazier, N. W. 1983. Occurrence of Pierce's disease bacteria in plants and vectors in California. *Phytopathology* 73:1309-1313.
- Redak, R. A., Purcell, A. H., Lopes, J. R. S., Blua, M. J., Mizell, R. F., III, and Andersen, P. C. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annu. Rev. Entomol.* 49:243-270.
- Severin, H. P. 1949. Life history of the blue-green sharpshooter, *Neokolla circellata*. *Hilgardia* 19:187-189.
- Severin, H. P. 1949. Transmission of the virus of Pierce's disease of grapevines by leafhoppers. *Hilgardia* 19:190-206.
- Severin, H. P. 1950. Spittle-insect vectors of Pierce's disease virus. *Hilgardia* 19:357-381.
- Turner, W. F., and Pollard, H. N. 1959. Life history and behavior of five vectors of Phony Peach disease. *U.S. Dep. Agric. Tech. Bull.* 1188.
- Zimmermann, M. H. 1983. Xylem structure and the ascent of sap (Springer series in wood science, v.1). Springer-Verlag, Berlin. pp. 31, 76.