Supplemental plant hosts for *Xylella fastidiosa*

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Objective

Survey weeds, woody plants, and ornamentals for supplemental plant hosts of *Xylella fastidiosa*

Procedures

In 2003, we collected weeds and woody plants at four Texas Hill Country vineyards and a few woody ornamentals and trees from urban settings. Two of the vineyard sites (Gillespie and Llano Counties) had a history of Pierce's Disease (PD) (Fig. 1) and two vineyards (Gillespie and Travis Counties) had no history of the disease.

Leaves and/or stems were collected in the field with emphasis on plants and plant parts with symptoms (dead leaf margins, yellow leaves), older leaves low on the plant, plants near a permanent water source (riparian habitat), and species in the composite, grass and grape plant families. Samples were placed in plastic bags and kept cool in insulated cool containers for transport to the laboratory. Additional plant material for species of uncertain or unknown botanical identification was pressed and dried for later examination. Fresh plant tissue was stored at 5oC until testing was completed.

Approximately 526 plant samples representing 49 plant families were tested one or more times with serology (DAS ELISA, polyclonal antisera for protein from all known *X. fastidiosa* strains; Agdia, Inc., Elkhart, IN). Priorities for selecting tissue (0.5 g/sample) for sap extraction were petioles first, then leaf mid-veins, then stem tissue. Xylem-containing tissue was twice rinsed with distilled water before sap was extracted with mortar and pestle (Fig. 2) or Polytron PT 10-35 homogenizer fitted with a Positron PTA 10 S generator probe (Fig. 3). Tween 20 was added after homogenization (Fig 4). Spectrophotometer readings were arbitrarily interpreted as ? 0.300 positive; ? 0.200 to 0.299 questionable; and <0.200 negative (Fig. 5).

Dilution plating was used to confirm positive serology reactions and estimate *X. fastidiosa* concentrations in plants. Xylem-containing plant tissue (0.1 g/sample) was homogenized in SCP buffer. The original sap extract and two dilutions (1:100 and 1:10,000) were duplicate plated on PWG semi-selective microbiological culture medium. Dilution plates were incubated for several weeks at approximately 25 C in plastic containers with loose fitting lids. More than 80 plant specimens were processed with this labor-intensive technique in 2003.

Bacterial colonies typical of *X. fastidiosa* were counted periodically for several weeks and estimates were made for c.f.u./g xylem-containing plant tissue. Identification on selected plates was confirmed with

serology.

Results

The minimum detection threshold for *X. fastidiosa* with ELISA is approximately 104 c.f.u./g of xylem-rich plant tissue. Bacterial concentrations lower than 104 c.f.u./g were not detected (possible false negative reactions), but insect acquisition of *X. fastidiosa* has been estimated by A. H. Purcell et al. to occur at ?104 c.f.u./g. Unfortunately, ELISA kits sometimes react to plant sap components that are not related to *X. fastidiosa* (false positive reactions). Therefore, ELISA was used to process large numbers of samples from which we selected fewer specimens for the more labor intensive but more reliable dilution plating technique. Plant samples that reacted in the positive range for *X. fastidiosa* were from 12 plant families, but dilution plating confirmed the bacterium in only eight families (Table 1). Some plant families had no positive serology reactions (Table 2). It is interesting that native wild grapes and two other native Vitaceae species were never positive with either technique in 2003.

Dilution plating minimum *X. fastidiosa* detection threshold is approximately 103 c.f.u./g, which is more sensitive than ELISA. This technique allowed us to estimate the numbers of bacteria in grape, five weeds, and woody ornamentals (Table 3).

X. fastidiosa colonies on sap dilution plates developed earlier for grape and redbud compared to sycamore and oleander. There were either too few samples for us to categorize colony growth rates, or results were mixed among sample dates and locations for Mexican hat, western ragweed, hierba del marrano, western soapberry, cedar elm, giant ragweed, and common sunflower.

Five weed species were of great interest. Some specimens of these five species were positive for *X. fastidiosa* for both serology and dilution plating. All five species are in the Asteraceae (composites); two are perennials, and three are annuals (Table 4).

X. fastidiosa was detected in weeds at three of the four vineyards (two with a history of PD and one with no history of PD). One vineyard had no history of PD and no detectable *X. fastidiosa* in weeds.

Three weed species were found at all four vineyards (Mexican hat, western ragweed, hierba del marrano). Two weed species were found only at the two vineyards with a history of PD (giant ragweed, common sunflower). The vineyard with no PD history but *X. fastidiosa* in nearby weeds had good weed control in the vineyard blocks and vineyard perimeters were closely mowed.

This bacterium was also found in urban trees and shrubs in urban landscape situations in Fredericksburg, Uvalde and San Antonio (Table 1). We expect to add more broadleaf and woody plants to the known host list in 2004.

Significance

Within *X. fastidiosa* are several strains (variations). These strains are referred to as “grape strain,”

“oleander strain,” “citrus strain,” “phony peach,” “periwinkle wilt strain,” etc. A strain has some degree of specialization to be more pathogenic on certain plants and less pathogenic on others. When wine grape plants were inoculated with “citrus strain,” thought to be most different from “grape strain,” J. S. Hartung et al. observed symptoms on grape similar to PD symptoms.

Until we have more information on Texas isolates, we remain suspicious that the *X. fastidiosa* isolates we recovered from weeds, shrubs, and trees may be pathogenic on grape (Table 5).

