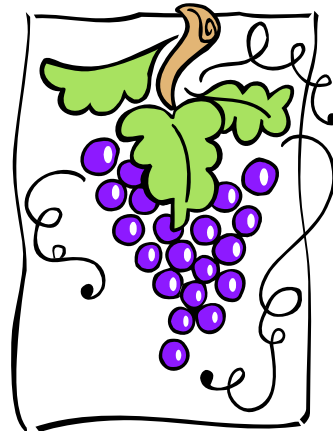


# Texas Pierce's Disease Program Semi-annual Report March 31, 2005



## **Overview**

The Texas Pierce's Disease Program seeks to find answers and potential solutions to the disease for growers in Texas, California and other parts of the world. Entomological efforts seek to identify the numerous insect vectors of the disease, understand their population dynamics and seasonality. This knowledge will help Texas growers more efficiently manage vectors and reduce the economic impact of Pierce's disease throughout the state. This baseline data will also prove beneficial should any of the other native Texas vectors become established in California growing regions. Vectors will be screened this year which strains of *Xylella fastidiosa* each vector can carry. Additional efforts are underway to explore natural enemies of glassy-winged sharpshooter and other large sharpshooters. These natural enemies may hold promise should GWSS become established in northern California growing areas and for sharpshooter suppression efforts in urban environments.

Plant pathologists are using greenhouse and field studies to better understand how quickly *Xylella fastidiosa* moves within vines and in vineyards under Texas climatic conditions. Work continues in the Texas Hill Country and the Gulf Coast areas to ascertain which plants serve as supplemental hosts of the bacterium and to better understand the relationships between different strains of *Xylella fastidiosa*. Collaborative work seeks to explore the genetic diversity of *Xylella* found in wild vines from Coastal areas where few cold temperature events inhibit bacterial growth. New detection techniques are being developed which will provide rapid, cost-effective diagnostics and help to understand variability in host specificity and virulence.

Work also continues in an effort to map all commercial vineyards in Texas to better understand the incidence and severity of Pierce's disease. GIS and analysis of spatial geographical data may shed light on factors associated with disease risk.

Work is also being initiated to investigate the impact of soils of the Hickory Sands region of the Hill Country on incidence of Pierce's disease. In this region, known vectors are abundant while *Xylella fastidiosa* is rare or absent in known supplemental hosts. The

specific attributes of this region's soil chemistry may be impacting xylem chemistry inhibiting *Xylella* growth and development.

## **Plant Pathology Effort**

### **I. Supplemental Plant Hosts of *Xylella fastidiosa***

### **II. Plant Communities Near Vineyards With and Without PD**

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## **Objectives**

1. Survey weeds, woody plants, and ornamentals for supplemental plant hosts of *Xylella fastidiosa*.
2. Compare botanical diversity near selected vineyards with and without Pierce's disease.

## **Procedures**

We collected weeds and woody plants at four Texas Hill Country vineyards and a few woody ornamentals and trees from selected urban settings. Two of the vineyard sites had a history of Pierce's Disease (PD)(Gillespie Co. PD; Llano Co. PD) and two had no history of the disease (Gillespie Co. no PD; Travis Co. no PD). Bacterial leaf scorch symptoms were present on urban ornamental species tested.

ELISA was used to process large sample numbers from which we selected fewer specimens (those ELISA positive) for the more labor intensive and more definitive dilution plating technique. 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. We tested roots and low stems from the two perennial Asteraceae hosts throughout 2004 to check for seasonal detection and culture recovery differences. Two or three sub-samples from each were tested first with ELISA, and positives were then dilution plated.

Samples were placed in plastic bags and kept cool in insulated 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 5-12 C until testing was completed. Plant samples were tested one or more times with serology (DAS ELISA, polyclonal antisera for protein from several *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 a Polytron PT 10-35 homogenizer fitted with a Positron PTA 10 S generator probe. Tween 20 was added after homogenization. Spectrophotometer readings were arbitrarily interpreted as  $\geq 0.300$  positive;  $\geq 0.200$  to  $0.299$  questionable; and  $<0.200$  negative.

Dilution plating was used in attempts 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 or three dilutions (1:10, 1:100, 1:10,000) were duplicate plated on PWG semi-selective microbiological culture medium. Dilution plates were incubated for several weeks at 28 C in plastic containers with loose fitting lids. 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. Several cultures were suspended in glycerol + PW broth for long-term storage at low temperature in liquid nitrogen. In early 2005, several cultures from various plants were sent to Blake Bextine, University of California Riverside for strain characterization.

After a preliminary stem inoculation test with an isolate from western ragweed, we detected *X. fastidiosa* with ELISA in *Nicotiana repanda* and *Catharanthus rosea* (3-5 cm cuttings) growing in recycled Sunshine Mix#1 (mostly peat moss), but not in *V. vinifera* 'Chardonnay' in SuperSoil or *Arachis hypogaeae* in recycled Sunshine Mix#1. Symptoms were absent from all these plants. Therefore, we used selected residual plant extracts in SCP buffer from samples positive with ELISA for inoculation attempts on greenhouse grown grape (stem slits; Chardonnay) in SuperSoil (mostly redwood bark) and/or *Nicotiana repanda* (petiole slits), *Arachis hypogaeae* (seedling with tip of taproot removed before submersion, or 3-5 cm cuttings with basal end submersed for 24 hr), or *Catharanthus rosea* (3-5 cm cuttings with basal end submersed ca. 24 hr) in recycled Sunshine Mix#1 (mostly peat moss). No consistent symptoms were observed through December 2005. These plants will be assayed for *X. fastidiosa* in spring 2005.

In autumn 2004, we attempted to identify all vascular plant species near the four test vineyards along four 100-meter transect lines from arbitrary starting points and running approximately N, E, S, W, or arbitrary directions with bias for the greatest plant diversity. We recorded each plant species at or near 1-meter intervals and/or pressed samples for later identification all plant species. Additional plant species observed near these transects were also recorded. Numerous pressed specimens were later mounted on herbarium sheets at Uvalde for future reference.

## Results

We recovered *X. fastidiosa* isolates with dilution plating in 2003 and 2004 from some of the ELISA-positive specimens of six native Asteraceae (composite) species. Two are perennials, and four are annuals (Tables 1,2). *X. fastidiosa* ELISA tests were positive from one to numerous plant species from all four sites (Llano PD > Gillespie PD > Travis no PD > Gillespie no PD), but the bacterium was cultured only from three sites (Llano PD > Gillespie PD > Travis no PD). To date, we have not recovered a culture of *X. fastidiosa* from wine grape or native plants at the Gillespie-no-PD site.

Three weed host species were found at all four vineyards (Mexican hat, western ragweed, hierba del marrano). Three weed host species were found only at the two vineyards with

a history of PD (giant ragweed, common sunflower, seacoast sumpweed)(Table 3). 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.

We did not recover a *X. fastidiosa* culture from native *Vitis* species. *V. mustangensis* was present at all four sites. *V. cinerea* var. *helleri* (syn. *V. berlandieri*) was at three sites, including both PD sites and the Travis no PD site. *V. vulpina* and a suspected *V. vulpina* hybrid was only found at the Llano PD site (Table 3).

This bacterium was also found in urban trees and shrubs in urban landscape situations in Fredericksburg, Uvalde and San Antonio (Table 4). Colony growth rates were usually slower and colony appearances were usually distinct from isolates from wine grape and annual Asteraceae weeds near the two vineyards with PD histories.

In general, we observed a strong seasonal affect on detection and recovery of *X. fastidiosa*, and specifically on two perennial Asteraceae species (Table 5). Both cool season and plant maturity (e.g. annuals) clearly affects this bacterium, because we did not start recovering isolates from weeds until the fall. We got isolates from symptomatic wine grape starting in early summer.

## Discussion

Within *X. fastidiosa* are several strains (variations) 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. Until we have more information about Texas isolates, we remain concerned that *X. fastidiosa* isolates recovered from weeds, shrubs, and trees may be pathogenic on grape.

Populations of *X. fastidiosa* in weed hosts apparently increase late in the season. This may provide acquisition opportunities for individual sharpshooters and other xylem-feeding insects that will overwinter.

Poaceae was the most represented family at all sites, followed by Asteraceae (Table 6). There were trends among the four sites for decreasing PD risk as percent grasses increased and total number of plant species increased (Tables 6, 7).

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  $\geq 10^4$  c.f.u./g of *X. fastidiosa* in xylem-containing plant tissue.

Management of PD risk should consider the following:

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. Riparian habitats often have up to three annual weed hosts unique to the two PD sites in this study.

2. Weed control. Based on circumstantial evidence to date, common sunflower, giant ragweed, and seacoast sumpweed may be useful indicators of PD risk. Broadleaf weed control within and near vineyards should be a high priority with emphasis on six *X. fastidiosa* weed hosts in Asteraceae. A frequently mowed perimeter around vineyards is suggested, and selected species control in the vicinity may be in order.

3. Rogueing. Symptomatic grape vines contain *X. fastidiosa* at very high c.f.u./g. Early detection while incidence is still low, and immediate rogueing may help reduce vine-to-vine spread.

4. Tolerant varieties. Vines of tolerant varieties in the high PD risk areas of Texas may harbor high populations of *X. fastidiosa* but have none-to-mild symptoms. Infected planting stock may have potential for spreading this pathogen to new areas. Tolerant varieties should not be planted adjacent to highly susceptible European wine grapes in the warm parts of Texas.

### Acknowledgements

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**Table 1.** ELISA and dilution plating for *Xylella fastidiosa* and six Asteraceae weeds collected near all four vineyard locations in summer and fall of 2003 and 2004.

Common name	Scientific name	Longevity	Percent positive			
			Serology (ELISA)		Dilution plating	
			2003	2004	2003	2004
Perennial (western) ragweed	<i>Ambrosia psilostachya</i> DC.	P <sup>w</sup>	33%, N=54 <sup>x</sup>	58%, N=39	65% <sup>y</sup> , N=17	17% N=24
Red-spike Mexican hat	<i>Ratibida columnifera</i> (Nutt.) Woot. & Standl.	P	19%, N=48	62%, N=40	89%, N=9	8%, N=24
Hierba del marrano (slim aster)	<i>Symphytotrichum divaricatum</i> (Nutt.) Nesom	A	21%, N=14	21%, N=32	100%, N=3	11%, N=9
Great (giant)	<i>Ambrosia trifida</i> L.	A	57%, N=7	85%, N=28	75%, N=4	29%, N=17

ragweed

Marsh sumpweed	<i>Iva annua</i> L.	A	.	33%, N=15	.	20%, N=5
Common sunflower	<i>Helianthus annuus</i> L.	A	25%, N=12	55%, N=22	33%, N=3	9%, N=11

<sup>w</sup>P=Perennial, A=Annual

<sup>x</sup>Number of specimens tested.

