Final Progress Reports due **April 14, 2006**

USDA APHIS – Texas Pierce's Disease Research and Education Program

Title of project: Pierce's disease risk factors in Texas (FY05-06)

Principal investigator: Mark C. Black (collaborating with J. Kamas, P. Adams, L. Moreno, B. Bextine, D. Vickerman)

Major accomplishments to date (April 1, 2005 through March 31, 2006):

1. **Surveys near selected Texas vineyards with and without PD histories for supplemental plant hosts of *Xylella fastidiosa* (*Xf*) using ELISA and dilution plating to recover bacterial isolates (cultures).**

Known (ELISA+, *Xf* isolation in culture) and suspected (ELISA+, isolation unsuccessful) *Xf* hosts lists were expanded in 2005. *Xf* was isolated for the first time in 2005 in/near PD vineyards from *Vitis vulpina*, *V. mustangensis* (abandoned rootstock experiment), *Iva angustifolia*, *Morus alba*, and *Lavandula* sp. *Xf* was previously isolated (2003, 2004) near vineyards from *Ambrosia psilostachya*, *A. trifida*, *Ratibida columnifera*, *Symphyotrichum divaricatum*, *I. annua*, and *Helianthus annuus*.

Based on several plants at multiple locations, two of three native *Vitis* spp. never (*V. cinerea* var. *helleri*) or rarely (*V. mustangensis*) had PD based on leaf symptoms, ELISA testing, and pathogen isolation. One population of *V. mustangensis* previously grafted with infected scions of wine grape had ca. 30% plants with symptoms, ELISA+'s, and yielded *Xf* isolates. One population of native *V. vulpina* had PD symptoms in 2005, ELISA+'s, and yielded *Xf* isolates. After mechanical inoculation on rooted cuttings in the greenhouse, all three species had some localized infections based on ELISA+'s. Testing for systemic infections of the wild species is scheduled for 2006.

2. **Compare plant communities near Texas vineyards with and without PD histories with plant surveys.**

Plant species identified to date that indicate risk of PD are *Ambrosia trifida*, *Helianthus anuus*, *Iva annua*, and *I. angustifolia*. Study of more populations are needed for another candidate, *V. vulpina*. These four species can flourish at seasonally riparian and adjacent sites, and in wet years at additional sites far from streams and reservoirs. Rigorous control efforts for these species are suggested near existing vineyards and new vineyards should not be established near large populations of these species. At distances of 0.5 to 3 miles from two non-PD vineyards (highway rights-of-way; farm pens, outbuildings, drainage ditches, stream banks), *A.trifida* and *H. annuus* were found and tested positive for Xf. This suggests that even non-PD vineyards have some risk during seasons of high vector populations. More work is needed before we can form conclusions about the Jeff Davis vineyard.

3. **Characterize *X. fastidiosa* strains recovered from supplemental plant hosts near vineyards, and from urban landscape hosts.**

Some isolates apparently are capable of infecting wine grape and certain weeds with insect inoculation. We established that three grape isolates can infect *A. trifida* and that one *A. psilostachya* isolate did not infect winegrape. Work by collaborators (Moreno, Bextine, Vickerman) with a small subset of our isolates found that one isolate from *A. trifida* is genetically similar to known grape strains, and one isolate from grape is genetically similar to non-grape strain.

4. **Study soils from vineyards with and without PD under controlled screen-house conditions by inoculating a susceptible grape variety with a grape isolate.**

There was no indication with the first run of this experiment that soil type affects PD in susceptible Chardonnay after mechanical inoculation. This experiment should be repeated with more aggressive inoculation technique because success was low in all soils.

5. **Screening rootstocks commonly used in TX for reaction to natural insect-mediated inoculation with *X. fastidiosa*.**

All seven Apr05-planted stocks and two of five Aug05-planted stocks had some PD by Nov05, suggesting that most commercial stocks used in TX are susceptible to some degree to insect-inoculated *Xf*. Most skips have been planted and two more years of data will be collected.

