SUPPLEMENTAL PLANT HOSTS FOR *XYLELLA FASTIDIOSA* NEAR FOUR TEXAS HILL COUNTRY VINEYARDS

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ABSTRACT
Floras near four Texas Hill Country vineyards were surveyed *for Xylella fastidiosa* from late 2003 through mid 2004. Two vineyards had histories of Pierce's disease (Gillespie County, Llano County) and two did not (Gillespie County; Travis County). In 2003, 526 plant samples representing 49 plant families were tested one or more times with serology (DAS ELISA) and 80 specimens were dilution plated in attempts to confirm positive serology reactions and estimate *X. fastidiosa* concentrations in plant tissue. Two perennial Asteraceae species were then surveyed in winter, spring, and early summer and serological detection was lowest in spring. Bacterial strain characterizations are underway. This study has implications for site selection, weed control in and near vineyards, rogueing of vineyards, and the need for pathogen-free planting stock.

INTRODUCTION
Pierce's disease (PD), caused by the bacterial pathogen *Xylella fastidiosa*, is the greatest limiting factor for growing *Vitis vinifera* in most of Texas. Associations of *X. fastidiosa*, known vector glassy wing sharpshooter *Homalodisca coagulata*, other xylophagous insects, and numerous host plant species in warmer climates of Texas are apparently ancient and complex. Widespread death of European grape plants has been a common occurrence in much of Texas, perhaps since the first of many plant introductions 400 years ago. There are numerous scientific advantages to studying a biological system where pathogen, vectors, and host plants are native and endemic. However, little is known about the diversity of plants and the bacterium, or potential biocontrol agents in warmer regions of Texas.

In the mid 90's, the incidence and severity of Pierce's disease escalated in the Texas Hill Country (west of Austin and north of San Antonio). While this area of Texas was once thought to be a PD risk transition zone, many established Hill Country vineyards have seen increased vine mortality due to PD. It is speculated that a series of warm winters allowed the pathogen to become more widely distributed throughout the native plant community, providing the initial inoculum for vine infections. While the disease is not known to occur in the northern Panhandle of the state, recent outbreaks at higher elevations in far-west Texas raise questions about pathogen survival and transport into commercial grape plantings.

Variation exists within and among strains of *X. fastidiosa* with some degree of specialization to be more pathogenic on certain plants and less pathogenic on others (Hopkins 1984, Purcell and Hopkins 1996). However, wine grape plants inoculated with "citrus strain," thought to be most different from "grape strain," PD-like symptoms developed on grape (Li et al., 2002). Questions abound regarding plasticity of bacterial strains in response to changes in insect vectors, climate, plant species composition near vineyards, and grape cultivars.

The greatest genetic variations within species of pathogens, vectors, and potential biocontrol agents typically occur where the species first evolved or coexisted. The *X. fastidiosa* center of origin probably includes the coastal areas of the U.S. near the Gulf of Mexico, including large areas of Texas. Various supplemental hosts may harbor diverse strains of *X. fastidiosa*, perhaps even mixed infections within a single plant. A non-native and highly susceptible species (e.g., *V. vinifera*) growing nearby may be repeatedly challenged by bacteria carried by xylem-feeding insects feeding on both weeds and the introduced plant. Numerous *X. fastidiosa* strains may have potential for some reproduction in European grape (Hopkins 1984, Li et al., 2002, Purcell and Hopkins, 1996), but the highly pathogenic populations that reproduced the most rapidly in wine grape xylem fluids and were vectored most efficiently quickly become predominant.

OBJECTIVES
Our objectives were to survey annuals, perennials, woody plants, and ornamentals near vineyards for colonization by *X. fastidiosa* using serology (ELISA) and dilution plating, and to collect isolates for European grape pathogenicity studies and other strain characterization.

