FATE OF *XYLELLA FASTIDIOSA* IN ALTERNATE HOSTS

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ABSTRACT

Investigations of the fate of the Pierce's disease bacterium *Xylella fastidiosa* (*Xf*) in alternate hosts from which sharpshooters might acquire *Xf* identified bacterial hosts among vineyard weeds, cover crops, field crops and adjacent vegetation common to vineyards in California's San Joaquin Valley. Work for the past year focused on completing additional replications of 15 high-priority weeds to compare mechanical and insect vector inoculation efficiencies, and to determine the fate of bacterial populations over a nine-week period after inoculation with *Xf*. Four things are required for a plant to be a good source of *Xylella* for vector acquisition: it must be an attractive food host to sharpshooters, develop *Xylella* infections frequently when fed on by infective insects, allow systemic growth of the bacteria beyond the inoculation site, and support population growth above 1,000,000 cfu of *Xf* per gram of plant tissue. Although insect survival varied considerably between replications, it averaged 77% on all plants for two days. Ten of the 35 weed species examined were colonized by *Xf* more than 50% of the time in greenhouse studies when plants were maintained in ideal conditions for bacterial growth. Sixteen species supported *Xylella* populations above 1,000,000 cfu/g, thirteen had populations between 1,000,000 and 10,000 cfu/g, and three had populations below 10,000 cfu/g. Finally, 13 species had *Xf* recovery immediately distal to the inoculation site in greater than half of successfully inoculated plants. The plants that had greater than 60% insect survival during inoculation, more than 50% of sites inoculation sites infected in greenhouse tests, that supported bacterial populations of 1,000,000 cfu/g or larger, and had systemic *Xf* movement beyond the inoculation site in more than half the infected inoculation sites were: black nightshade, common sunflower, annual bur-sage, morning glory, poison hemlock and fava bean.

INTRODUCTION

Understanding the fate of *Xylella fastidiosa* in its various hosts is important because of the wide range of plants that the insect vectors feed on (Adlerz, 1980; Purcell, 1976 and unpublished data). Additionally, the bacteria itself survives and multiplies in an unusually large number of plants (Freitag, 1951; Raju *et al.* 1983; Hopkins, 1988). Studies of the fate of *Xf* in four plant species highly preferred by various insect vectors revealed that blackberry, mugwort, and watergrass were propagative hosts but only blackberry allowed systemic movement (within-plant spread) of *Xf* (Hill and Purcell, 1995). In grape and two other hosts, vector transmission occurred only from plants with population densities exceeding 10,000 cells per gram, with efficient transmission occurring only near 10,000,000 (log 7) *Xf* cells per gram. Further studies of the fate of *Xf* in 33 species of riparian plants commonly found in Napa valley revealed that most species were propagative but non-systemic hosts (infected only the inoculated plant cells) of the bacteria and suggested that *Xylella* eventually disappeared from non-systemic hosts (Purcell and Saunders, 1999). Previously reported (Wistrom and Purcell, 2002) data in this study identified 9 alternate hosts that developed *Xf* infections greater then 50% of the time. Seven species supported *Xf* populations over 1,000,000 colony-forming units [cfu] per gram of plant tissue. Bacterial survival in field conditions was tested with 5 previously identified alternate hosts in summer and winter field studies. *Xylella* was recovered less frequently (at 26% of inoculation sites compared to 46% in winter, and 35% of inoculation sites compared to 21% in the summer) and at approximately 10-fold lower populations from field-grown plants from Bakersfield, CA, as compared to greenhouse-grown plants, in ideal conditions for *Xf* growth (Feil and Purcell, 2001) during the first three weeks after inoculation. In general, most non-systemic hosts of *Xf* developed highest populations within 1 to 3 weeks that thereafter decreased, while *Xf* populations in systemic hosts continued to increase or remain at the highest densities attainable in a particular host.

OBJECTIVES

Evaluate the fate of *Xylella fastidiosa* in Central Valley weeds and other crops of interest.