Goals Achieved:

1. **Surveys near selected Texas vineyards with and without PD histories for supplemental plant hosts of *Xylella fastidiosa* (*Xf*) using ELISA and dilution plating to recover bacterial isolates (cultures).**

Plants were collected for ELISA and dilution plating for *Xf* isolates and estimates of cfu/g from vineyards from: Gillespie (PD), Travis (originally no PD, but PD new in '05), Llano (PD), Gillespie (no PD), Val Verde (PD), Jeff Davis (PD) and a few samples from vicinity of a McCulloch Co. vineyare (no PD).

Pressed plant specimens representing plants of unknown or uncertain identification collected in 2005 for ELISA and dilution plating for *Xf* isolates were identified to genus and species or referred to others with more taxonomic expertise (Poaceae to R. Lyons, TCE TAMU AREC Uvalde; Cyperaceae and Juncaceae to D. Kruse, Tracy Herbarium, TAMU College Station; selected dicots to M. Reed, Dept. Biology TAMU College Station, and G. Nesom, BRIT Fort Worth). Plants for ID were from Becker (PD), Flat Creek (PD new in '05), Fall Creek (PD), Granite Hills (no PD), Val Verde (PD), Certenberg (no PD), and Blue Mountain (PD).

Began work at a Jeff Davis Co. vineyard (previously reported to be PD positive) with Kevin Stark, Ed Hellman's M.S. student at TTU-Lubbock. Only 1 of 8 suspect grape samples was ELISA+, and we did not recover *Xf* from that sample with dilution plating. A few weeds (including an Asteraceae sp.) and grasses were ELISA+ but none yielded *Xf* cultures.

Field collections (petioles, wood) of *V. mustangensis* (the most common native grape species in most of Texas (Fig. 1)) and *V. cinerea* var. *helleri* (syn. *V. berlandieri*) were always negative for numerous ELISA tests and several *Xf* isolation attempts in 2003, 2004, and 2005 except for *V. mustangensis* at Val Verde Co. Symptomatic *V. vulpina* (with GWSS egg masses) samples from near the PD-vineyard in Llano Co. were positive in 2004 and 2005 for Xf with ELISA; *Xf* isolation attempts were unsuccessful in 2004 on very few asymptomatic samples, and successful on some 2005 symptomatic samples. Rooted cuttings of all three species were mechanically inoculated with *Xf* in the greenhouse in 2005 (Table 1).

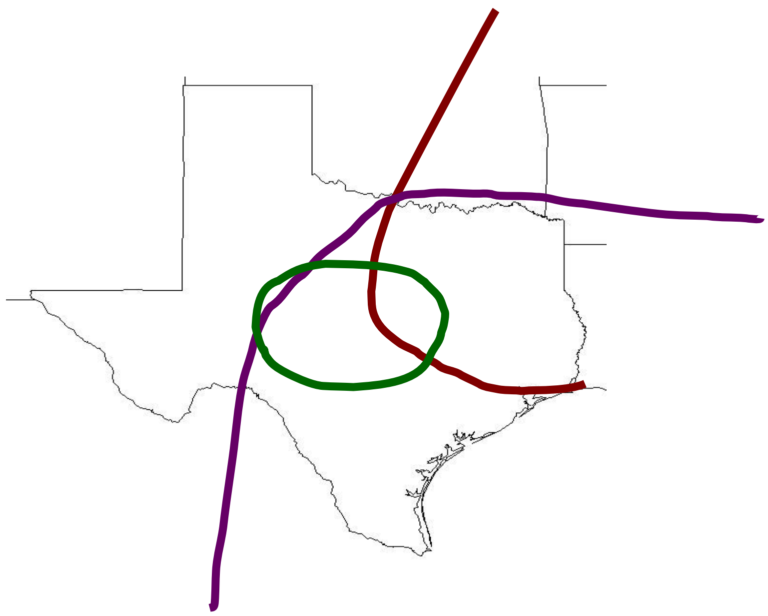

Fig. 1. Approximate ranges of *Vitis vulpina* (red), *V. cinerea* var. *helleri* (green), and *V. mustangensis* (purple) in Texas (Hatch et al., 1990).

Table 1. ELISA[1] response on 19Dec05 of rooted cuttings of three native *Vitis* species to 24Aug05 greenhouse inoculation[2] with *Xylella fastidiosa* isolate 5074 from *Vitis vinifera* 'Viognier' (Gillespie County, TX).