<sup>y</sup>Dilution plating with samples positive or questionable positive with serology.

<sup>z</sup>Not tested in 2003.

**Table 2.** *Xylella fastidiosa* c.f.u./g<sup>x</sup> estimates in 2003-2004 for wine grape and six Asteraceae weed species at four locations in the Texas Hill Country.

Plant species	Vineyard location, history, and year							
	Llano PD		Gillespie PD		Travis no PD		Gillespie no PD	
	'03	'04	'03	'04	'03	'04	'03	'04
Wine grape	10 <sup>6</sup> -10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>3</sup> -10 <sup>7</sup>	<sup>y</sup>	0	-	0
Perennial (western) ragweed	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>3</sup>	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>4</sup>	10 <sup>3</sup> -10 <sup>6</sup>	10 <sup>3</sup>	0	0
Mexican hat	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>3</sup> -10 <sup>6</sup>	0	0	0
Hierba del marrano	10 <sup>7</sup>	0	-	10 <sup>7</sup>	10 <sup>4</sup>	0	0	0
Great (giant) ragweed	10 <sup>6</sup>	10 <sup>3</sup>	-	10 <sup>6</sup> -10 <sup>7</sup>	<sup>z</sup>	.	.	.
Marsh sumpweed	-	0	-	10 <sup>8</sup>	.	.	.	.
Common sunflower	10 <sup>5</sup>	10 <sup>4</sup>	-	0	.	.	.	.

<sup>x</sup>Colony forming units per gram of xylem-rich plant tissue.

<sup>y</sup>Species found but not sampled, or ELISA-negative sample not dilution plated.

<sup>z</sup>Species not found.

**Table 3.** Contrasts of species present and absent in autumn 2004 near two vineyards with a history of PD and two vineyards with no history of PD.

Family Species and common name(s)	County and PD history			
	Llano PD	Gillespie PD	Travis no PD	Gillespie no PD
Asteraceae <sup>x</sup> -----				
<i>Ambrosia psilostachya</i> DC. Perennial (western) ragweed	+	+	+	+
<i>Ratibida columnifera</i> (Nutt.) Woot. & Standl. Red-spike Mexican hat	+	+	+	+
<i>Symphyotrichum divaricatum</i> (Nutt.) Nesom Hierba del marrano (slim aster)	+	+	+	+
<i>Ambrosia trifida</i> L. Great (giant) ragweed	+	+	-	-
<i>Iva annua</i> L. Marsh sumpweed	+	+	-	-

<i>Helianthus annuus</i> L. Common sunflower	+	+	-	-
Vitaceae-----				
<i>Vitis mustangensis</i> Buckl. <sup>y</sup> Mustang grape	+	+	+	+
<i>Vitis cinerea</i> (Engelm.) Millard var. <i>helleri</i> (Bailey) M.O. Moore <sup>y</sup> Winter grape, roundleaf grape, Spanish grape	+	+	+	-
<i>Vitis vulpina</i> L. <sup>z</sup> Frost grape, chicken grape	+	-	-	-

<sup>x</sup>Numerous samples of each species strong ELISA positive for *X. fastidiosa* and some yielded cultures.

<sup>y</sup>Rare sample of numerous for these species was ELISA weak positive but *X. fastidiosa* never recovered by plating.

<sup>z</sup>One of two samples strong ELISA positive but *X. fastidiosa* was not recovered by plating.

**Table 4.** Plant families with one or more species **positive** for *Xylella fastidiosa* serology **and** dilution plating in 2003-04.

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

**Table 5.** Seasonal 2004 ELISA survey for *Xylella fastidiosa* colonization, as a combined summary for Mexican hat and perennial (western) ragweed, near four Texas Hill Country vineyards. Dilution platings on PWG semi-selective medium were sometimes successful only in autumn.

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)	<sup>z</sup>	43% (N=37)
Spring (Apr, May)	9% (N=33)	5% (N=41)	.	20% (N=41)
Summer (Jun-Aug)	0% (N=6)	10% (N=10)	25% (N=4)	83% (N=5)
Fall (Oct-Nov)	88% (N=17)	53% (N=19)	50% (N=22)	57% (N=21)

<sup>z</sup>Site not sampled.

**Table 6.** The ten most frequent plant families (% of total specimens observed) in autumn 2004 near two vineyards with a history of PD and two vineyards with no history of PD.

Family	County and PD history			
	Llano PD	Gillespie PD	Travis no PD	Gillespie no PD
Poaceae	27.8% (363)	29.5 (474)	30.8% (377)	36.8% (540)
Asteraceae	21.6% (282)	14.6% (235)	11.6% (142)	19.4% (285)
Smilacaceae	9.2% (120)	8.0% (129)		3.4% (50)
Fagaceae	7.4% (97)	7.2% (116)	8.8% (108)	2.5% (36)
Euphorbiaceae	4.1% (54)	2.7% (43)	2.6% (32)	2.7% (39)
Aquifoliaceae	2.8% (36)			
Ulmaceae	2.8% (36)	5.6% (90)		3.1% (46)
Fabaceae	2.7% (35)	12.2% (196)		9.2% (135)
Menispermaceae	2.6% (34)	2.2% (35)		
Oxalidaceae	2.2% (29)			
Vitaceae		2.9% (47)	2.8% (34)	
Acanthaceae		2.1% (33)		
Cactaceae				3.8% (56)
Polygonaceae				2.7% (39)
Convolvulaceae				2.0% (30)
Bromeliaceae			4.3% (53)	
Cyperaceae			4.2% (51)	
Cupressaceae			3.6% (44)	
Scrophulariaceae			3.2% (39)	
Platanaceae			2.8% (34)	

**Table 7.** Estimated number of families and species in autumn 2004 near two vineyards with a history of PD and two vineyards with no history of PD.

	County and PD history			
	Llano PD	Gillespie PD	Travis no PD	Gillespie no PD
Number of families	48	41	56	42
Number of species	121	117	169	128

**Table 8.** Plants at Llano Co PD site (transects and supplemental observations), autumn 2004.

Family	Species	Frequency	Percent <sup>z</sup>
Acanthaceae	Ruellia sp.	3	0.23
Anacardiaceae	Toxicodendron pubescens P. Mill.	1	0.08
Apiaceae	Pastinaca sativa L.	2	0.15
Aquifoliaceae	Ilex decidua Walt.	36	2.76
Asteraceae	Achillea millefolium L.	4	0.31
	Ambrosia psilostachya DC.	145	11.11
	Amphiachyris dracunculoides (DC.) Nutt.	28	2.15
	Artemisia ludoviciana Nutt.	4	0.31
	Aster sp.	6	0.46
	Baccharis salicifolia (Ruiz & Pavon) Pers.	6	0.46
	Baccharis sp.	1	0.08
	Bidens laevis (L.) B.S.P.	1	0.08
	Helianthus annuus L.	7	0.54



	<i>Iva angustifolia</i> Nutt. ex DC.	1	0.08
	<i>Iva annua</i> L.	20	1.53
	<i>Parthenium hysterophorus</i> L.	1	0.08
	<i>Ratibida columnifera</i> (Nutt.) Woot. & Standl.	21	1.61
	<i>Symphytotrichum divaricatum</i> (Nutt.) Nesom	3	0.23
	<i>Verbesina virginica</i> L.	22	1.69
	<i>Xanthium strumarium</i> L.	6	0.46
	unknown composite	1	0.08
Brassicaceae	<i>Capsella bursa-pastoris</i> (L.) Medik.	2	0.15
Bromeliaceae	<i>Tillandsia recurvata</i> (L.) L.	28	2.15
Cactaceae	<i>Opuntia leptocaulis</i> DC.	5	0.38
	<i>Opuntia</i> sp.	2	0.15
Caprifoliaceae	<i>Viburnum rufidulum</i> Raf.	1	0.08
Convolvulaceae	<i>Ipomoea cordatotriloba</i> Dennst. var. <i>torreyana</i> (Gray) D. Austin	1	0.08
Cucurbitaceae	<i>Cucurbita foetidissima</i> Kunth	1	0.08
	<i>Melothria pendula</i> L.	1	0.08
Cupressaceae	<i>Juniperus ashei</i> Buchh.	9	0.69
Cyperaceae	<i>Carex</i> sp.	15	1.15
	<i>Eleocharis</i> sp. R. Br.	1	0.08
Ebenaceae	<i>Diospyros texana</i> Scheele	17	1.3
Euphorbiaceae	<i>Acalypha gracilens</i> Gray var. <i>monococca</i> Engelm. ex Gray	3	0.23
	<i>Acalypha gracilens</i> Gray	1	0.08
	<i>Chamaesyce maculata</i> (L.) Small	1	0.08
	<i>Chamaesyce nutans</i> (Lag.) Small	1	0.08
	<i>Croton capitatus</i> var. <i>lindheimeri</i> (Engelm. & Gray) Muell.-Arg.	1	0.08
	<i>Croton monanthogynus</i> Michx.	1	0.08
	<i>Croton</i> sp.	21	1.61
	<i>Euphorbia marginata</i> Pursh	1	0.08
	<i>Euphorbia dentata</i> Michx.	2	0.15
	<i>Tragia glanduligera</i> Pax & K. Hoffmann	21	1.61
	Unknown euphorbia	1	0.08
Fabaceae	<i>Lupinus texensis</i> Hook.	1	0.08
	<i>Prosopis glandulosa</i> Torr.	17	1.3
	<i>Rhynchosia senna</i> Gillies ex Hook.	12	0.92
	<i>Sophora affinis</i> Torr. & Gray	3	0.23
	<i>Vicia</i> sp.	1	0.08
	annual legume weed	1	0.08
Fagaceae	<i>Quercus stellata</i> Wangenh.	4	0.31
	<i>Quercus virginiana</i> P. Mill.	93	7.13
Juglandaceae	<i>Carya illinoensis</i> (Wangenh.) K. Koch	17	1.3
Juncaceae	<i>Juncus</i> sp.	8	0.61
Lamiaceae	<i>Marrubium vulgare</i> L.	1	0.08
	<i>Monarda</i> sp.	1	0.08