RESULTS

Experiments in the past year focused on repeating inoculations of 15 high-priority weeds to obtain larger sample sizes and comparisons of inoculation efficiency between glassy-winged sharpshooters, blue-green sharpshooters, and mechanical inoculation. Three new host species were tested; curly dock, lambsquarters, 'Moapa' alfalfa. To date, 35 species of common vineyard weeds, crops and other plants have been evaluated. All plants were inoculated with STL, a Pierce's disease strain of

Xf originally isolated from Napa valley. Plants were inoculated with *Xf*-carrying sharpshooters by confining groups of 2 to 4 infective sharpshooters to a 3-cm length of stem, petiole or leaf (depending on plant morphology) in a foam-and-mesh cage for 2 days. Only results from sharpshooter groups capable of successfully transmitting *Xf* were used. Plants were mechanically inoculated by placing 5 µl of turbid (8 to 9 $\log_{10}$ cfu/ml) *Xf* cell suspension in SCP buffer on the stem, leaf or petiole of the plant, which was probed with a #2 insect pin until drawn into the plant. Inoculations were performed on sunny days to maximize uptake of the *Xf* suspension via transpiration. Plants were assayed for presence and population of *Xf* by culture on semi-selective PWG media using the techniques of Davis (1983) and Hill (1995).

Host range experiments with plants inoculated in the greenhouse identified 10 species of weeds that were infected greater than 50% of the time with *Xf*. These were black nightshade, common sunflower, common cocklebur, annual bur-sage, morning glory, marestail, silverleaf nightshade, sacred datura, poison hemlock and fava bean. Another 18 species become infected 20 to 50% of the time when inoculated with *Xf*; johnsongrass, cheeseweed, field bindweed, yellow nutsedge, purple nutsedge, prickly lettuce, southwestern cupgrass, whitestem filaree, curly dock, common purslane, California burclover, black mustard, quinoa, tree tobacco, red gum, 'Ace' tomato, 'Violeta Lunga' eggplant, and 'Moapa' alfalfa. Plants that became infected less than 20% of the time in greenhouse tests were: jojoba, annual sowthistle, prostrate pigweed, watergrass, and blue gum. Only white clover (0 of 3 sites) and red clover (0 of 5) had no *Xf* recovery from any inoculation sites tested. A total of 54 and 74 sites were tested respectively, but either were contaminated with bacteria other than *Xf* or inoculated with non-infective sharpshooters. California burclover, black mustard and alfalfa did not have infections move beyond inoculation sites, however the three species had small sample sizes due to contamination and poor transmission of the sharpshooters. Johnsongrass had *Xf* populations of $\log_{10}5$ cfu/g at the inoculation site, but only developed systemic infections at 2 of 14 infected sites. Other species with fewer than 20% of infections moving beyond the inoculation site were annual sowthistle and yellow nutsedge.

Populations of *Xf* in alternate hosts followed three main patterns, either increasing over nine weeks, remaining steady over the sampling period, and decreasing. *Xf* populations increased in 6 plants, including fava bean, field bindweed, and annual morning glory. Seven plant species had steady populations of *Xf* at 3 and 9 weeks after inoculation. *Xf* populations decreased in 3 species, johnsongrass, sowthistle, and eggplant population from three weeks after inoculation. Species with populations less than 10,000 cfu/ gram of plant material were cheeseweed, jojoba, annual sowthistle, and whitestem filaree. Sharpshooters cannot acquire *Xf* from plants with populations lower than 10,000 cfu/g (Hill and Purcell, 1997).

Some plants other than white clover had a large proportion of contaminated inoculation sites. This appeared to be a function of the plant rather than deficiencies in technique, as contamination problems were consistent across replications and other species tested on the same day were not contaminated. Black nightshade was tested three times, but all samples were contaminated in two replications. Prostrate pigweed had 10 of 19 sites contaminated, and red clover had 10 of 11 sites contaminated. Other complications were low infectivity (poor acquisition of *Xf*) of blue-green sharpshooters used in experiments from October 2002 to February 2003. Only 30% (46 of 152 groups) were infective compared to 94% (119 of 126 groups) from April to July 2003. This was not the case the previous winter (November 2001 to January 2002), where 122 of 128 (95%) of sharpshooter groups transmitted *Xf*. Probable causes for the drop were the condition of the Pierce's disease-infected plants from which the sharpshooters acquired *Xf*, or reduced feeding by sharpshooters.