Vitis species	Plant population	No. plants inoculated	No. positive	No. check[3] plants	No. check plants positive
V. cinerea var. *helleri*	Llano Co. 2595	3	3	0	0
	Gillespie Co. 1391	1	1	0	0
	Total	4	4	0	0
V. mustangensis	Travis Co. Lago Vista	2	0	0	0
	Llano Co. 2564B	15	4	2	0
	Llano Co. 2594	1	0	0	0
	Uvalde Co. So. Getty	15	1	3	0
	Llano Co. 2564	5	2	1	0
	Total	38	7	6	0
V. vulpina	Llano Co. 2564	5	2	1	0

[1]Threshold for ELISA positive was ≥0.300 O.D.
[2]Bacterial cells from 7-day-old plates of PWG medium were suspended in SCP buffer for inoculating an internode of water-stressed plants with two 10-µl droplets followed by two pin-pricks to xylem depth.
[3]Check plants were mock inoculated with SCP buffer.

2. Compare plant communities near Texas vineyards with and without PD histories with plant surveys.

We added new species to the lists of plant species at our experimental sites. This improved our characterization of botanical diversity at sites with and without PD, affirming our suggestions to growers to manage vegetation as one part of a PD management strategy.

We collected, pressed, and identified plant specimens in the spring from no-PD McCulloch Co. (additional study vineyard in 2005), no-PD Gillespie, and PD Jeff Davis Co. (additional study vineyard in 2005 with K. Stark and E. Hellman) to continue our study of plant communities near vineyards with and without PD.

At stream and drainage areas near (0.5 to 3 mi) two no-PD vineyards with no weed *Xf* in or immediately adjacent to the vineyard, we detected and recovered *Xf* isolates from *A. trifida* and/or *H. annuus*. Low PD risk in vineyards that we have attributed to absence of certain weed species is tenuous if those species encroach on rights-of-way, seasonal riparian habitats, and farmyards.

Table 2. Presence or absence of selected *Xf* host plant species near vineyards with and without Pierce's disease.

	PD sites				No PD sites	
Species	Llano	Gillespie	Val Verde	Travis[1]	Gillespie	McCulloch
Vitis mustangensis, mustang grape	+	+	+	+	+	-
V. cinerea var. *helleri*, winter grape	+	+	-	+	-	-
V. vulpina, frost grape	+	-	-	-	-	-
Ambrosia psilostachya, western ragweed	+	+	+	+	+	+
A. trifida, giant ragweed	+	+	+	-[2]	-[2]	-[2]
Helianthus annuus, common sunflower	+	+	+	-	-	-
Ratibida columnifera, res-spike Mexican hat	+	+	+	+	+	+
Symphyotrichum divaricatum, hierba del marrano (slim aster)	+	+	-	+	+	-
Iva annua, seacoast sumpweed	+	+	-	-	-	-
I. angustifolia, narrowleaf sumpweed	+	+	-	+	-	-
Morus alba, white mulberry	+	-	+	+	-	-
Carya illinoisnensis, pecan[3]	+	+	+	+	+	-

[1]Travis site had no PD when project began in 2003; PD was diagnosed for the first time in 2005.
[2]Species was found 1-3 miles from vineyard.
[3]Pecan never diagnosed with *Xf* in TX, but is implicated as GWSS overwintering host.

3. Characterize *X. fastidiosa* strains recovered from supplemental plant hosts near vineyards, and from urban landscape hosts.

V. vinifera 'Chardonay' grape, greenhouse grown giant ragweed (*A. trifida*), cuttings of *V. mustangensis, V. berlandier*, and *V. vulpina* rooted winter 2004-05 were inoculated in greenhouse with grape isolate to compare reactions with field data. We evaluated these greenhouse experiments with ELISA after inoculating wine grape (with 1 western ragweed and 2 wine grape isolates)(Fig. 2), *A. trifida* (with 3 wine grape isolates)(Fig. 3), and three native grape species with wine grape isolates (Table 1). We showed a low percentage of mustang grapes were successfully inoculated, but do not yet know if infection became systemic.