RESULTS

Some plant families had no positive serology reactions and two native grape species and two other native Vitaceae species were never positive with either technique in 2003 (Table 1). Plant samples that reacted serologically for *X. fastidiosa* in 2003 were from 12 plant families, but dilution plating (Hill and Purcell, 1995) with SCP buffer (Hopkins 1988) confirmed the bacterium in specimens from only eight families (Table 2). Identification of selected colonies was confirmed with serology.

Xylella fastidiosa was detected in and cultured from weeds at three (two with PD histories, one with no PD history) of the four vineyards in 2003 (Tables 3, 4). Three weed host species were found at all four vineyards (Mexican hat, western ragweed, hierba del marrano). Two weed host species were found only at the two vineyards with PD histories (giant ragweed, common sunflower). Near one no-PD-history vineyard (Travis County), *X. fastidiosa* was in some nearby weeds, but weed control in the vineyard blocks was good and vineyard perimeters were closely and often mowed.

Supplemental hosts of particular interest were five species in Asteraceae (Table 3). Two are perennials and three are annuals. Serological detection rates for two Asteraceae perennials were higher in summer and fall 2003 (aboveground plant parts, Table 3) and winter 2004 (belowground and soil surface-level plant parts) than in spring 2004 (belowground and soil surface, Table 5). Serology was not consistent among plant parts when petiole and root (Mexican hat) and underground stem, horizontal root and vertical root (perennial [western] ragweed) were tested separately. Overwintering *X. fastidiosa* may not be highly systemic on these species through winter and spring. Spittlebug nymphs (Cercopoidea) were frequently found on these two Asteraceae species in the spring, especially in riparian habitats. Fungal and bacterial contamination of dilution plates were much more pronounced in winter and spring from plant parts belowground or near the soil surface and *X. fastidiosa* concentrations could not be estimated.

This bacterium was also detected and cultured from certain urban trees and shrubs in urban landscape situations in Fredericksburg, Uvalde and San Antonio in summer and fall (Table 1). Colonies of *X. fastidiosa* on sap dilution plates developed earlier for grape and redbud compared to sycamore and oleander in 2003. There were either too few positive samples for us to compare 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.

CONCLUSIONS

Knowledge of PD epidemics in Texas increases prospects for disease control in other wine grape production regions. This work focused on surveys for supplemental *X. fastidiosa* host plants at diverse vineyard sites. Future work will utilize the bacterial isolates and plant community data at PD and non-PD vineyards to explore new control strategies.

A. H. Purcell described four requirements for a plant species to be an important source for *X. fastidiosa* acquisition by xylem-feeding insects: 1) frequently inoculated with *X. fastidiosa*; 2) attractive food host for the insect carrier; 3) *X. fastidiosa* spreads beyond the inoculation site [systemic spread]; and 4) $\geq 10^4$ c.f.u./g of *X. fastidiosa* in xylem-containing plant tissue.

Education efforts related to PD risk in European wine grapes grown in the Texas Hill Country include:

A. Site selection. Avoid locating vineyards near riparian habitats because more weeds found there probably meet the four requirements listed above for important bacterial sources.
B. Plant species composition. Based only on circumstantial evidence to date, presence of common sunflower and great (giant) ragweed may indicate higher site risk. This may be because of insect behavior on these two weeds.
C. Weed control. Until Texas *X. fastidiosa* strains are characterized, broadleaf weed control within and near vineyards should remain a priority, including frequently mowed perimeters.
D. Rogueing. Infected and symptomatic *V. vinifera* vines contain *X. fastidiosa* with high c.f.u./g. Early PD detection while incidence in still low, and immediate rogueing should be considered to help reduce vine-to-vine spread.
E. Planting stock. Infected tolerant (few if any acute symptoms) cultivars grown in Texas and other southern states, including *V. aestivalis*, can be reservoirs of X. fastidiosa (L. Moreno, unpublished). Infected planting stocks of these varieties are potential sources of inoculum if planted adjacent to *V. vinifera* and in previously PD-free areas.