Lemnaceae	Lemna sp.	3	0.23
Liliaceae	Nothoscordum bivalve (L.) Britt.	1	0.08
Lythraceae	Lagerstroemia X faurei	1	0.08
Malvaceae	Abutilon abutiloides (Jacq.) Garcke ex Britt. & Wilson	1	0.08
	Abutilon fruticosum Guill. & Perrottet	2	0.15
	Abutilon sp.	1	0.08
	Callirhoe (Torr. & Gray) Gray	1	0.08
	Hibiscus moscheutos L.	1	0.08
	Rhynchosida physocalyx (Gray) Fryxell	8	0.61
Meliaceae	Melia azedarach L.	1	0.08
Menispermaceae	Cocculus carolinus (L.) DC.	34	2.61
Moraceae	Morus alba L.	3	0.23
Nyctaginaceae	Boerhavia sp.	1	0.08
Oleaceae	Forestiera reticulata Torr.	2	0.15
Onagraceae	Guara suffulta Engelm. Ex Gray	2	0.15
	Ludwigia octovalvis (Jacq.) Raven	1	0.08
Oxalidaceae	Oxalis drummondii Gray	10	0.77
	Oxalis stricta L.	19	1.46
Phytolaccaceae	Phytolacca americana L.	1	0.08
Poaceae	Bothriochloa barbinodis (Lag.) Herter	1	0.08
	Bothriochloa ischaemum(L.)Keng	28	2.15
	var.songarica(Rupr.exFisch.&C.A.Mey.)Cel.&Har.		
	Bouteloua curtipendula (Michx.) Torr.	1	0.08
	Cenchrus spinifex Cav.	1	0.08
	Chloris X subdolichostachya Muell. [cucullata X verticillata]	2	0.15
	Chloris verticillata Nutt.	1	0.08
	Cynodon dactylon (L.) Pers.	27	2.07
	Dichanthelium oligosanthos(J.A.Schultes)Gould	30	2.3
	var.scribnerianum(Nash)Gould		
	Digitaria californica (Benth.) Henr.	1	0.08
	Echinochloa frumentacea Link	1	0.08
	Elymus virginicus L.	2	0.15
	Eragrostis curtipedicellata Buckl.	1	0.08
	Eragrostis sp.	32	2.45
	Eragrostis spectabilis (Pursh.) Steud.	1	0.08
	Grass	5	0.38
	Leptochloa fusca (L.) Kunth. ssp. fascicularis (Lam.) N. Snow	1	0.08
	Muhlenbergia sp.	34	2.61
	Panicum coloratum L.	95	7.28
	Panicum obtusum Kunth	8	0.62
	Panicum virgatum L.	1	0.08
	Paspalum dilatatum Poir.	21	1.61
	Paspalum pubiflorum Rupr. ex Fourn	5	0.38

	Paspalum sp.	1	0.08
	Paspalum urvillei Steud.	1	0.08
	Setaria parviflora (Poir.) Kerguelen	1	0.08
	Setaria leucopila (Scribn. & Merr.) K. Schum.	2	0.15
	Setaria scheelei (Steud.) A.S. Hitchc.	1	0.08
	Sorghum halepense (L.) Pers.	39	2.99
	Sporobolus cryptandrus (Torr.) Gray	1	0.08
	grass	2	0.15
Polygonaceae	Rumex crispus L.	1	0.08
	Rumex sp.	1	0.08
Ranunculaceae	Ranunculus sp.	2	0.15
Rhamnaceae	Frangula caroliniana (Walt.) Gray	1	0.08
Rosaceae	Prunus angustifolia Marsh	2	0.15
Rubiaceae	Cephalanthus occidentalis L.	1	0.08
	Lonicera japonica Thunb.	1	0.08
Salicaceae	Salix babylonica L.	1	0.08
	Salix sp.	1	0.08
Sapindaceae	Sapindus saponaria L. var. drummondii (Hook. & Arn.) L. Benson	5	0.38
Sapotaceae	Sideroxylon lanuginosum Michx.	7	0.54
Scrophulariaceae	Verbascum thapsas L.	1	0.08
Smilacaceae	Smilax renifolia Small	120	9.2
Solanaceae	Solanum elaeagnifolium Cav.	10	0.77
Ulmaceae	Celtis sp.	16	1.23
	Ulmus crassifolia Nutt.	20	1.53
Verbenaceae	Aloysia gratissima (Gillies & Hook.) Troncoso	3	0.23
	Lantana urticoides Hayek	1	0.08
	Phyla sp.	3	0.23
	Verbena halei Small	2	0.15
	Vitex agnus-castus L.	5	0.38
Vitaceae	Cissus trifoliata (L.) L.	5	0.38
	Parthenocissus quinquefolia (L.) Planch.	1	0.08
	Parthenocissus sp.	4	0.31
	Vitis cinerea (Engelm.) Millard var. helleri (Bailey) M.O. Moore	1	0.08
	Vitis cinerea x vulpina	1	0.08
	Vitis vulpina	2	0.15
	Vitis mustangensis Buckl.	10	0.77

<sup>z</sup>Percent of points on transects (+ supplementals) where this species occurred.

**Table 9.** Plants at Gillespie Co PD site (transects and supplemental observations), autumn 2004.

Family	Species	Frequency	Percent <sup>z</sup>
Acanthaceae	Ruellia sp.	33	2.05
Agavaceae	Yucca sp.	2	0.12

Anacardiaceae	<i>Rhus aromatica</i> Ait. var. <i>serotina</i> (Greene) Rehd.	3	0.19
	<i>Rhus lanceolata</i> (Gray) Britt.	2	0.12
Aquifoliaceae	<i>Ilex decidua</i> Walt.	1	0.06
Asteraceae	<i>Achillea millefolium</i> L.	6	0.37
	<i>Ambrosia psilostachya</i> DC.	59	3.67
	<i>Ambrosia trifida</i> L.	1	0.06
	<i>Amphiachyris dracunculoides</i> (DC.) Nutt.	33	2.05
	<i>Artemisia ludoviciana</i> Nutt.	17	1.06
	<i>Baccharis salicifolia</i> (Ruiz & Pavon) Pers.	1	0.06
	<i>Baccharis</i> sp.	1	0.06
	<i>Brickellia eupatorioides</i> (L.) Shinnery	1	0.06
	<i>Calyptocarpus vialis</i> Less.	8	0.5
	<i>Carduus nutans</i> L. ssp. <i>macrocephalus</i> (Desf.) Nyman	1	0.06
	<i>Grindelia nuda</i> Wood var. <i>nuda</i>	3	0.19
	<i>Helianthus annuus</i> L.	5	0.31
	<i>Heterotheca subaxillaris</i> (Lam.) Britt. & Rusby	1	0.06
	<i>Heterotheca subaxillaris</i> var. <i>latifolia</i> (Buckl.) Gandhi & Thomas	1	0.06
	<i>Iva annua</i> L.	1	0.06
	<i>Jefea brevifolia</i> (Gray) Stother	9	0.56
	<i>Lactuca serriola</i> L.	1	0.06
	<i>Ratibida columnifera</i> (Nutt.) Woot. & Standl.	59	3.67
	<i>Rudbeckia hirta</i> L. var. <i>pulcherrima</i> Farw.	1	0.06
	<i>Symphotrichum divaricatum</i> (Nutt.) Nesom	1	0.06
	<i>Symphotrichum ericoides</i> (L.) Nesom	10	0.62
	<i>Verbesina encelioides</i> (Cav.) Benth. & Hook. f. ex Gray	10	0.62
	<i>Verbesina virginica</i> L.	3	0.19
	<i>Xanthium strumarium</i> L.	1	0.06
Buddlejaceae	<i>Polypremum procumbens</i> L.	2	0.12
Cactaceae	<i>Opuntia leptocaulis</i> DC.	2	0.12
	<i>Opuntia</i> sp.	9	0.56
Caprifoliaceae	<i>Viburnum rufidulum</i> Raf.	1	0.06
Chenopodiaceae	<i>Chenopodium</i> sp.	1	0.06
Commelinaceae	<i>Commelina erecta</i> L. var. <i>angustifolia</i> (Michx.) Fern.	7	0.44
Convolvulaceae	<i>Dichondra</i> sp.	1	0.06
	<i>Evolvulus sericeus</i> Sw.	2	0.12
	<i>Ipomoea cordatotriloba</i> Dennst.	2	0.12
Cucurbitaceae	<i>Cucurbita foetidissima</i> Kunth	1	0.06
Cupressaceae	<i>Juniperus ashei</i> Buchh.	2	0.12
Cyperaceae	<i>Carex</i> sp.	3	0.19
Ebenaceae	<i>Diospyros texana</i> Scheele	23	1.43
Euphorbiaceae	<i>Acalypha phleoides</i> Cav.	1	0.06
	<i>Acalypha virginica</i> L.	2	0.12

	<i>Chamaesyce nutans</i> (Lag.) Small	1	0.06
	<i>Croton monanthogynus</i> Michx.	9	0.56
	<i>Euphorbia dentata</i> Michx.	4	0.25
	<i>Euphorbia marginata</i> Pursh	2	0.12
	<i>Tragia glanduligera</i> Pax & K. Hoffmann	24	1.49
Fabaceae	<i>Desmanthus velutinus</i> Scheele	3	0.19
	<i>Medicago lupulina</i> L.	1	0.06
	<i>Prosopis glandulosa</i> Torr.	100	6.23
	<i>Rhynchosia</i> sp.	43	2.68
	<i>Sophora affinis</i> Torr. & Gray	37	2.3
	<i>Trifolium</i> sp.	3	0.19
	<i>Vicia</i> sp.	6	0.37
Fagaceae	<i>Quercus stellata</i> Wangenh.	80	4.98
	<i>Quercus virginiana</i> P. Mill.	36	2.24
Liliaceae	<i>Nothoscordum bivalve</i> (L.) Britt.	1	0.06
Linaceae	<i>Linum imbricatum</i> (Raf.) Shinnery	1	0.06
Malvaceae	<i>Abutilon abutiloides</i> (Jacq.) Garcke ex Britt. & Wilson	4	0.25
	<i>Callirhoe digitata</i> Nutt.	4	0.25
	<i>Sida abutilifolia</i> P. Mill.	11	0.68
Menispermaceae	<i>Cocculus carolinus</i> (L.) DC.	35	2.18
Oleaceae	<i>Ligustrum</i> sp.	8	0.5
Onagraceae	<i>Gaura brachycarpa</i> Small	3	0.19
	<i>Gaura mollis</i> James	1	0.06
	<i>Gaura suffulta</i> Engelm. ex Gray	5	0.31
	<i>Ludwigia peploides</i> (Kunth) Raven	1	0.06
Oxalidaceae	<i>Oxalis drummondii</i> Gray	2	0.12
	<i>Oxalis stricta</i> L.	11	0.68
Plantaginaceae	<i>Plantago aristata</i> Michx.	4	0.25
Poaceae	<i>Aristida oligantha</i> Michx.	6	0.37
	<i>Aristida</i> sp.	4	0.25
	<i>Bothriochloa hybrida</i> (Gould) Gould	14	0.87
	<i>Bothriochloa ischaemum</i> (L.) Keng	52	3.24
	var. <i>songarica</i> (Rupr. ex Fisch. & C. A. Mey.) Cel. & Har		
	<i>Bothriochloa saccharoides</i> (Sw.) Rydb.	7	0.44
	<i>Bouteloua curtipendula</i> (Michx.) Torr. var. <i>curtipendula</i>	5	0.31
	<i>Bouteloua rigidiseta</i> (Steud.) A. S. Hitchc.	1	0.06
	<i>Bromus catharticus</i> Vahl	1	0.06
	<i>Cenchrus spinifex</i> Cav.	2	0.12
	<i>Chloris verticillata</i> Nutt.	3	0.19
	<i>Cynodon dactylon</i> (L.) Pers	115	7.16
	<i>Dichanthelium linearifolium</i> (Scribn. ex Nash) Gould	66	4.11
	<i>Dichanthelium oligosanthes</i> (J.A. Schultes) Gould	4	0.25
	<i>Dichanthelium oligosanthes</i> (J.A. Schultes) Gould	13	0.81