Morano, Bextine, and Vickerman completed genetic analysis of some isolates in our collection at Uvalde. At least one weed species can apparently harbor *Xf* grape-strain.

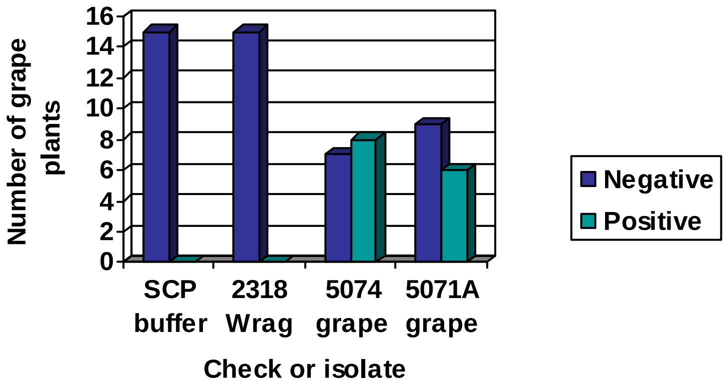

Fig. 2. ELISA reactions following greenhouse *Xf* inoculations of susceptible Chardonnay grape with isolates from grape and western ragweed (*A. psilostachya*).

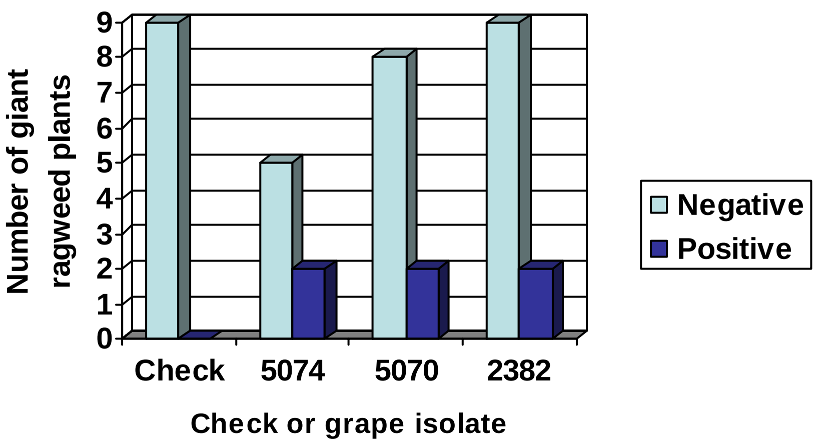

Fig. 3. ELISA reactions following greenhouse *Xf* inoculations of giant ragweed (*Ambrosia trifida*) with three grape isolates.

Table 3. Xylella fastidiosa isolates characterized genetically by L. Morano, B. Bextine, and D. Vickerman.

Isolate	Host plant source	Strain
1313	Vitis vinifera, wine grape	Grape
1318	V. vinifera, wine grape	Grape
1318B	**V. vinifera, wine grape**	**Non-grape**
2325	Ambrosia trifida, giant ragweed	Non-grape
3032	Ratibida columnifera, red-spike Mexican hat	Non-grape
5025	Ulmus crassifolia, cedar elm	Non-grape
5070A	V. vinifera Cabernet Sauvignon	Grape
5071A	V. vinifera Cabernet Sauvignon	Grape
5071B	V. vinifera Cabernet Sauvignon	Grape
5075A	V. aestivalis, Black Spanish	Grape
1459A&B	**A. trifida, giant ragweed**	**Grape**
2318	A. psilostachya, western ragweed	Non-grape

4. Study soils from vineyards with and without PD under controlled screen-house conditions by inoculating a susceptible grape variety with a grape isolate.

Soils from two vineyards (granite-based; Gillespie Co., McCulloch Co.) with no history of Pierce's disease (PD) and two vineyards (limestone-based; Gillespie Co., Blanco Co.) with PD histories were collected in the spring of 2005. These were compared to a commercial peat moss-based potting medium (Metro-Mix 366) in a white shaded screenhouse to exclude vectors (*ca.* 62% shade) in black plastic pots (0.082 m^3) irrigated as needed with distilled water. PD-susceptible Chardonnay (own-rooted) was inoculated 22-23 August 2005 with log-phase Xf cells isolated from *Vitis vinifera* in Gillespie Co (SCP buffer control). Overall condition of grape vines in the 5 soil types was poorest condition in the high pH clayey soils, intermediate in the granite sands, and best in the peat-based mix.