Results are pending from 2004 greenhouse wine grape plant inoculations with *X. fastidiosa* isolates from grape, weeds and woody ornamentals to determine pathogenicity. Work in progress includes estimating frequency of selected plant species at four vineyards to learn more about high and low risk sites, and strain characterization in this and another laboratory.

Table 1. Selected plant families negative for *Xylella fastidiosa* in one or more species with ELISA and in some cases, also with dilution plating in 2003.

Family	Number of plant specimens
Cupressaceae	2
Cyperaceae	14
Euphorbiaceae	12
Juncaceae	3
Onagraceae	12
Poaceae	43
Solanaceae	16
Taxodiaceae	7
Vitaceae[z] (excluding *Vitis vinifera, V. aestivalis*)	31

[z]*Cissus trifoliata* (L.) L., *Parthenocissus* spp., *V. mustangensis* Buckl., *V. berlandieri* Planch.

Table 2. Plant families with one or more species positive for *Xylella fastidiosa* with serology and dilution plating in 2003.

Family	Species
Apocynaceae	Oleander (*Nerium oleander* L.)
Asteraceae	[five species, see Table 3]
Fabaceae	Redbud (*Cercis canadensis* L.)
Fagaceae	Red oak (*Quercus* sp.)[y]
Platanaceae	Sycamore (*Platanus occidentalis* L.)
Sapindaceae	Western soapberry (*Sapindus saponaria* L.)
Ulmaceae	Cedar elm (Ulmus crassifolia Nutt.)
Vitaceae	European grape (*Vitis vinifera* L.)[z]

[y]D. Appel, T. Kurdyla and M.Vest, unpublished data.
[z]Also in 2004, *V. aestivalis* Michx L. Moreno, cv. Cynthiana/Norton, by serology and immuno-fluorescence; M. Black, cv. Lenoir (uncertain parentage) by serology with dilution plating pending.

Table 3. Five weed species in Asteraceae collected near four vineyards and positive for *Xylella fastidiosa* with serology and dilution plating in summer and fall 2003.

			Percent positive			
			Serology (ELISA)		Dilution plating	
Common name	Scientific name	Longevity				
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 positive with serology.

Table 4. *Xylella fastidiosa* c.f.u./g[u] estimates for wine grape and five Asteraceae weed species at four locations in the Texas Hill Country in 2003.

	Vineyard location and history			
Plant species	Llano PD[v]	Gillespie PD[w]	Travis no PD[v]	Gillespie no PD[x]
Wine grape	10^6-10^8	10^6-10^7	-[y]	-
Perennial (western) ragweed	10^4-10^6	10^6-10^7	10^3-10^6	0
Mexican hat	10^6-10^7	10^3	10^3-10^6	0
Great (giant) ragweed	10^6	-	.[z]	.
Common sunflower	10^5	-	.	.
Hierba del marrano	10^7	-	10^4	0

[u]Colony forming units per gram of xylem-rich plant tissue.
[v]Near riparian habitats.
[w]Near smaller, varied, somewhat seasonal riparian habitats.
[x]Not near significant riparian habitat.
[y]Species found but not sampled, or ELISA-negative sample not dilution plated.
[z]Species not found.

Table 5. Winter, spring and summer 2004 survey of Mexican hat and perennial (western) ragweed for colonization by *Xylella fastidiosa* near four Texas Hill Country vineyards. Results of dilution plating on PWG semi-selective medium were all negative through August 2004.

Season	Location and PD history			
	Gillespie PD	Llano PD	Gillespie No PD	Travis No PD
	---------- Positive samples, % (N=total number of samples) ----------			
Winter (Feb, Mar)	17% (N=30)	20% (N=40)	.[z]	43% (N=37)
Spring (Apr, May)	9% (N=33)	5% (N=41)	.	20% (N=41)
Summer (Jun-Aug)	0% (N=6)	10% (N=10)	20% (N=4)	83% (N=5)

[z]Site not sampled.

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