Five of the 6 plant species that had been inoculated both with blue-green sharpshooters and mechanically had a greater percentage of sites became infected with *Xf* when the plants were insect-inoculated. Poison hemlock was the sole exception (Table 1). Most notably, annual morning glory had 93% (28 of 30 stems) become infected with *Xf* when fed on by infective insects, but 0 of 20 when mechanically inoculated. It is unclear why this is the case; however, it is not due to the disruption of *Xf* infection by excretion of protective latex as results for prickly lettuce show that *Xf* can be mechanically introduced into plants that produce latex (12 of 53 sites infected).

Four other plants had varying results when mechanically inoculated as compared to infected with glassy-winged sharpshooters (GWSS). Cheeseweed developed infections in 29 of 57 (51%) needle-inoculated sites but only in 1 of 12 (8%) insect-inoculations. Similar results were seen for sacred datura (needle inoculation: 43 of 56, compared with 1 of 7 for GW), and red gum (needle inoculation: 12 of 33; 0 of 5 for GW). In previous tests, 30% of GWSS groups transmitted bacteria to seedling grapes, compared to 96% of blue-green sharpshooter groups. It appeared that GWSS were even less efficient at transmitting *Xf* to alternate hosts than they were to grapes.

Table 1. Comparison of insect (BG: blue-green sharpshooter) and mechanical (NI: needle) inoculation.

Plant	Inoc.	**Sites Infected**	% Sites w/ *Xf*	Median [*Xf*]	Systemic Infections	% Sys w/ *Xf*	Systemic [*Xf*]
jojoba	BG	5/41	12	log 4	1/5	20	log 4
jojoba	NI	3/74	4	log 6	2/3	67	log 6
prickly lettuce	BG	35/56	63	log 6	8/33	24	log 3
prickly lettuce	NI	13/54	24	log 6	7/13	54	log 6
cocklebur	BG	54/71	76	log 5	33/54	61	log 4
cocklebur	NI	11/32	34	log 4	7/11	64	log 6
ann. bur-sage	BG	21/27	78	log 6	17/21	81	log 6
ann. bur-sage	NI	10/19	53	log 5	9/10	90	log 4
m. glory	BG	36/43	84	log 5	19/36	84	log 4
m. glory	NI	12/51	24	log 5	7/17	42	log 4
hemlock	BG	14/22	64	log 6	11/14	79	log 6
hemlock	NI	10/15	67	log 4	5/10	50	log 5
quinoa	BG	12/24	50	log 5	2/12	17	log 4
quinoa	NI	13/49	27	log 3	6/13	46	log 4

Sites infected are the *Xylella fastidiosa (Xf)*-infected sites over total number inoculated. Median *Xf* population is in $\log_{10}$ colony-forming-units per gram of plant material (cfu/g) as determined by culture. Systemic infections are *Xf* infections distal to the inoculation site, over number of sites infected, showing the tendency for *Xf* to colonize the plant. Systemic [*Xf*] is the population of *Xf* at distal to the inoculation site in $\log_{10}$ cfu/g.

CONCLUSIONS

For a plant to be a good source from which sharpshooters can acquire *Xf*, the plant must be an attractive food host to sharpshooters, capable of being inoculated with *Xf,* allow the spread of the bacteria beyond the inoculation site, and support population growth above well 10,000 cfu of *Xf* per gram of plant tissue, the threshold for bacterial acquisition by sharpshooters when feeding on a host plant. While most of the plants examined supported some degree of bacterial growth, few have all the traits that are needed to play a role in the propagation of Pierce's disease by serving as inoculum sources for large numbers of sharpshooters.