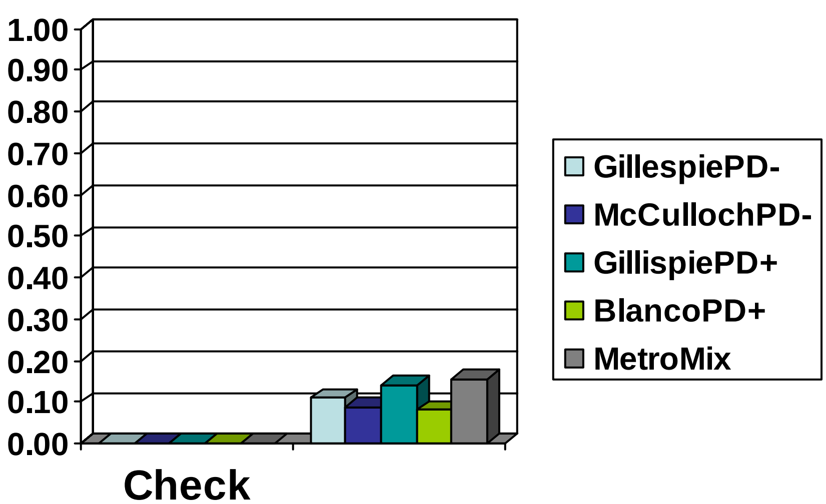

Fig. 4. Proportion of own-rooted Chardonnay plants in four vineyard soils and a commercial peat-based potting mix that were ELISA+ 86 to 91 days after inoculation with *Xylella fastidiosa* (isolate 5074 from Viognier, Gillespie Co., TX).

5. **Screening rootstocks commonly used in TX for reaction to natural insect-mediated inoculation with *X. fastidiosa*.**

A 3-yr rootstock study was planted in 2005 in Llano County, TX at a site where two previous plantings of *V. vinifera* cultivars were lost to PD. Entries are 5BB, 5C, 110R, 1103P, 1613C, 1616C, Champanel, Dog Ridge, Freedom, Harmony, Salt Creek and SO4 (five plants/plot, five replications). Leaves with PD symptoms in cv. Black Spanish adjacent to this test were positive with ELISA in September 2005 and *Xylella fastidiosa* (*Xf*) was isolated. Composite samples from five plants/plot were assayed with ELISA at the end of the season. Significant PD has already occurred in this field test, with all five replications of Freedom already infected. Most skips in the rootstock experiment were planted in March 06, with a few more due when irrigation is in place. The west border of Chardonnay was replaced with Black Spanish in March06. We are in year 2 of this 3-year study. Data to be collected includes symptoms and ELISA reactions in 2006 and 2007.

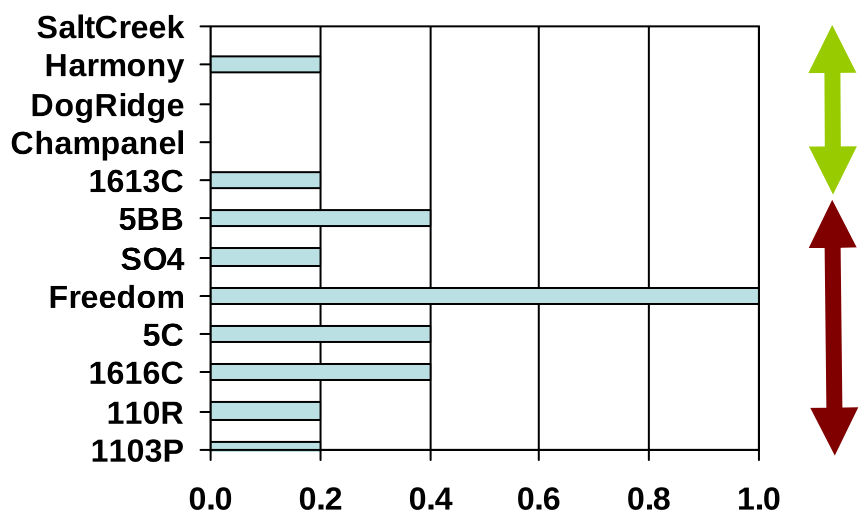

Figure 5. Evidence for initiation of Pierce's disease (PD) soon after planting in a rootstock trial at a site with a history of severe PD. Proportion of rootstock plots (one composite sample per plot) that were ELISA+ for *Xylella fastidiosa* on 7Nov2005. Planting dates were 28Mar-12Apr05 (**red arrow**) or 11Aug05 (**green arrow**) as plants became available.