	var. scribnerianum(Nash)Gould		
	<i>Elymus canadensis</i> L.	3	0.19
	<i>Eragrostis intermedia</i> A.S. Hitchc.	7	0.44
	<i>Muhlenbergia</i> sp.	3	0.19
	<i>Nassella leucotricha</i> (Trin. & Rupr.) Pohl	22	1.37
	<i>Panicum coloratum</i> L.	101	6.29
	<i>Panicum obtusum</i> Kunth	8	0.5
	<i>Paspalum dilatatum</i> Poir.	5	0.31
	<i>Paspalum setaceum</i> Michx.	2	0.12
	<i>Pennisetum ciliare</i> (L.) Link var. <i>ciliare</i>	2	0.12
	<i>Pharlaris caroliniana</i> Walt.	1	0.06
	<i>Sorghum halepense</i> (L.) Pers.	6	0.37
	<i>Sporobolus compositus</i> (Poir.) Merr.	1	0.06
	<i>Sporobolus compositus</i> (Poir.) Merr. var. <i>compositus</i>	3	0.19
	<i>Sporobolus</i> sp.	12	0.75
	<i>Urochloa texana</i> (Buckl.) R. Webster	1	0.06
Polygonaceae	<i>Polygonum pensylvanicum</i> L.	2	0.12
	<i>Rumex</i> sp.	5	0.31
Portulacaceae	<i>Portulaca oleracea</i> L.	1	0.06
Rhamnaceae	<i>Ziziphus obtusifolia</i> (Hook. ex Torr. & Gray) Gray var. <i>obtusifolia</i>	1	0.06
Rubiaceae	<i>Galium texense</i> Gray	1	0.06
Rutaceae	<i>Zanthoxylum hirsutum</i> Buckl.	13	0.81
Sapindaceae	<i>Sapindus saponaria</i> L. var. <i>drummondii</i> (Hook. & Arn.) L. Benson	23	1.43
Sapotaceae	<i>Sideroxylon lanuginosum</i> Michx.	16	1
Scrophulariaceae	<i>Agalinis</i> sp.	2	0.12
Smilacaceae	<i>Smilax renifolia</i> Small	129	8.03
Solanaceae	<i>Cnidoscolus texanus</i> (Muell.-Arg) Small	1	0.06
	<i>Physalis angulata</i> L.	3	0.19
	<i>Solanum dimidiatum</i> Raf.	1	0.06
	<i>Solanum elaeagnifolium</i> Cav.	17	1.06
Ulmaceae	<i>Celtis</i> sp.	76	4.73
	<i>Ulmus crassifolia</i> Nutt.	14	0.87
Urticaceae	<i>Parietaria pensylvanica</i> Muhl. ex Willd.	1	0.06
Verbenaceae	<i>Glandularia bipinnatifida</i> (Nutt.) Nutt.	1	0.06
	<i>Phyla</i> sp.	1	0.06
	<i>Verbena halei</i> Small	1	0.06
Vitaceae	<i>Cissus trifoliata</i> (L.) L.	34	2.12
	<i>Vitis cinerea</i> (Engelm.) Millard var. <i>helleri</i> (Bailey) M.O. Moore	13	0.81
	<i>Vitis mustangensis</i>	1	0.06

<sup>z</sup>Percent of points on transects (+ supplementals) where this species occurred.

**Table 10.** Plants at Travis Co no PD site (transects and supplemental observations), autumn 2004.

Family	Species	Frequency	Percent <sup>z</sup>
Acanthaceae	Justicia ovata (Walt.) Lindau var. lanceolata (Chapman) R.W. Long	2	0.16
	Ruellia nudiflora (Engelm. & Gray) Urban	7	0.57
	Ruellia sp.	1	0.08
Agavaceae	Yucca sp.	5	0.41
Amaranthaceae	Amaranthus blitum L.	1	0.08
Anacardiaceae	Rhus lanceolata (Gray) Britt.	1	0.08
	Rhus virens Lindheimer ex Gray	5	0.41
	Toxicodendron pubescens P. Mill.	7	0.57
Apocynaceae	Catharanthus roseus (L.) G. Don	1	0.08
	Vinca minor L.	1	0.08
Aquifoliaceae	Ilex decidua Walt.	4	0.33
Asclepiadaceae	Cynanchum racemosum (Jacq.) Jacq. var. unifarium (Scheele) E. Sundell	5	0.41
Asteraceae	Ambrosia psilostachya D.C.	18	1.47
	Amphiachyris dracunculoides (DC.) Nutt.	3	0.25
	Baccharis sp.	2	0.16
	Bidens laevis (L.) B.S.P.	1	0.08
	Brickellia cylindracea Gray & Engelm.	1	0.08
	Calyptocarpus vialis Less.	25	2.04
	Centaurea sp.	1	0.08
	Chrysanthemum sp.	1	0.08
	Conyza canadensis (L.) Cronq. var. glabrata (Gray) Cronq.	1	0.08
	Eupatorium serotinum Michx.	1	0.08
	Helianthus sp.	6	0.49
	Heterotheca subaxillaris (Lam.) Britt. & Rusby	8	0.65
	Hymenopappus sp.	2	0.16
	Iva angustifolia Nutt. ex DC.	5	0.41
	Jefea brevifolia (Gray) Stother	8	0.65
	Lactuca serriola L.	2	0.16
	Liatris sp.	6	0.49
	Melampodium sp.	1	0.08
	Palafoxia callosa (Nutt.) Torr. & Gray	2	0.16
	Pluchea odorata (L.) Cass.	1	0.08
	Ratibida columnifera (Nutt.) Woot. & Standl.	6	0.49
	Solidago petiolaris Ait.	2	0.16
	Symphotrichum divaricatum (Nutt.) Nesom	14	1.14
	Symphotrichum pratense (Raf.) Nesom	1	0.08
	Taraxacum officinale Webber ex. Wiggers	1	0.08
	Thymophylla tenuiloba (DC.) Small	1	0.08
Verbesina virginica L.	3	0.25	
Vernonia baldwinii Torr. ssp. baldwinii	14	1.14	

	Zinnia sp.	1	0.08
	trans 4 yellow	3	0.25
	unk plant	1	0.08
Berberidaceae	Mahonia trifoliolata (Moric.) Fedde	2	0.16
Bromeliaceae	Tillandsia recurvata (L.) L.	53	4.33
Cactaceae	Opuntia leptocaulis DC.	1	0.08
	Opuntia sp.	1	0.08
Caprifoliaceae	Lonicera sp.	1	0.08
Commelinaceae	Commelina erecta L. var. angustifolia (Michx.) Fern.	1	0.08
	Commelina sp.	1	0.08
	Tradescantia sp.	1	0.08
Convolvulaceae	Dichondra sp.	1	0.08
	Ipomoea cordatotriloba Dennst. var. torreyana (Gray) D. Austin	1	0.08
	Ipomoea sp.	5	0.41
Cornaceae	Cornus drummondii C.A. Mey	2	0.16
Crassulaceae	Sedum sp.	1	0.08
Cupressaceae	Juniperus ashei Buchh.	44	3.59
Cyperaceae	Carex sp.	5	0.41
	Cyperus elegans L.	1	0.08
	Cyperus strigosus L.	1	0.08
	Eleocharis geniculata (L.) Roemer & J.A. Schultes	1	0.08
	Eleocharis sp.	34	2.78
	Unknown Sedge	8	0.65
	Unknown sedge (shade)	1	0.08
Ebenaceae	Diospyros texana Scheele	5	0.41
Euphorbiaceae	Chamaesyce angusta (Engelm.) Small	2	0.16
	Chamaesyce maculata (L.) Small	3	0.25
	Croton capitatus Michx. var. lindheimeri ( Englem. & Gray) Muell.-Arg.	3	0.25
	Croton sp.	2	0.16
	Euphorbia dentata Michx.	6	0.49
	Euphorbia sp.	1	0.08
	Stillingia texana I.M. Johnston	1	0.08
	Tragia glanduligera Pax & K. Hoffmann	14	1.14
Fabaceae	Acacia sp.	1	0.08
	Desmodium sp.	11	0.9
	Eysenhardtia sp.	1	0.08
	Indigofera miniata Ortega	2	0.16
	Prosopis glandulosa Torr.	2	0.16
	Sophora secundiflora (Ortega) Lag. ex DC.	1	0.08
	unk legume	1	0.08
Fagaceae	Quercus shumardii Buckl.	24	1.96
	Quercus virginiana P. Mill	84	6.86
Iridaceae	Iris sp.	1	0.08



Juglandaceae	<i>Carya illinoensis</i> (Wangneh.) K. Koch	27	2.21
	<i>Juglans microcarpa</i> Berl.	3	0.25
	<i>Juglans nigra</i> L.	1	0.08
Juncaceae	<i>Juncus</i> sp.	2	0.16
	<i>Juncus torreyi</i> Coville	1	0.08
Lamiaceae	<i>Lavandula angustifolia</i> P. Mill.	1	0.08
	<i>Ocimum basilicum</i> L.	1	0.08
	<i>Rosmarinus officinalis</i> L.	1	0.08
	<i>Salvia coccinea</i> P.J. Buchoz ex Etlinger	1	0.08
	<i>Salvia greggii</i> Gray	1	0.08
	<i>Salvia</i> sp.	2	0.16
	<i>Teucrium</i> sp.	1	0.08
	<i>Thymus vulgaris</i> L.	1	0.08
Liliaceae	<i>Allium sativum</i> L.	1	0.08
	<i>Allium schoenoprasum</i> L.	1	0.08
	<i>Lilium</i> sp.	1	0.08
	<i>Nolina texana</i> S. Wats.	2	0.16
	<i>Tulbaghia violacea</i> W. Harvey	1	0.08
Malvaceae	<i>Abutilon abutiloides</i> (Jacq.) Garcke ex Britt. & Wilson	4	0.33
	<i>Abutilon fruticosum</i> Guill. & Perrottet	1	0.08
	<i>Callirhoe involucrata</i> (Torr. & Gray) Gray	4	0.33
	<i>Sida abutifolia</i> P. Mill.	1	0.08
Moraceae	<i>Morus alba</i> L.	13	1.06
Najadaceae	<i>Najas</i> sp.	3	0.25
Nyctaginaceae	<i>Boerhavia</i> sp.	1	0.08
Oleaceae	<i>Fraxinus texensis</i> (Gray) Sarg.	1	0.08
Onagraceae	<i>Calylophus berlandieri</i> Spach	5	0.41
Oxalidaceae	<i>Oxalis drummondii</i> Gray	1	0.08
	<i>Oxalis stricta</i> L.	3	0.25
Platanaceae	<i>Platanus occidentalis</i> L.	34	2.78
Poaceae	<i>Andropogon glomeratus</i> (Walt.) B.S.P.	22	1.8
	<i>Bothriochloa bladhii</i> (Retz.) S.T. Blake	1	0.08
	<i>Bothriochloa ischaemum</i> (L.) Keng	122	9.97
	var. <i>songarica</i> (Rupr. ex Fisch. & C.A. Mey.) Cel. & Har.		
	<i>Bothriochloa saccharoides</i> (Sw.) Rydb.	2	0.16
	<i>Bouteloua hirsuta</i> Lag. var. <i>pectinata</i> (Featherly)	1	0.08
	<i>Cory</i>		
	<i>Cenchrus</i> sp.	3	0.25
	<i>Chasmanthium latifolium</i> (Michx.) Yates	5	0.41
	<i>Chloris cucullata</i> Bisch.	21	1.72
	<i>Cortaderia selloana</i> (J.A. & J.H. Schultes) Aschers. & Graebn.	1	0.08
	<i>Cynodon dactylon</i> (L.) Pers.	67	5.47
	<i>Dichanthelium oligosanthos</i> (J.A. Schultes) Gould	16	1.31
	<i>Eragrostis trichodes</i> (Nutt.) Wood	1	0.08