Although insect survival varied considerably between replications, it averaged 77% on all plants for two days, confined to a small foam-and-mesh cage. All plants studied had insect survival above 60% for all insect-inoculated replications except for southwestern cupgrass, marestail, purslane, sacred datura, tree tobacco and blue gum. Even in this artificial situation, sharpshooters fed on a wide range of plants and potentially exposed them to *Xylella*.

Ten of the 35 weed species examined were inoculated more than 50% of the time in greenhouse studies. Plants were maintained in ideal conditions for bacterial growth in the greenhouse following the two-day inoculation access period with 2 to 4 insects per inoculation site. When similarly inoculated plants were placed outside for the nine-week evaluation period in the previous years' field study, many fewer *Xf* infections survived. As noted in the introduction, 20% fewer infections developed field-grown plants in winter, and 14% less in the summer. Thus it is unlikely that the plant species that had low inoculation efficiencies in the greenhouse will develop infections in the field.

Study results will assist researchers by identifying the biology of *Xf* in various hosts, while grape growers may use this information to determine which types of vegetation are potential inoculum sources, and when to eliminate them.
Sixteen species supported *Xylella* populations above 1,000,000 cfu/g, thirteen had populations between 1,000,000 and 10,000 cfu/g, and three had populations below 10,000 cfu/g. Since acquisition efficiency by sharpshooters increases with the bacterial population in the host plant (Hill 1997), plants with bacterial populations below 1,000,000 cfu/g are unlikely to be major inoculum sources. Finally, the bacteria must move beyond the inoculation site throughout the plant to colonize it. The plants that had all the traits; greater than 60% insect survival during inoculation, were infected greater than 50% of the time in greenhouse tests, supported bacterial populations of 1,000,000 cfu/g or larger, and had systemic *Xf* movement beyond the inoculation site at more than 50% of plants where *Xylella* was recovered at the inoculation site, were black nightshade, common sunflower, annual bur-sage, morning glory, poison hemlock and fava bean.

REFERENCES

Adlerz, W.C. 1980. Ecological observations on two leafhoppers that transmit the Pierce's disease bacterium. Proceedings of the Florida State Horticultural Society 93:1980.

Davis, M.J., *et al.* 1983. Periwinkle wilt bacterium: axenic culture, pathogenicity, and relationships to other gram-negative, xylem-inhabiting bacteria. Phytopathology 73: 1510-1515.

Feil, H., and A.H. Purcell. 2001. Temperature-dependant growth and survival *of Xylella fastidiosa in vitro* and in potted grapevines. Plant Disease 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.

Hill, B.L., and A.H. Purcell, 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology 97: 1197-1201.

Hill, B.L., and A.H. Purcell. 1995. Multiplication and movement of *Xylella fastidiosa* within grape and four other plants. Phytopathology 85: 1368-1372.

Hopkins, D.L., and W.C. Adlerz. 1988. Natural Hosts of *Xylella fastidiosa* in Florida. Plant Disease 72: 429-431.

Purcell, A.H. 1976. Seasonal changes in host plant preference by the blue-green sharpshooter (*Hordinia circellata*). Pan-Pacific Entomology 52:33-37.

Purcell, A.H., and S.R. Saunders. 1999. Fate of Pierce's disease strains *of Xylella fastidiosa* in common riparian plants in California. Plant Disease 83: 825-830.

Raju, B.C., A.C. Goheen,, N.W. Frazier. 1983. Occurrence of Pierce's disease bacteria in plants and vectors in California. Phytopathology 73: 1309-1313.

Wistrom, C.W., and A.H. Purcell. 2002. Fate of *Xylella fastidiosa* in alternate hosts. *In*: Proceedings, Pierce's Disease Research Symposium, M. Athar Tariq, S. Oswalt, P. Blincoe, and T. Esser, (eds.), December 15-18, 2002, San Diego, California, California Department of Food and Agriculture, Sacramento, CA.

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