Relevance to the USDA APHIS – Texas Pierce's Disease Research and Education Program:

1. Knowledge of *Xf* plant reservoirs near vineyards with PD in multiple years has increased our understanding of high risk sites, provided growers with criteria for selecting low-risk sites for future vineyards, and allowed growers to target certain weed species for control efforts.

Supplemental hosts collected to date near vineyards are mostly in Asteraceae; the fact that five annual and two perennial Asteraceae spp. hosts have no symptoms suggests long-term ecological vector/plant/pathogen relationships in Texas that may be unique from CA. Non-Asteraceae hosts near vineyards had symptoms.

Except for some ELISA+ data from two grasses in Jeff Davis Co., no supplemental hosts have been identified in Texas to date in Poaceae, Cyperaceae, Juncaceae, and Iridaceae. There is potential use/management of monocots (grasses, sedges, rushes, and native irises) in and around vineyards for erosion control, prevention of soil compaction, water conservation, and broadleaf weed control through competition.

Earlier reports of PD in the Jeff Davis vineyard were not confirmed and more work is needed. One possible explanation is that *X.f.* in this environment (rainy and relatively cool through Sept. 2005) may be at low populations, decreasing serological detection and plating success. Compared to Central TX ELISA data, detection in Asteraceae was consistent, detections in Poaceae and Celtis (Ulmaceae) were not consistent. Weed control implications in and near vineyards is consistent for PD management with Central TX data.

Entomologists should include known X. fastidiosa supplemental hosts in their studies on sharpshooter/leafhopper/spittlebug feeding/reproduction hosts. Beneficial insect research should include searches for egg mass parasitism on supplemental hosts.

An isolate collection for X. fastidiosa was be expanded for use in this project and for collaboration with other researchers in TX and CA. We added new species to the list of *Xf* supplemental hosts near vineyards in fall '05: *V. vulpina, V. mustangensis* (rare event, thought to have been inoculated by grafting infected scions), *Iva angustifolia, Morus alba*, and *Lavandula* sp. The find in *V. mustangensis* corroborates our concerns about infected rootstocks as *Xf* reservoirs.

2. This work is related to **Goal 1** and complements the supplemental Xf host work by characterizing botanical diversity at sites with and without PD.

Poaceae species are more frequent at the two original no-PD sites (Travis Co. site was diagnosed with PD in 2005) than at the two PD sites. USDA ARS personnel need these data and plant lists to assess potential for beneficial insect releases to survive on endemic plants. Entomologists needs these data and our expertise/herbarium sheets to identify plants where various sharpshooters are observed feeding-reproducing and where egg masses are parasitized. Entomologists have expressed interest in our lists of plant species by site as background information for their work with beneficial insects, etc. These data contribute to our estimates of PD risk in Central and SW TX for site selection and vegetation management practices. We have proposed plant species composition in and near vineyards as an indicator of site PD risk for new vineyard establishment. Species consistently associated with PD vineyards helps us prioritize weed species for control at existing vineyards.

3. We need pathogenicity comparisons for *Xf* isolates from wine grape and weeds in grape and weeds to compare to genetic work (L. Moreno, B. Bextine) on *Xf* strains we collected previously. The *Xf* isolate from *A. psilostachya* did not infect grape, but wine grape isolates did. All three wine grape isolates infected *A. trifida*. From genetic analysis completed to date, one isolate from *A. trifida* is “grape” strain, and one isolate from wine grape is “non-grape” strain. Some *Xf* isolates can apparently move among certain weeds and wine grape. These data support our suggestions that growers select vineyard sites in part on certain PD-risk indicator plant species, and that certain weed species be targeted for control in and near existing vineyards. Mustang grape can become infected to some extend under artificial conditions, corroborating our detection of *Xf* in mustang grape in a vineyard where the plants were once used as rootstock. If infection does not go systemic in our greenhouse work (results pending), this may point to a resistance mechanism such as limited movement from the infection site.