	<i>Leersia oryzoides</i> (L.) Sw.	13	1.06
	<i>Muhlenbergia X involuta</i> Swallen (pro sp.)	4	0.33
	<i>Muhlenbergia</i> sp.	3	0.25
	<i>Nassella leucotricha</i> (Trin. & Rupr.) Pohl	1	0.08
	<i>Panicum coloratum</i> L.	2	0.16
	<i>Panicum virgatum</i> L.	1	0.08
	<i>Paspalum dilatatum</i> Poir.	1	0.08
	<i>Paspalum notatum</i> Fluegge	7	0.57
	<i>Paspalum pubiflorum</i> Rupr. ex Fourn.	6	0.49
	<i>Paspalum urvillei</i> Steud.	1	0.08
	<i>Schizachyrium scoparium</i> (Michx.) Nash	35	2.86
	<i>Setaria leucopila</i> (Scribn. & Merr.) K. Schum.	1	0.08
	<i>Sorghastrum nutans</i> (L.) Nash	8	0.65
	<i>Sorghum halepense</i> (L.) Pers.	2	0.16
	<i>Tridens flavus</i> (L.) A.S. Hitchc.	4	0.33
	<i>Tridens texanus</i> (S. Wats.) Nash	13	1.06
Polemoniaceae	<i>Ipomopsis rubra</i> (L.) Wherry	1	0.08
	<i>Ipomopsis</i> sp.	1	0.08
Polygalaceae	<i>Polygala lindheimeri</i> Gray	1	0.08
Polygonaceae	<i>Polygonum densiflorum</i> Meisn.	8	0.65
Potamogetonaceae	<i>Potamogeton diversifolius</i> Raf.	3	0.25
Primulaceae	<i>Samolus ebracteatus</i> Kunth ssp. <i>cuneatus</i> (Small) R. Knuth	2	0.16
Pteridaceae	<i>Adiantum capillus-veneris</i> L.	2	0.16
Ranunculaceae	<i>Clematis</i> sp.	1	0.08
Rosaceae	<i>Prunus serotina</i> Ehrh.	14	1.14
	<i>Rosa</i> sp.	1	0.08
Rubiaceae	<i>Cephalanthus occidentalis</i> L.	11	0.9
	<i>Diodia virginiana</i> L.	5	0.41
Rutaceae	<i>Ptelea trifoliata</i> L. ssp. <i>trifoliata</i> var. <i>mollis</i> Torr. & Gray	3	0.25
Salicaceae	<i>Salix nigra</i> Marsh.	11	0.9
Sapotaceae	<i>Sideroxylon lanuginosum</i> Michx.	4	0.33
Scrophulariaceae	<i>Bacopa monnieri</i> (L.) Pennell	38	3.1
	<i>Leucophyllum</i> sp.	1	0.08
Smilacaceae	<i>Smilax renifolia</i> Small	17	1.39
Solanaceae	<i>Physalis cinerascens</i> (Dunal) A.S. Hitchc. var. <i>cinerascens</i>	1	0.08
	<i>Solanum elaeagnifolium</i> Cav.	2	0.16
	<i>Solanum lycopersicon</i> L.	1	0.08
	<i>Solanum triquetrum</i> Cav.	4	0.33
Thelypteridaceae	<i>Thelypteris kunthii</i> (Desv.) Morton	4	0.33
Typhaceae	<i>Typha</i> sp.	7	0.57
Ulmaceae	<i>Celtis</i> sp.	6	0.49
	<i>Ulmus crassifolia</i> Nutt.	7	0.57
	<i>Ulmus rubra</i> Muhl.	1	0.08

Verbenaceae	<i>Aloysia gratissima</i> (Gill & Hook)	1	0.08
	<i>Callicarpa americana</i> L.	4	0.33
	<i>Lantana urticoides</i> Hayek	2	0.16
	<i>Phyla</i> sp.	14	1.14
	<i>Verbena</i> sp.	1	0.08
	<i>Vitex agnus-castus</i> L.	1	0.08
Vitaceae	<i>Cissus trifoliata</i> (L.) L.	1	0.08
	<i>Parthenocissus quinquefolia</i> (L.) Planch.	1	0.08
	<i>Vitis cinerea</i> (Engelm.) Millard var. <i>helleri</i> (Bailey) M.O. Moore	4	0.33
	<i>Vitis mustangensis</i> Buckl.	28	2.29

<sup>z</sup>Percent of points on transects (+ supplementals) where this species occurred.

**Table 11.** Plants at Gillespie Co no PD site (transects and supplemental observations), autumn 2004.

Family	Species	Frequency
Acanthaceae	<i>Ruellia</i> sp.	1
Agavaceae	<i>Yucca glauca</i> Nutt.	1
Amaranthaceae	<i>Froelichia gracilis</i> (Hook.) Moq.	4
Anacardiaceae	<i>Toxicodendron pubescens</i> P. Mill.	3
Asclepiadaceae	<i>Asclepias</i> sp.	1
	<i>Cynanchum unifarium</i> (Scheele) Woods	2
Asteraceae	<i>Achillea millefolium</i> L.	14
	<i>Ambrosia psilostachya</i> DC.	28
	<i>Amphiachyris dracunculoides</i> (DC.) Nutt.	20
	<i>Artemisia ludoviciana</i> Nutt.	1
	<i>Carduus nutans</i> L. ssp. <i>macrocephalus</i> (Desf.) Nyman	1
	<i>Conyza canadensis</i> (L.) Cronq. var. <i>glabrata</i> (Gray) Cronq.	3
	<i>Coreopsis tinctoria</i> Nutt. var. <i>tinctoria</i>	9
	<i>Gaillardia pulchella</i> var. <i>pulchella</i> Foug.	4
	Genus unknown	1
	<i>Gnaphalium</i> sp.	7
	<i>Grindelia nuda</i> Wood var. <i>nuda</i>	7
	<i>Heterotheca subaxillaris</i> (Lam.) Britt. & Rusby	3
	<i>Iva angustifolia</i> Nutt. ex DC.	1
	<i>Pluchea odorata</i> (L.) Cass.	3
	<i>Pseudognaphalium obtusifolium</i> (L.) Hilliard & Burttt ssp. <i>obtusifolium</i>	1
	<i>Ratibida columnifera</i> (Nutt.) Woot. & Standl.	112
	<i>Symphotrichum divaricatum</i> (Nutt.) Nesom	17
	<i>Symphotrichum ericoides</i> (L.) Nesom	34
	<i>Symphotrichum patens</i> (Ait.) Nesom var. <i>patens</i>	1
	<i>Thelesperma simplicifolium</i> Gray	1
<i>Verbesina encelioides</i> (Cav.) Benth. & Hook. f. ex Gray	1	
<i>Verbesina virginica</i> L.	4	
<i>Xanthisma texanum</i> DC.	1	

	Xanthium strumarium L. var. canadense (P. Mill.) Torr. & Gray	7
Brassicaceae	Lepidium densiflorum Schrad.	1
Cactaceae	Opuntia leptocaulis DC.	41
	Opuntia sp.	15
Chenopodiaceae	Chenopodium leptophyllum (Moq.) Nutt. ex S. Wats.	1
Cistaceae	Helianthemum georgianum Chapman	1
Commelinaceae	Commelina erecta L. var. angustifolia (Michx.) Fern.	2
	Tradescantia sp.	7
Convolvulaceae	Dichondra sp.	30
Cupressaceae	Juniperus ashei Buchh.	12
Cyperaceae	Cyperus hystericinus Fern.	4
	Cyperus retrorsus Chapman	1
	Eleocharis sp.	3
	Juncus marginatus Rostk.	1
	Unkn. Sedge	12
Ebenaceae	Diospyros texana Scheele	1
Euphorbiaceae	Acalypha gracilens Gray var. monococca Engelm. ex Gray	2
	Chamaesyce maculata (L.) Small	6
	Croton capitatus Michx. var. lindheimeri (Engelm. & Gray) Muell.-Arg.	10
	Croton glandulosus L.	1
	Croton monanthogynus Michx.	8
	Euphorbia dentata Michx.	1
	Euphorbia marginata Pursh	1
	Stillingia texana I.M. Johnston	1
	Tragia glanduligera Pax & K. Hoffman	9
Fabaceae	Desmanthus velutinus Scheele	3
	Lathyrus sp.	1
	Prosopis glandulosa Torr.	94
	Rhynchosia sp.	28
	Unknown legume Vine	1
	Vicia sp.	5
Fagaceae	Quercus marilandica Muenchh.	12
	Quercus sp.	1
	Quercus stellata Wangenh.	23
Juncaceae	Juncus sp.	25
	Juncus torreyi Coville	1
Lamiaceae	Marrubium vulgare L.	4
Liliaceae	Cooperia pedunculata Herbert	1
Malvaceae	Callirhoe involucrata (Torr. & Gray) Gray	14
	Sida lindheimeri Engelm. & Gray	1
Menispermaceae	Cocculus carolinus (L.) DC.	1
Molluginaceae	Mollugo verticillata L.	1
Nyctaginaceae	Mirabilis nyctaginea (Michx.) MacM.	3
Oleaceae	Forestiera reticulata Torr.	1

Oxalidaceae	<i>Oxalis stricta</i> L.	23
Poaceae	<i>Andropogon glomeratus</i> (Walt.) B.S.P	1
	<i>Aristida oligantha</i> Michx.	24
	<i>Aristida purpurea</i> Nutt.	10
	<i>Aristida purpurea</i> Nutt. var. <i>wrightii</i> (Nash) Allred	1
	<i>Bothriochloa hybrida</i> (Gould) Gould	3
	<i>Bothriochloa ischaemum</i> (L.) Keng	75
	var. <i>songarica</i> (Rupr. ex Fisch. & C.A. Mey.) Cel. & Har.	
	<i>Bothriochloa saccharoides</i> (Sw.) Rydb.	1
	<i>Bothriochloa</i> sp.	1
	<i>Bouteloa hirsuta</i> Lag.	4
	<i>Bouteloua curtipendula</i> (Michx.) Torr. var. <i>caespitosa</i> Gould & Kapadia	2
	<i>Bromus japonicus</i> Thunb. ex Murr.	1
	<i>Bromus</i> sp.	1
	<i>Cenchrus spinifex</i> Cav.	40
	<i>Chloris verticillata</i> Nutt.	6
	<i>Coelorachis cylindrica</i> (Michx.) Nash	1
	<i>Cynodon dactylon</i> (L.) Pers.	146
	<i>Dichanthelium linearifolium</i> (Scriben. ex Nash) Gould	34
	<i>Dichanthelium oligosanthes</i> (J.A. Schultes) Gould	2
	<i>Dichanthelium oligosanthes</i> (J.A. Schultes) Gould var. <i>scribnerianum</i> (Nash) Gould	38
	<i>Dichanthelium</i> sp.	2
	<i>Digitaria californica</i> (Benth.) Henr.	3
	<i>Digitaria ciliaris</i> (Retz.) Koel.	6
	<i>Distichlis spicata</i> (L.) Greene	1
	<i>Elymus canadensis</i> L.	1
	<i>Eragrostis capillaris</i> (L.) Nees	1
	<i>Eragrostis curvula</i> (Schrad.) Nees	2
	<i>Eragrostis intermedia</i> A.S. Hitchc.	3
	<i>Eragrostis secundiflora</i> J. Presl ssp. <i>oxylepis</i> (Torr.) S.D. Koch	3
	<i>Eragrostis spectabilis</i> (Pursh) Steud.	3
	<i>Eragrostis trichodes</i> (Nutt.) Wood	1
	<i>Leptochloa fusca</i> (L.) Kunth. ssp. <i>fascicularis</i> (Lam.) N. Snow	2
	<i>Nassella leucotricha</i> (Trin. & Rupr.) Pohl	22
	<i>Paspalum dilatatum</i> Poir.	44
	<i>Paspalum pubiflorum</i> Rupr. ex Fourn.	6
	<i>Schizachyrium scoparium</i> (Michx.) Nash var. <i>scoparium</i>	21
	<i>Setaria parviflora</i> (Poir.) Kerguelen	1
	<i>Sporobolus compositus</i> (Poir.) Merr.	1
	<i>Sporobolus</i> sp.	5
	<i>Tridens flavus</i> (L.) A.S. Hitchc.	1
	<i>Tridens flavus</i> (L.) A.S. Hitchc. var. <i>flavus</i>	14

	Tridens strictus (Nutt.) Nash	1
	Unkn. Fine grass	1
	Unkn. grass	1
Polygonaceae	Eriogonum annuum Nutt.	1
Portulacaceae	Rumex pulcher L.	38
	Portulaca oleracea L.	8
Pteridaceae	Cheilanthes tomentosa Link	1
Ranunculaceae	Anemone berlandieri Pritz.	6
Rosaceae	Rubus trivialis Michx.	6
Rubiaceae	Richardia tricocca (Torr. & Gray) Standl.	1
Rutaceae	Zanthoxylum hirsutum Buckl.	2
Smilacaceae	Smilax renifolia Small	50
Solanaceae	Physalis cinerascens (Dunal) A.S. Hitchc. var. cinerascens	3
	Solanum elaeagnifolium Cav.	12
Typhaceae	Typha sp.	1
Ulmaceae	Celtis laevigata Willd. var. Reticulata (Torr.) L. Benson	1
	Celtis sp.	19
	Ulmus crassifolia Nutt.	26
Verbenaceae	Aloysia gratissima (Gillies & Hook.) Troncoso	4
	Phyla sp.	9
	Verbena halei Small	10
Viscaceae	Phoradendron tomentosum (DC.) Engelm. ex Gray	10
Vitaceae	Cissus trifoliata (L.) L.	3
	Vitis mustangensis Buckl.	3

<sup>z</sup>Percent of points on transects (+ supplementals) where this species occurred.

### ***Xylella* genetics- Lisa Morano**

During Fall of 2004 and Spring of 2005 my lab has been evaluating the genetics of *X. fastidiosa* strains in Texas. Recently, we have successfully sequenced the gyrase B gene from four strains and compared them to the database sequences in California. Three strains from grape are PD strains with various levels of sequence variation and one strain from *Baccharis* shrub is closest to a periwinkle strain in Florida. It is our hope to have all 20 of our strains from Texas sequenced by the end of the semester. This will allow us to evaluate the phylogenetic similarities in Texas and to compare the strains to those in California. Identification of the strains from weeds around vineyards as compared to strains from vineyards will also indicate whether weeds are serving as a direct reservoir for PD strains.

## **Entomology Effort**

### **Entomology Research Program on Pierce's Disease- Forrest Mitchell**

Winter work was focused mainly on finishing the counts of remaining sticky traps and getting field plots ready for the spring. The protocols for conducting research have changed with the added power of having APHIS assist with the statewide sampling. More research will be done on vector behavior and host plant selection, insecticide efficacy and interactions between the vector and the pathogen *Xylella fastidiosa*.

Vineyards across the state were selected for counts by APHIS. The main criteria used for selection was location of the vineyard relative to its distance from the coast in order that an environmental gradient be sampled by the traps placed in them. Traps collected by APHIS will be forwarded to Isabelle Lauzierre for counts and identification. Insects selected by her will then be sent to the Stephenville entomology lab for ELISA analysis for presence or absence of *Xylella fastidiosa*. The goal is to determine seasonal abundance of both insects and disease in the environment and to directly test the risk assessment that has been the focus of research and extension recommendations on planting location across the state.

Approximately two acres of grape will come into production this year and another acre has been planted, all directed toward entomological field plot research on the Pierce's Disease issue. Two varieties of grape, Cabernet Sauvignon and Chardonnay, will be planted on half an acre each. Grapevines obtained from cuttings will also be planted in complete rows this spring, including Merlot and Blanc duBois varieties. Approximately 16 species of wild grapes are also being planted, approximately a dozen plants of each. The taxonomy of wild grape is uncertain, therefore the exact number of species is also uncertain. Seven more Munson varieties have been requested from the Grayson County College in Denison, TX. These varieties were selected for their vigor in the north-central Texas area. The projected experiments will attempt to determine if the vigor is due to the lack of insect feeding pressure. Assistance from horticulturalists and plant pathologists will be sought if the resistance appears to be genetic. Similar approaches are planned for the wild species as well.

A sampling program using an adjustable counting frame is being developed that will allow direct counts of leafhoppers on grapevine. These counts may then be used to compare the density of trap catches with the density of vine counts. The frame is one meter square and constructed of inexpensive lumber. It slides up and down on adjustable legs to conform to the height of the vines to be sampled. The color it will be painted is as yet undetermined. A neutral color is necessary to prevent both attraction and repulsion of the insects. The frame will be placed in position before the counts in order to allow the insects to recover from any disturbance. Insects that can be seen on the vine within the boundaries of the frame will be recorded and the number per unit area (probably a volume) determined.

### **Behavior and Reproductive Biology of Different Xylem Feeding Leafhoppers & Their Natural Enemies in Texas- Isabelle Lauziere**

Xylem feeding insects represent a great challenge for the American viticulture. These small insects have the ability to acquire, carry and transmit the bacterium *Xylella fastidiosa*, causal agent of Pierce's disease of grapevine. This disease has had serious economic consequences to this country's multibillion dollar grape industry. In the past 100 years, grape production in California has experienced several epidemics of Pierce's disease. Similarly, growers in the state of Texas have been struggling with this disease for the past 25 years. Susceptible plants infected with *X. fastidiosa* die relatively slowly from restriction of sap movement in the plant's conductive tissue. Until those vines are

detected and removed, they represent reservoirs of the bacterium that insects feeding on infested xylem can spread to healthy vines. As a result of infection, growers have to re-plant vines over areas lost to the disease.

Compounding this problem are the broad host range of xylem feeding insects and broad host range of the bacterium. To date the most studied xylem feeding insect is the glassy-winged sharpshooter, *Homalodisca coagulata* (Hemiptera: Cicadellidae), which is known to feed and reproduce on over 200 plants species pertaining to over 35 plant families. Alfalfa dwarf, phony peach, almond, oleander, elm, oak, sycamore, maple, plum, mulberry leaf scorch and citrus variegated chlorosis are all diseases caused by *X. fastidiosa*. This bacterium is native to the Gulf coast states in the U.S. and historical record for the glassy-winged sharpshooter in Texas suggests it has inhabited this area for quite some time. The opportunity to study this insect in its area of origin, in a natural environment, provides researchers with the opportunity to unravel the host plant - insect vector - plant disease triangle.

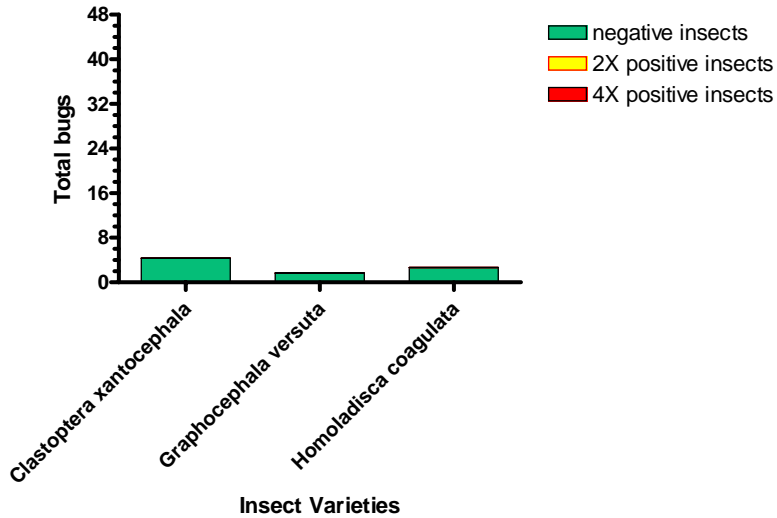
Recent exploration for xylem feeding insects associated with vineyards in Texas have shed new light on the complex system that exists in this state. In Central Texas, several vineyards have been monitored since the spring 2004 to identify the key players in this host plant - insect vector - plant disease triangle and to understand their biology. Preliminary data indicated the presence of xylem feeding leafhoppers (including the glassy-winged sharpshooter), spittlebugs and cicadas. In Central Texas, a total of 19 species were identified as xylem feeders, all with the potential to carry and transmit *X. fastidiosa* when feeding on susceptible host plants. Captured insects pertaining to the family Cicadellidae, or leafhoppers, are the most abundant with a total of 15 species. Predominant species were *H. coagulata*, *Graphocephala versuta* and *Clastoptera xanthocephala*.

Throughout January and first two weeks of February took place the relocation of this program from the former location at Moore Air Base, Edinburg, to a new location in Fredericksburg. The relocation effort was made possible through the help for USDA-APHIS-PPQ-Facility Management Services of Moore Air Base who provided support with the transportation of the equipment listed in the property inventory updated by Lauzière last February. The leased facility, an ex-nursery, is currently being retrofitted to allow the laboratory and field research activities to take place. The greenhouse structures need many repairs, particularly the structure, as well as electrical and plumbing upgrades which will take place over the next several months. Personnel are being hired to carry out the activities described for year 3 in the five-year work plan.

### **Insects as PD vectors- Lisa Morano**

Preliminary work on general infection level among Texas sharpshooters was conducted in Fall of 2004. Three insect species from four vineyards from the Hill Country were compared. Two species of leaf hoppers *Graphocephala versuta* and *Homalodisca coagulata* and one species of spittle bug, *Clastoptera xanthocephala* were evaluated for both their frequency and level of *Xylella fastidiosa* for a two week period in July of 2004. Vineyards with low insect levels also had few or no positive insects (Figure 1).

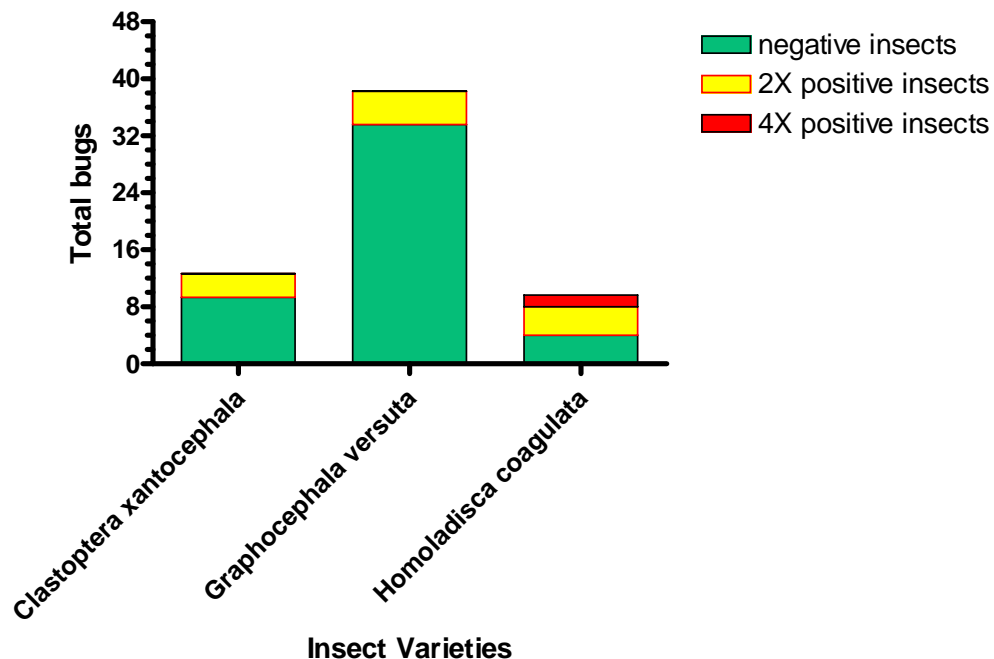




Three potential insect vectors during two weeks in July of 2004 showing low frequency and no insects tested positive for *X. fastidiosa*

Several vineyards tested had high insect frequency. For some vineyards the highest insect frequency was *G. versuta* (Figure 2) and in some cases it was the spittle bug *C. xantocephala* or the glassy-winged sharpshooter *H. coagulata* (data not shown). This variability in frequency suggests small differences in habitat are selecting for one xylem feeder over another at the different vineyard locations.

Positive insects (4 times the cut-off established in negative insects) was detected in some insects of all three species suggesting that all these insect species are able to serve as reservoirs for this bacterium. It seems likely that in addition to *H. coagulata*, *G. versuta* and *C. xantocephala* could vector Pierce's disease. The vectoring efficiency of these and other insects should be investigated.



## **Statewide Vineyard Survey and Geographic Information System- Ed Hellman, Laura Stretch & Jim Kamas**

The Texas vineyard survey was initiated in 2004 and has thus far collected data from 46 commercial vineyards in 26 different counties. Surveyed vineyards are located in 6 distinct growing regions representing different climatic conditions and ecosystems. For each vineyard, the survey has recorded grape acreage by variety, soil type, proximity to bodies of water, extent of weed control, surrounding vegetation, use of the insecticide imidicloprid, and presence or absence of the following: Pierce's disease (PD), sharpshooters, and supplemental hosts for *Xylella fastidiosa*.

Preliminary examination of the survey dataset shows that glassy-winged sharpshooters (GWSS) have been observed in 25 vineyards and PD is present or was previously observed in 19 of the 46 vineyards surveyed. GWSS has been observed in all 19 vineyards with PD, but 6 vineyards in the Hill Country had GWSS with no apparent PD at the time of the survey. GWSS was seen in 8 vineyards that grow PD-susceptible varieties and do not use imidicloprid; five of these vineyards have experienced PD. Vineyards in the Gulf Coast region, a high-risk area for PD, primarily grow PD-resistant grape varieties and therefore do not use imidicloprid.

Pierce's disease was related to geographic location and was observed in the eastern half of the state. It should be noted that two vineyards in west Texas are known to have PD, but these have not yet been surveyed. These preliminary survey results were reported at the second annual Texas Pierce's Disease Research Symposium.

The survey database has been incorporated into a geographic information system (GIS) that will enable spatial analysis of relationships of Pierce's disease and sharpshooters with geographical and environmental factors and vineyard attribute data collected from the survey. The GIS currently contains limited statewide elevation and climatic data; additional datasets will be added in 2005 to include topography, soils, and satellite imagery. The survey will continue to collect data from vineyards not yet included in the dataset. Additionally, insect trapping locations and data will be incorporated into the GIS.

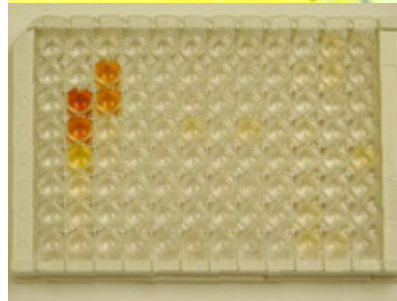
Completion of the GIS and spatial analysis of the layers of data may reveal patterns that provide insight into factors that favor development of Pierce's disease. Greater knowledge of these risk factors will lead to improved recommendations to producers on management practices and site selection to reduce losses from PD.

### **Information Transfer- Jim Kamas & Ed Hellman**

In an effort to relay research findings to growers, colleagues and the general public, several vehicles of information transfer have been employed. The following screen capture represents the home page of the Texas Pierce's Disease Program Web Site. As this site continues to grow, insect vector diagnostics, area wide insect activity overviews and access to research reports will become increasingly available to the public. Growers

will also have links to the APHIS server where participating growers will have access to real-time insect activity in their own vineyards. Efforts are underway to include information from related ARS research studies in these reports.

<http://piercedisease.tamu.edu>



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### **Second Annual Texas Pierce's Disease Research Symposium- Jim Kamas**

To relay relevant research findings to the grape growing community, a Research Symposium was held in the heart of the Texas Hill Country. Dr. Sandy Purcell served as keynote speaker and growers were given research overviews from all members of the Texas Research Team and ARS researchers. Once again, grower support and feedback validates both the research program and the educational outreach portion of this effort.

## *Program Participants*

### Keynote Speaker

Dr. Alexander Purcell  
Division of Insect Biology  
University of California, Berkeley

Dr. David Appel  
Dept. of Plant Pathology  
Texas Agricultural Experiment Station  
College Station, TX

Dr. Ed Hellman  
Dept. of Horticultural Sciences  
Texas Cooperative  
Extension/ Texas Tech  
Lubbock, TX

Jim Kamas  
Dept. of Horticultural Sciences  
Texas Cooperative Extension  
Fredericksburg, TX

Dr. Jesse de Leon  
USDA/ARS  
Weslaco, TX

Dr. Lisa Morano  
Dept. of Biology & Microbiology  
University of Houston-Downtown

Dr. Josh Stephenson  
College of Arts & Sciences  
Texas A&M International  
Laredo, TX

Dr. Mark Black  
Dept. of Plant Pathology  
Texas Cooperative Extension  
Uvalde, TX

Dr. Frank Gilstrap  
Dept. of Entomology  
The Texas A&M System  
College Station, TX

Dr. Isabelle Lauziere  
Dept. of Entomology  
Texas Ag. Experiment Station  
College Station, TX

Dr. Forrest Mitchell  
Dept. of Entomology  
Texas Ag. Experiment Station  
Stephenville, TX

Dr. Mamoudou Setamou  
USDA/ARS  
Weslaco, TX

Laura Stretch  
USDA/APHIS  
Ft. Collins, CO



### **Wine Business Monthly Article- Kamas, Hellman, Morano, Appel & Black-**

In order to heighten public awareness about the Texas Pierce's disease research effort, the following article was printed in the December, 2004 edition of *Wine Business Monthly*:

# Unraveling Pierce's Disease in Its Ancient Environment

With hopes of decreasing the widespread death of wine grapes, Texas researchers seek answers in the vectors and bacterial pathogens that fuel Pierce's disease in their region.

By the Texas Pierce's Disease Task Force

For most of Texas, Pierce's disease (PD) is the greatest limiting factor in cultivating *Vitis vinifera* and most hybrid wine grapes. Widespread death of wine grapes has been a common occurrence in Texas since the first introduction of old-world varieties brought by European settlers over four hundred years ago. Although the scientific knowledge of Pierce's disease has grown significantly in recent years, little was known about the variety of vectors or how the bacterial pathogen (*Xylella fastidiosa*) colonizes the plants and xylem-feeding insects in Texas ecosystems where the disease has long been endemic.

In the 1970s and early 1980s, PD risk was thought to be strongly correlated with proximity to the Gulf of Mexico. Mild winters and a diversity of plants suitable for sharpshooter feeding and reproduction made the coastal areas of Texas ideal for both vector and pathogen.

There are numerous scientific advantages to studying a disease where it is highly endemic, including: survey of plant reservoirs, evaluation of resistance in native grape species and hybrid varieties, and genetic diversity of the pathogen. Of equal importance, entomological studies can evaluate vector diversity and population levels in a natural environment (as opposed to California where the Glassy-winged sharpshooter is still in outbreak mode), and discover potential vector parasites and pathogens.

In the mid 1990s, the incidence and severity of Pierce's disease escalated dramatically in the Texas Hill Country (west of Austin and north of San Antonio). While this area of Texas was once thought to be a transition zone between high- and low-disease probabilities, 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 vineyard infection. While the disease is not known to occur in the northern Panhandle of the state, recent outbreaks in areas thought to be at relatively low risk in far-west Texas have changed the thinking on where the pathogen and vectors can ultimately survive and move into commercial grape plantings.

## Project Background

From its modest beginnings in 1988, members of the Texas Pierce's Disease Task Force started to investigate how the disease and its vectors interact in their native habitat. Initially, limited industry and university funding provided the first insight into the diversity of sharpshooters active in and around Texas vineyards.

In 2003, Texas A&M University entered into a cooperative agreement with USDA and APHIS (Animal and Plant Health Inspection Service) that resulted in the formation of a multi-disciplinary and multi-institutional research team. The team broadened the scope of previous work to include identification of supplemental *Xylella* plant hosts, spread of PD within commercial vineyards, year-round insect surveys across the state to identify potential vectors and gain an understanding of their seasonality, and utilization of GPS/GIS (global positioning system/geographic information system) to gain an understanding of where the disease occurs and why. Program budgets grew in 2004, and a targeted annual budget of \$2 million is anticipated by 2006.

## *Xylella fastidiosa* in Its Center of Origin

The native range of *Xylella fastidiosa*, the bacterial pathogen that causes Pierce's disease, includes the Gulf Coast of the US which may also be the center of origin for this bacterium. The Texas Gulf Coast region has warmer temperatures and much higher humidity than other grape-growing regions and subsequently has enormous disease pressure for Pierce's disease. Evaluation of specific questions in this PD hot zone allows for a better understanding of the ecology and epidemiology of the disease.

With respect to plant reservoirs for PD, we are establishing which plants routinely test positive for *X. fastidiosa*. The advantage of testing plants in an area with intense disease pressure is that it allows for evaluation of plants serving as persistent reservoirs rather than sporadic positives during an outbreak, as those positives may not survive. By testing plants from more than 45 different plant families in the area, we also will be determining trends in structure or evolutionary relatedness among tolerant plant reservoirs.

Initial evaluation of the genetic diversity of *Xylella fastidiosa* strains across Texas is underway. Partial sequencing of several strains suggests homology to other published strains of *X. fastidiosa*, but preliminary work on strain diversity suggests that there are multiple strains within Texas vineyards and within the wild plant reservoirs. *X. fastidiosa* isolates from grape, other crops, ornamentals and weeds have some visible differences in the laboratory on culture media (fast- vs. slow-growing isolates; large vs. small colonies).

Big unknowns include the stability of *X.f.* strains and potential for strains to exchange genes. Does genetic recombination occur when two or more *X.f.* strains colonize an insect or plant?

Prior to the establishment of the current Pierce's disease project, the only thing really known about the pathogen in Texas was that vineyards were sustaining alarming losses of vines.

Valuable research in California has illustrated that many factors are responsible for influencing Pierce's disease epidemiology; however, conditions in Texas are different, requiring further research to understand how the pathogen behaves in this environment.

Growers who have successful vineyards along the Gulf Coast have done so by planting American hybrid varieties, including Black Spanish (Lenoir), Blanc du Bois, and Cynthiana (Norton). We have done an extensive evaluation of the bacterial levels of these hybrids in this intense disease area. Specifically, we have evaluated bacterial levels across the growing season and over several years and are analyzing the effect of bacterial load on yield in these varieties. It appears that hybrids vary in the level of resistance to PD and potentially in the mechanism as well.

One such variety, Cynthiana (*Vitis aestivalis*), has been reported to be tolerant and has been planted in some parts of the state. Current research however shows that Cynthiana is not as resistant as might be expected. Cynthiana vines growing in greenhouses were artificially inoculated with *Xylella fastidiosa*, only to find that the bacterium could grow and thrive throughout the entire vine. Levels of colonization resembled those of the known susceptible varieties, Cabernet Sauvignon and Chardonnay (*Vitis vinifera*). The difference was that Cynthiana showed no signs of infection, unlike the known susceptible varieties.

In the following year, Cynthiana did decline in vigor, a sign that *Xylella* was indeed affecting the vines. Field observations confirm this finding in that

Cynthiana plantings have reduced yields and vigor over time in spite of not showing classical PD symptoms. In fact, these results illustrate that growers need to be aware that such varieties could indeed produce while enduring high levels of infection in the vineyard, but they could also serve as sources of inoculum that could destroy more vulnerable varieties nearby.

### Diagnosics

One troubling part of the Pierce's disease scenario has been the uncertainty associated with decisive, reliable and rapid diagnosis of the disease. Each method seems to have advantages and disadvantages in such areas as cost, speed and consistency.

One of those methods, ELISA (Enzyme Linked Immunosorbent Assay), has been regularly used for many years, but criticized for consistency and sensitivity. On the other hand, a new technique named RT-PCR (Real Time Polymerase Chain Reaction) holds great promise to overcome the ELISA limitations. In side-by-side tests on the same plant tissues, there was fairly consistent agreement between the two techniques, with a similarity rate of 75 percent on samples assumed infected with the pathogen. Yet the RT-PCR did perform more reliably on the tissues, and had a lower rate of false negatives and false positives on uninfected tissues.

With repeated use, the RT-PCR may eventually come to be the diagnostic tool of choice, but cost of the machine and the per-sample reagent costs are very high when compared to other methods. Two conclusions were clear from these studies: no diagnostic technique is completely reliable, and the consistency of the results depends on sampling routines as much as on the technique being implemented.

The diversity of phyto-chemicals in some plants is commonly blamed for false positive ELISA results or inhibition of PCR enzymes creating false negatives. Although steps can be taken to reduce these risks, the novel use of indirect immuno-fluorescence has been employed in Texas.

The technique indirectly attaches fluorescent antibodies to *X. fastidiosa* cells within the plant sap. A small amount of xylem fluid from wild plants (or grapevines) can be evaluated under a fluorescent microscope to confirm

either a positive ELISA or PCR result. This immuno-fluorescence technique allows for the visualization of cells from a variety of plants, and its application to multiple *X. fastidiosa* strains suggests differential expression of antigen (brighter signal) and different cell morphologies among strains.

### Vector Diversity and Behavior

Glassy-winged sharpshooter (GWSS) and *X. fastidiosa* are almost certain to have had a long history of coexistence in much of Texas. While incidence and severity of Pierce's disease in Texas appears to be strongly correlated with GWSS numbers, by no means is that the only vector responsible for the spread of the disease.

Of about 110 Homoptera species captured over the past two seasons, a total of 20 species were identified as xylem feeders, all with the potential to carry and transmit *X. fastidiosa*. In 2003, insect surveys were conducted in 20 vineyards across the state, and in 2004, sampling was increased to 40 vineyards with an emphasis on the Texas Hill Country. Work continues to assay insects for their ability to acquire *Xylella* and to infect grapevines.

Initially, GWSS was thought to have been introduced into California from Florida. Two recent, independent studies have shown that the genetic footprint of the California introduction is most closely related to that of populations found in Texas. Over the past two seasons, Texas insect surveys have pointed out irregularities in GWSS behavior and population densities. One striking difference is that in California, GWSS can be readily found feeding on warm winter days while in central Texas, the insect is almost entirely absent from late fall through late spring.

Throughout the fall and winter, surveys will be initiated in an attempt to unravel the behavior of these insects during overwintering (reproductive diapause). Understanding this migratory or diapausal behavior may provide insight as to what degree the insect can ultimately establish itself in different climates.

In early 2005, additional surveys will take place to identify natural enemies of GWSS and other large sharpshooter species. Very low population numbers in areas where winter temperatures

regularly drop into the single digits could indicate that biological control agents may be playing a role in limiting GWSS populations. If confirmed, there may well be application for biological control in northern California should GWSS become established.

Preliminary observations of GWSS egg-masses show a high degree of parasitism in many areas of the state suggesting there may be parasitoids that would enhance the current proposed long-term sustainable strategy in California.

### The Anomalies

The Texas Hill Country is the state's fastest growing wine region. Over the past eight years, many established vineyards have been hit hard by Pierce's disease, but interest in new vineyards and wineries continues. In the northern part of the Hill Country, there are numerous vineyards where vectors, including Glassy-winged sharpshooter, are routinely trapped, but where PD is not known to occur.

While most of these vineyards have been established in the last 10 years, one vineyard that is over 25 years old has never had a confirmed case of PD. One aspect of this research program focuses on how this area remains disease free in the presence of abundant numbers of vectors. In this northern part of the Hill Country, we are intrigued by absence or rarity of *Xylella fastidiosa* in weeds and brush around some vineyard sites within this anomalous zone, and by the high frequency in many of the same plant species around other vineyards to the south and east. With an endemic disease and abundant vectors, investigations on what is breaking the disease triangle at these locations may have nation-wide implications.

### Geographical Information System

A geographic information system (GIS) is under development to improve our understanding of the distribution of Pierce's disease and sharpshooters in Texas, in relation to geographical, environmental and viticultural factors. The GIS will combine layers of broader-scale geographical data on state maps with site-specific data collected at all vineyard locations within the state. Geographical data will include climatic factors, topography, and vegetation.

The vineyard surveys will obtain data on cultivars, acreage, weed management, surrounding vegetation, presence of supplemental hosts of *Xylella*, proximity to water, imidacloprid use, and presence/absence of PD and sharpshooters.

Completion of the GIS and spatial analysis of the layers of data may reveal patterns that provide insight into factors that favor development of Pierce's disease. Greater knowledge of these risk factors will lead to improved recommendations to producers on management practices and site selection to reduce losses from PD.

### Much More Than Just a Regional Program

While this research has already paid dividends on how to manage the disease for Texas growers, several aspects of this work have nationwide implications. Identification of robust parasitoids or vector pathogens, the understanding of the nature of disease tolerance in hybrids and native species, and a greater understanding of *Xylella fastidiosa* populations in its native range may provide important knowledge that could ultimately provide a breakthrough in Pierce's disease management. wbm

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**Invited Presentation at CDFA National Pierce's Disease Research Symposium-  
Kamas  
Poster Presentations at Same- Black & Morano**

Oral and poster presentations at the national meetings delivered research findings, relayed program focus and goals and served to build collaborative relationships between researchers in Texas, California and other areas of the country currently involved in Pierce's disease research

## Pierce's Disease Research in Texas



**A Cooperative  
Program Between  
USDA/APHIS &  
Texas A&M**