Very often, the greatest genetic variation within a species occurs at/near the place where that species first evolved. The *X. fastidiosa* center of origin may be the coastal areas near the Gulf of Mexico. Conversely, less genetic variation usually is found in distant regions where the species was more recently introduced. One interpretation our 2003 data is that certain weeds may harbor diverse strains of *X. fastidiosa*, perhaps even mixed infections within a single plant. An exotic and highly susceptible plant (e.g. European wine grape) growing nearby may be repeatedly challenged by bacteria carried into the vineyard by xylem-feeding insects that can feed on both weeds and the introduced plant. All *X. fastidiosa* strains may grow to some extent in grape, but the disastrous strain that can reproduce the most rapidly in wine grape xylem fluids may quickly become predominant.

There are four requirements for a particular plant species to be an important sources for *Xylella fastidiosa* acquisition by sharpshooters, according to A. H. Purcell et al. The plant must 1) be frequently inoculated with *X. fastidiosa*; 2) be an attractive food host for the insect carrier; 3) allow *X. fastidiosa* to spread beyond the inoculation site [systemic spread]; and 4) support ? 104 c.f.u./g of *X. fastidiosa* in xylem-containing plant tissue.

Our interpretation of 2003 data point to four very important factors related to Pierce’s Disease risk in wine grapes in the Texas Hill Country.

1. **Site selection**. Avoid locating vineyards near riparian habitats because several weeds found there probably meet the four requirements listed above for important bacterial sources.
2. **Plant species composition**. Based only on circumstantial evidence to date, common sunflower and giant ragweed may contribute to risk, perhaps because of insect behavior on these two weeds.
3. **Weed control**. Broadleaf weed control within and near vineyards should remain a very high priority with new emphasis on five weeds in Asteraceae. A frequently mowed perimeter around vineyards is suggested.
4. **Rogueing**. Symptomatic grape vines contain *X. fastidiosa* at very high c.f.u./g. Early detection while incidence in still low, and immediate rogueing should help reduce vine-to-vine spread.

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David Forbes, Carrie Hensarling, and Allen McGinty. Maria Beltran and Linda Brochu provided office support. We also gratefully acknowledge the cooperation of several Texas vineyards and their owners.

Planned work (outline for 2004)

We will continue surveying plants (annuals, perennials, woody plants) near selected infested and non-infested grape vineyards in the Texas Hill Country for *X. fastidiosa* to confirm weed hosts and add to our list. Detection techniques will again be serology (ELISA) on large numbers of field samples to identify suspect samples for dilution plating. Our sample storage temperature was too low in 2003, so we plan to hold samples at approximately 17 C for as short a time as possible in 2004. Our dilution plate incubation temperature was also too low in 2003, and we will use 28 C in 2004.

In 2004, we will challenge greenhouse grape plants of a highly susceptible variety with our *X. fastidiosa* isolates from weeds and woody ornamentals to determine pathogenicity in wine grape.

We will also estimate in 2004 the frequency of selected plant species at multiple sites to learn more about high and low risk sites.

Table 1. Plant families with one or more species positive for *Xylella fastidiosa* with both serology and plating.

Family	Species
Apocynaceae	Oleander
Asteraceae	[five species]
Fabaceae	Redbud
Fagaceae	Red oak [Appel, Kurdyla, Vest]
Platanaceae	Sycamore
Sapindaceae	Western soapberry
Ulmaceae	Cedar elm
Vitaceae	Wine grape only

Table 2. Selected plant families negative for *Xylella fastidiosa* with serology (ELISA) and in some cases, also with dilution plating.

Family	Number of plant specimens

Cupressaceae	2
Cyperaceae	14
Euphorbiaceae	12
Juncaceae	3
Onagraceae	12
Poaceae	43
Solanaceae	16
Taxodiaceae	7
Vitaceae (excluding V. vinifera)	31

Table 3. *Xylella fastidiosa* c.f.u./gx estimates for wine grape and five Asteraceae weed species at four locations in the Texas Hill Country.

	Vineyard			
Plant species	1	2	3	4
Wine grape	106-108	106-107	-y	-
Western ragweed	104-106	106-107	103-106	0
Mexican hat	106-107	103	103-106	0
Giant ragweed	106	-	.z	.
Common sunflower	105	-	.	.
Hierba del marrano	107	-	104	0

[x]colony forming units per gram of xylem-rich plant tissue
[y]species found but not sampled, or ELISA-negative sample not plated
[z]species not found

Table 4. Five weed species in Asteraceae (composites) positive for *Xylella fastidiosa* with both serology and dilution plating.

Common name	Scientific name	Longevity	Percent positive			
			Serology (ELISA)		Dilution plating	
Perennial (western) ragweed	*Ambrosia psilostachya* DC.	Perennial	33%	N=54[y]	65%[z]	N=17
Red-spike Mexican hat	*Ratibida colunifera* (Nutt.) Woot. & Standl.	Perennial	19%	N=48	89%	N=9
Hierba del marrano (slim aster)	*Symphyotrichum divaricatum* (Nutt.) Nesom	Annual	21%	N=14	100%	N=3
Great (giant) ragweed	*Ambrosia trifida* L.	Annual	57%	N=7	75%	N=4
Common sunflower	*Helianthus annuus* L.	Annual	25%	N=12	33%	N=3

[y]Number of specimens tested.
[z]Dilution plating usually done only with samples positive or questionable with serology.

Table 5. Comments on *Xylella fastidiosa* related to strains.

Quote	Source
Strain differences are minor compared to strain similarities	J. S. Hartung
X. fastidiosa is a New World bacterium	J. S. Hartung
X. fastidiosa is an endophyte in New World plants	J. S. Hartung
X. fastidiosa is a pathogen of Old World plants grown in the New World	J. S. Hartung
All plants could probably be hosts of *X. fastidiosa* with artificial inoculation	A. H. Purcell
X. fastidiosa strain mixtures occur in insects; evidence of strain recombination	L. Nunney
Two *X. fastidiosa* colony types recovered from some weeds	M. Black