We need to determine which supplemental plant hosts harbor *Xf* strains pathogenic to grape. If certain weeds harbor only *Xf* that are not pathogenic to grape (*Ratibida columnifera* and *A. psilotachya* may be in this category), weed control can concentrate on the weeds infected with grape strains. Some weeds probably harbor both grape and non-grape strains, so numerous isolates from each supplemental host should be characterized.

In CA, isolates from urban sites were usually not pathogenic to grape. If urban landscape species in TX harbor *Xf* pathogenic to grape, we should caution vineyard/winery owners about landscape plant choice.

4. If we find differences, future research plans would seek to determine nature of the soil factor(s), and if it could be affected by vineyard management. If no direct soil differences occur, site differences may be related to plant communities associated with soil type, e.g. some supplemental hosts do not readily colonize well drained sands. This will help answer questions about site selection, vineyard management, and vegetation management in high-risk areas of TX and CA.

5. An integrated approach will be necessary to manage PD at high risk sites, because intensive insecticide applications are unlikely to be successful. We are hoping to identify rootstocks adapted to TX that are less susceptible to *Xf*.
Certain rootstock clones may provide advantages in PD areas: if rootstocks vary for *X.f.* populations (cfu/g) and vigor, those more resistant and more vigorous may be better choices for Texas vineyards at risk for PD. Rootstocks vary in reaction to X. fastidiosa based on work by Cornell Univ. researchers in FL, and in CA. We need information on those used in TX, because some are unique, e.g. need for resistance to *Phymatotrichopsis omnivorum* (cotton root rot) and summer stress in TX. Peter Cousins, Cornell U. speculates that rootstock vigor may be correlated with PD resistance or tolerance.

Publications submitted/published; presentations/posters presented at national technical meetings/conferences:

Abstracts/proceedings for posters/presentations:

M. C. Black, A. M. Sanchez, J. L. Davis, J. S. Kamas, and S. E. Ortiz. 2005. Supplemental *Xylella fastidiosa* hosts found near four central Texas vineyards with or without Pierce's disease histories. Phytopathology 95:S10 (abstr.). Presented poster at American Phytopathological Society national annual meeting in Austin, TX 29July-3Aug.

M. C. Black, J. S. Kamas, A. M. Sánchez, J. L. Davis and P. S. Adams. 2005. Aspects of Pierce's Disease Risk in Texas: I. Screenhouse inoculations of wine grape grown in soils from vineyards with or without Pierce's Disease histories. II. Field Evaluation of grape rootstocks most commonly used in Texas vineyards. III. *Xylella fastidiosa* tests on native Vitis species. Proceedings of Pierce's Disease Research Symposium, San Diego, CA, 5-7Dec05 (poster and invited oral presentation)(summary at: pages 150-151, http://www.cdfa.ca.gov/phpps/pdcp/ResearchSymposium/2005Section3.pdf).

Contributions to Newsletters:

Texas PD Notes Vol. 1 No. 1. Texas Cooperative Extension, September 15, 2005. http://piercesdisease.tamu.edu/news/newsletter/sept2005_pdNewsletter.pdf

Texas PD Notes Vol. 1 No. 2. Texas Cooperative Extension, November 15, 2005. Black provided article on p. 3 (photos by Penny Adams) http://piercesdisease.tamu.edu/news/newsletter/nov2005_pdNewsletter.pdf

Texas PD Notes Vol. 1 No. 3. Texas Cooperative Extension, January 15, 2005. http://piercesdisease.tamu.edu/news/newsletter/jan2006_pdNewsletter.pdf

Texas PD Notes Vol. 1 No. 4. Texas Cooperative Extension, March 20, 2005. http://piercesdisease.tamu.edu/news/newsletter/mar2006_pdNewsletter.pdf

Signature: Mark C. Black Date: April 17, 2006

If prepared by someone other than the Principal Investigator, please provide name and contact information: