

PATTERNS OF *XYLELLA FASTIDIOSA* INFECTION IN PLANTS AND EFFECTS ON ACQUISITION BY INSECT VECTORS

Project Leaders:

Alexander H. Purcell
ESPM – Division of Insect Biology
University of California
Berkeley, CA 94720

Steven E. Lindow
Plant and Molecular Biology
University of California
Berkeley, CA 94720

Researcher:

Christina Wistrom
ESPM – Division of Insect Biology
University of California
Berkeley, CA 94720

Reporting Period: The results reported here are from work conducted October 2004 to September 2005.

ABSTRACT

This project investigates the effects of host plant tolerance on insect vector acquisition and spread of *Xylella fastidiosa* (*Xf*) from plants with a range of susceptibility to Pierce's disease (PD). By characterizing vascular anatomy, bacterial populations, and sharpshooter acquisition of *Xf* from those plants, we may assess their ability to provide inoculum for PD spread. Previously reported data from paint infusion experiments did not identify significant anatomical measures as an explanation for tolerance or resistance differences among grape varieties. There were significant differences in vascular anatomy between alternate host species, and vessels of all alternate hosts were much shorter than in grapevines. In morning glory, 71% of vessels were less than 3cm, and the other species had between 40% and 63% of vessels <3cm. The longest vessel measured in any alternate host was 32cm (sunflower), and on average, sunflower and quinoa had longer vessels than mugwort and morning glory. Mugwort had roughly twice as many vessels at the stem base than morning glory, quinoa or sunflower stems of comparable diameter and age. Sunflower, mugwort and quinoa had vascular tissues in evenly distributed bundles with wide regions of parenchyma between them. Annual morning glory had a smaller number of large vessels distributed evenly along the cambium, compared to large numbers of small vessels in mugwort. As grapevines, most susceptible to *Xf* infection, had the longest vessels, and sunflower, the second most susceptible, had the next-longest vessels. The relationship between vascular anatomy and *Xf* host status appears complicated, and additional comparisons of overall vascular area and vessel distribution are required to generalize further.

Plants were inoculated with an *Xf* strain that continually expressed green fluorescent protein (Gfp-*Xf*), or, as a control, the wild-type parent strain. Six weeks after inoculation, *Xf*-free sharpshooters were placed on the inoculation site for a 4-day acquisition access period, and tested for bacterial acquisition. Next, the inoculation site was examined with confocal microscopy and bacterial presence determined by culture. Six percent of grapes and 17% of alternate hosts had Gfp-*Xf* infections, compared to 50% of grapevines and 16% of alternate hosts inoculated with wild-type *Xf*. There was no difference in infection rate of wild-type *Xf* between grape cultivars. No colonized vessels were seen in plants infected with Gfp-*Xf*. Wild-type *Xf* populations were at least seven times lower in inoculated grape stems (10^5 to 10^6 CFU/g) compared to populations in distal petioles (10^6 to 10^8 CFU/g). Data analysis from those experiments is ongoing to determine the acquisition rate of *Xf* from inoculated stems.

INTRODUCTION

Plants with varying degrees of susceptibility to PD were assessed for their ability to provide inoculum for disease spread by characterizing their vascular anatomy, bacterial populations, and sharpshooter acquisition of *Xf* from them. Three grape cultivars and four alternate hosts were selected for their pattern of *Xf* colonization following vector inoculation, lack of stem lignification, morphology, and absence of autofluorescence. In previous experiments, *Xf*-carrying sharpshooters infected more than 80% of the morning glory and sunflower inoculated. *Xf* spread systemically throughout both plants and reached populations over 10^5 colony-forming units (CFU)/gram. Quinoa and mugwort were less-frequently infected (32% and 16%, respectively) by *Xf* and supported lower bacterial populations (10^3 CFU/g for quinoa, 10^6 CFU/g for mugwort). *Xf* moved systemically to a limited extent in quinoa, but not in mugwort (Hill and Purcell 1995b, Wistrom and Purcell 2005). Tolerant 'Sylvaner', moderately susceptible 'Cabernet Sauvignon' and highly susceptible 'Pinot Noir' cultivars of *Vitis vinifera* were selected for evaluation (Purcell 1981, Raju and Goheen 1981). Blue green sharpshooters (BGSS) were used to infect plants and assess insect acquisition efficiency (Almeida and Purcell 2003, Hill and Purcell 1995a, Hill and Purcell 1997, Purcell et al. 1979).

Vessel length was measured by infusion of diluted latex paint into cut stems at approximately 100kPa, pressure sufficient to displace sap in vessels but not strong enough to damage pit membranes between vessels (Ewers and Fisher 1989). Stems were infused ~48 hours, until displaced sap stopped exuding from the distal end. Stems were sectioned at regular intervals

and the number of vessels with paint counted at each interval. The number of vessels in each length class was determined from the raw vessel count by the double-difference correction (Ewers and Fisher 1989).

Wild type and transformed isolates of *Temecula Xf* were used for inoculations. The transformed isolate continually expressed green fluorescent protein (Gfp-*Xf*) when illuminated with blue light. In previous tests, Gfp-*Xf* was transmitted by BGSS, retained typical virulence in grape, and was visible in grape petioles via confocal microscopy. Gfp-*Xf* was observed individually and in large colonies, and passing through bordered pits between vessels of grape petioles (Newman et al. 2003). Electron and confocal microscopy with *in situ* DNA hybridization shows *Xf* densely packed in individual vessels, with adjacent vessels empty (Newman et al. 2003, Tyson et al. 1984). We hypothesize that alternate hosts or tolerant grape cultivars with low overall populations may have just a few vessels completely colonized with bacteria, leading to lower acquisition rates that are dependant upon sharpshooters encountering the colonized vessels while feeding. *Xf* inoculation, acquisition, and colonization were measured similarly in all plants. Groups of four BGSS inoculated a 3cm stem section with either wild-type or Gfp-*Xf*, and the plants were held in the greenhouse for six weeks to develop infections. New groups of four *Xf*-free BGSS were confined to the inoculation site for four days to acquire the bacteria, and then moved to a grape seedling for four days to determine their acquisition efficiency. After sharpshooter feeding, the stem site was examined with confocal microscopy. Three stem cross-sections per plant were suspended in 50% glycerol on a depression slide. Stem sections were illuminated with blue and ultraviolet light, to show green Gfp-*Xf* and blue vessel walls, and scanned for the presence of *Xf*. Bacterial populations were determined from remaining plant material of the inoculation site by culture on PWG media (Davis et al. 1983, Hill and Purcell 1995a).

One-way ANOVA was used to compare the number, length and distribution of xylem elements in grape varieties and alternate host species. Since acquisition efficiency has been related to bacterial populations (Hill and Purcell 1997), regression analysis will be used to qualitatively assess the contributions of bacterial distribution, proportion of colonized vessels, and bacterial population on acquisition.

OBJECTIVES

1. Describe the bacterial colonization of asymptomatic alternate host species and grape varieties of varying tolerance to PD.
2. Determine the relationship between the pattern of colonization of a plant by *Xf* and the ability of that plant to be a source for bacterial acquisition by sharpshooter vectors.

RESULTS

Anatomical comparisons between the various alternate hosts and grape cultivars included measurements of vessel length and number, and vascular bundle number and distribution (Table 1). The longest vessel measured in any alternate host was 15cm long (mugwort). In sunflower, 71% of vessels were less than 3cm long. Other species had between 63% and 40% of vessels <3cm. Mugwort had roughly twice as many vessels at the stem base than morning glory, quinoa or sunflower stems of comparable diameter and age. Sunflower, mugwort and quinoa had vascular tissues in evenly distributed vascular bundles with wide interfascicular regions of parenchyma.

In *Xf* inoculations by BGSS (Table 2), grapes were infected with Gfp-*Xf* less frequently than the parental wild type (11 of 22 grapes infected with Temecula, 1 of 16 infected with Gfp-*Xf*, $P < 0.01$, Chi-Square with Yates' correction). Infection rates of alternate hosts were similar (3 of 18 infected with Gfp-*Xf*, 3 of 19 infected with Temecula). Gfp-*Xf* was not observed in plant stems with low bacterial populations (10^2 CFU/g for grape, between 10^3 and 10^4 CFU/g in sunflower and mugwort, respectively). In all microscopy sessions, Gfp-*Xf* was observed in the symptomatic grape petioles used as positive controls to adjust microscope settings, which contained bacterial populations between 10^7 and 10^8 CFU/g. Insect-inoculated plants were used to compare sharpshooter acquisition and bacterial distribution from alternate host stems because the distribution of *Xf* in an insect-inoculated stem is likely different from a mechanically inoculated stem. Analysis of acquisition test plants is ongoing.

Table 1: Anatomical comparisons of canes/ stems of similar length, age, and diameter in four alternate hosts of *Xf*.

Species	Total # vessels at stem base (SE)	% Vessels < 3cm (SE)	Longest vessel (SE)	# Rays/ Bundles (SE)
<i>one-way ANOVA</i>	(n = 27, $P = 0.67$)	(n = 27, $P = 0.84$)	(n = 27, $P = 0.35$)	(n=27, $P = 0.01$)
Morning Glory	236 (24) c	71 (6) b	9 (1) a	84 (4) a
Mugwort	593 (58) a	63 (6) a,b	11 (1) a	19 (2) b
Quinoa	415 (29) b	40 (4) a	18 (2) b	26 (2) b
Sunflower	311 (25) b,c	41 (3) a	23 (3) b	19 (1) b
<i>one-way ANOVA*</i>	(n = 48, $P < 0.001$)	(n = 46, $P = 0.002$)	(n = 47, $P < 0.001$)	(n = 48, $P < 0.001$)

* Letters in bold indicate means are significantly different in pairwise comparisons with Tukey-Kramer HSD.

Table 2: BGSS transmission of wild-type (Temecula) and Gfp-expressing (Gfp-*Xf*) *Xf* to three grape cultivars and four alternate hosts.

Cultivar	<i>Xf</i> Isolate	No. Infected/ No. Inoculated	[<i>Xf</i>] Stem CFU/g ^a	% vessels colonized ^b	No. Systemic/ No. Infected ^c	[<i>Xf</i>] Systemic (petiole) ^d
Cabernet Sauvignon	Gfp- <i>Xf</i>	1/5	2.5×10^2	0	0/1	-
	Temecula	4/8	1.6×10^5	-	4/4	1.2×10^8
Sylvaner	Gfp- <i>Xf</i>	0/6	0	0	-	-
	Temecula	3/7	1.0×10^6	-	1/3	7.3×10^6
Pinot Noir	Gfp- <i>Xf</i>	0/5	0	0	-	-
	Temecula	4/7	6.0×10^6	-	3/4	9.2×10^7
Alternate Host Plant						
Morning Glory	Gfp- <i>Xf</i>	0/2	0	0	-	-
	Temecula	-	-	-	-	-
Mugwort	Gfp- <i>Xf</i>	1/4	4.5×10^4	0	0/1	-
	Temecula	1/6	3.2×10^2	-	0/1	-
Quinoa	Gfp- <i>Xf</i>	0/6	0	0	-	-
	Temecula	0/8	0	-	-	-
Sunflower	Gfp- <i>Xf</i>	2/4	1.2×10^3	0	0/2	-
	Temecula	2/5	1.7×10^5	-	1/2	4.1×10^3

^a *Xf* populations (colony-forming-units/ gram of plant material) in inoculated stems six weeks after vector inoculation

^b Proportion of vessels colonized by *Xf* in inoculated stems

^c Number of infections that moved beyond the inoculation site throughout the plant, as detected by culture of petioles 10-15cm distal from inoculation site

^d Population of *Xf* in distal petioles

CONCLUSIONS

Three things are required for the development of PD in grape: the pathogen *Xylella*, a sharpshooter insect vector, and a susceptible plant host. By systematically examining the interactions between plants and the pathogen, we may better understand the role that host resistance plays in the vector's ability to acquire *Xf* and spread PD. The vessels of alternate hosts were approximately 75% shorter than vessels of grapes, limiting the passive spread of *Xf* via xylem sap movement, and are found in bundles separated by parenchyma cells (Esau 1977), which may also limit the lateral spread of *Xf*. As grapevines, most susceptible to *Xf* infection, had the longest vessels, and sunflower, the second most susceptible, had the next-longest vessels, the relationship between vascular anatomy and *Xf* host status appears complicated. Additional comparisons of overall vascular area and vessel distribution are required before further generalization. Recent studies present conflicting data on whether *Xf* movement between bordered pits is an active or passive process (E. Thorne, G. Young, M. Matthews and T. Rost – *personal communication*; Newman et al 2003, Stevenson et al 2004); anatomical and biochemical differences in pit structure may explain differences between cultivar susceptibility to *Xf*.

Previous studies with symptomatic grape petioles, electron and confocal microscopy showed *Xf* densely packed in individual vessels, with adjacent vessels empty or containing a few cells (Newman et al. 2003, Stevenson et al. 2004). Alternate hosts or tolerant grape cultivars with low overall populations may have just a few vessels with bacteria, so acquisition would be highly variable and dependant upon sharpshooters encountering the few colonized vessels while feeding. In symptomatic grape petioles, 13% of vessels were colonized to some extent with Gfp-*Xf*, though only 2.1% of all vessels were completely blocked with bacteria (Newman et al. 2003). In this study, no Gfp-*Xf* was observed via confocal microscopy of the four infected stems; one grape, one mugwort, and two sunflower stems. The overall populations of Gfp-*Xf* in infected stems were at least 1,000-fold lower than Gfp-*Xf* populations in the symptomatic grape petioles used in previous experiments, and as positive controls in this experiment. It does not appear possible to detect Gfp-expressing *Xf* in infected stems at such low titers.

Though it is not known how many probes a sharpshooter makes in a given feeding session, glassy-winged sharpshooter can generate multiple salivary sheaths in one insertion, adjacent to vessels and xylem parenchyma cells (Leopold et al. 2003). Sharpshooter acquisition of *Xf* increased along with bacterial populations in infected grapes (Hill and Purcell 1997), and a similar positive relationship is expected if the proportion of colonized vessels increases insect acquisition of *Xylella*. Analysis of test grapes is ongoing to determine sharpshooter acquisition from the *Xf*-infected grapes and alternate hosts reported in Table 2.

REFERENCES

- Almeida, R.P.P. and A. H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata*. J. Econ. Entomology 96(2): 264-271.
- Davis, M. J., B. C. Raju, R. H. Brlansky et al. 1983. Periwinkle wilt bacterium: axenic culture, pathogenicity, and relationships to other gram-negative, xylem-inhabiting bacteria. Phytopathology 73: 1510-1515.
- Esau, K. 1977. Anatomy of Seed Plants. John Wiley and Sons, New York, NY. p. 308, 310.
- Ewers, F.W. and J. B. Fisher. 1989. Techniques for measuring vessel lengths and diameters in stems of woody plants. Am. J. Botany 76: 645-656.
- Hill, B.L. and A.H. Purcell. 1995a. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. Phytopathology 85: 209-212.
- Hill, B.L. and A.H. Purcell. 1995b. Multiplication and movement of *Xylella fastidiosa* within grape and four other plants. Phytopathology 85: 1368-1372.
- Hill B.L. and A.H. Purcell, 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology 87: 1197-1201.
- Leopold, R.A., T. P. Freeman, J. S. Bruckner, and D. R. Nelson. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*. Arthro. Struc. Devel. 32: 189-199.
- Newman, K.L., R.P.P Almeida, A. H. Purcell, and S.E. Lindow, 2003. Use of a green fluorescent strain of analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. App. and Env. Microbiol. 69: 7319-7327.
- 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., A. H. Finlay, and D.L. McLean. 1979. Pierce's disease bacterium: mechanism of transmission by leafhopper vectors. Science 206: 839-841.
- Purcell, A.H. and S.E. Lindow. 2004. Patterns of *Xylella fastidiosa* infection in plants and effects on acquisition by insect vectors, pp. 234-237. In Proc. of Pierce's Disease Res. Symposium, San Diego, CA, Dec. 7-10, 2004.
- Raju, B.C., and A.C. Goheen. 1981. Relative sensitivity of selected grapevine cultivars to Pierce's disease bacterial infections. Am. J. Enol. Vitic. 32: 155-158.
- Stevenson, J.F., M. A. Matthews, and T. L. Rost. 2004. Grapevine susceptibility to Pierce's disease I: relevance to hydraulic architecture. A. J. Enol and Vitic. 55: 228-237
- Tyson, G.E., B. J. Stojanovic, R. F. Kuklinski, T. J. DiVittorio, and M.L. Sullivan. 1984. Scanning electron microscopy of Pierce's disease bacterium in petiolar xylem of grape leaves. Phytopath. 75: 264-9.
- Wistrom, C., and A. H. Purcell. 2005. Fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts in California. Plant Disease. Plant Disease 89:994-999.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, and the College of Natural Resources, University of California, Berkeley.