RESPONSE OF UNGRAFTED GRAPE ROOTSTOCKS TO *XYLELLA FASTIDIOSA* AT A PIERCE'S DISEASE SITE IN TEXAS.

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ABSTRACT

There were highly significant differences in year 2 among 12 commonly used grape rootstocks in TX for Pierce's disease (PD) symptoms and *Xylella fastidiosa*-serology. The rootstocks Salt Creek, Dog Ridge, and Champanel were the most PD resistant and 1616C, Freedom, and Harmony were most susceptible. Growers in areas at high risk for PD should consider PD reactions with other traits when selecting rootstocks for specific vineyard sites. There were no correlations in year two between rootstock vigor and either PD symptoms or serology results.

INTRODUCTION

Pierce's disease (PD), caused by the bacterial pathogen *Xylella fastidiosa* (*Xf*), is the most limiting factor for growing grapes in much of TX and other U.S. gulf-coast states. Multiple management strategies are needed to improve PD control, including genetic resistance of scions and rootstocks, site selection, vegetation management, and vector management.

OBJECTIVES

1. Evaluate *Xf* reactions among commonly planted grape rootstocks in TX at a vineyard with a history of PD.

RESULTS

This report summarizes results midway through year two of a planned three-year rootstock study. Planting was initiated in 2005 in Llano County, TX at a site where two previous plantings of *V. vinifera* cultivars succumbed to PD. Planting continued as plants became available and to replace plants lost to transplant shock and PD (Table 1). Entries were 5BB, 5C, 110R, 1103P, 1613C, 1616C, Champanel, Dog Ridge, Freedom, Harmony, Salt Creek and SO4. There were five plants per plot and five replications. Leaves with PD symptoms in cv. Black Spanish (Le Noir) border rows consistently tested positive for *Xf* with ELISA and *Xf* was isolated. We anticipate collecting data on symptoms and ELISA reactions through 2007.

There were significant differences among entries for PD leaf scorch, proportion of plants positive with *Xf*-serology, mean optical density from *Xf*-ELISA, and plant vigor (Table 2). Harmony and 5C were least vigorous. SO4, 1613C, 1103P, 1616C and Salt Creek were most vigorous. PD symptoms were most prominent in 1616C, Freedom, and Harmony, but lowest in Salt Creek, Dog Ridge, Champanel and 110R. The proportion of plants positive for *Xf* with ELISA and the mean ELISA optical density were greatest for 1616C, Harmony, Freedom and SO4. Proportion of plants positive for *Xf* with ELISA and the mean ELISA optical density were lowest for Champanel, Salt Creek, and Dog Ridge. There were significant correlations between PD leaf scorch symptoms and both serology parameters (Table 3).

CONCLUSIONS

The rootstocks Salt Creek, Dog Ridge, and Champanel apparently have lower risk for PD than 1616C, Freedom, and Harmony. Growers in areas at high risk for PD should consider these PD reactions when selecting rootstocks for specific vineyard sites.

No rootstock entry in this trial was completely free of PD at mid-season of the second year of this study but there were highly significant differences among entries for symptoms and *Xf* serology. Serology (ELISA) is a general indicator of the concentration of *Xf* cells in grape tissue, and acquisition of *Xf* by vector insects is more efficient during feeding on plants with high populations (Hill and Purcell, 1997). The interaction of rootstock, scion and *Xf* has not been carefully studied, but our hypothesis is that vine mortality can be delayed or reduced if both scion and rootstock are not highly susceptible.

Genetic resistance in commercially-grown *Vitis* genotypes is useful in PD management in southern and southeastern U.S. (Hopkins and Thompson, 1984). Several rootstock cultivars derived from crosses with native *Vitis* species apparently have some resistance to *Xf* based on preliminary results from this study. Once we quantify resistance in the most commonly used rootstocks (ungrafted) in TX, we can assess the potential of certain rootstock X scion (grafted) interactions in PD management. Growers need multiple techniques, even if some have small effects, that can be combined to delay yield decline and plant death in vineyards at risk from PD. Grower strategies for PD management should include partial rootstock resistance along with site selection distant from riparian habitats, vector monitoring, timely insecticide applications, and selection of scion varieties adapted to the site.

Table 1. Planting dates[1] for rootstock plants rated and sampled on 14Aug06.

	Number of plants						
Entry	**28Mar05**	**12Apr05**	**11Aug05**	**26Sep05**	**14Mar06**	**20Apr06**	**31May06**
1103P	22					3	
110R	21						
1613C			20	4			
1616C	25						
5BB		23					2
5C	25						
Champanel			15		4	5	1
Dog Ridge			23			1	
Freedom	18						6
Harmony			23			1	
Salt Creek			25				
SO4	25						

[1]Planting date variation due to availability of planting materials and replanting due to transplant shock and PD mortality.

Table 2. Serology on samples collected 14Aug06, leaf scorch symptoms, and relative vigor of ungrafted grape rootstocks planted at a high PD-risk site in Llano County, TX.

Entry	**Serology reaction**[1]	**O.D.**[2]	**Scorch**[3]	**Vigor**[4]
1616C	1.0	1.7	2.8	1.9
Harmony	0.9	1.5	2.5	3.0
Freedom	0.9	1.7	2.7	2.4
SO4	0.8	1.4	2.0	1.8
1613C	0.7	1.1	1.9	1.8
1103P	0.6	1.0	1.6	1.9
5C	0.6	1.0	1.8	2.7
5BB	0.6	1.0	1.9	2.2
110R	0.5	0.8	1.3	2.1
Dog Ridge	0.3	0.4	1.2	2.3
Salt Creek	0.2	0.2	1.0	2.0
Champanel	0.1	0.1	1.2	2.3
Mean	**0.6**	**1.0**	**1.8**	**2.2**
LSD 0.05	**0.3**	**0.5**	**0.4**	**0.6**
CV, %	**37**	**35**	**18**	**21**

[1]Proportion of plants in a plot positive for *Xf* using DAS ELISA; OD<0.300 negative; OD≥0.300 positive.
[2]Mean optical density. Data analyzed were mean O.D. from individual plant samples in one plot.
[3]1 to 5 visual index of leaf scorch; 1=no symptoms, 4=plants with severe PD symptoms, 5=dead.
[4]1 to 5 visual index of plant vigor with 1=most vigorous, 4=very little growth, and 5=dead.

Table 3. Correlations of variables among rootstocks planted at a high risk PD site in Llano Co., TX.

	Serology reaction[1]	O.D.[2]	Vigor[3]
Leaf scorch index[4]	0.76 ***[5]	0.82 ***	0.2 N.S.[5]
Serology reaction		0.94 ***	-0.13 N.S.
O.D.			-0.06 N.S.

[1]Proportion of plants per plot positive with ELISA using O.D. threshold of ≥0.300 for positive reactions.
[2]Data analyzed were mean ELISA optical densities per plot.
[3]1 to 5 plant vigor index with 1=most vigorous, 4=very little growth, and 5=dead.
[4]1 to 5 leaf scorch index where 1=healthy, 4=plants with severe Pierce's disease symptoms, and 5=dead.
[5]*** indicates significant Pearson correlation at $P<0.001$; N.S. indicates not significant at $P=0.05$

REFERENCES

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EVALUATION OF GRAPEVINE ENDOPHYTIC BACTERIA FOR CONTROL OF PIERCE'S DISEASE

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ABSTRACT

In this reporting period we optimized our antagonism assay using our previously known antagonists. We found that a *Xylella fastidiosa* (*Xf*) lawn with a concentration of 10^5- 10^6 cfu/ml is optimal for visualizing zones of inhibition. Ten new isolates were shown to have antagonistic capability, including *Bacillus subtilis*, *Pseudomonas* sp., and *Pantoea agglomerans*.

Fourteen isolates chosen for their antagonistic or competitive ability *in vitro* were assayed *in planta* for systemic movement. Of these isolates, six were able to move upwards 30cm from the point of inoculation.

We continued our evaluation of Dr. Darjean-Jones' initial biological control experiment started in 2003. During fall of 2005, xylem sap extracted from these vines with a pressure bomb yielded approximately 220 new isolates. Of these, 169 are from vines inoculated with isolate #169 *Bacillus subtilis* and isolate #161*Bacillus* sp. Eighty-five of our new isolates were sequenced and 44 of these are *Bacillus* sp. Nine isolates had the same sequence as the *Bacillus* species that was originally inoculated into the vines. In February, bud-wood from Dr. Darjean-Jones' biological control experiment was collected from each treatment and propagated in the greenhouse. Ten vines per original treatment along with young Cabernet Sauvignon vines purchased from a nursery were inoculated with Stagg's Leap strain of *Xf* in mid July, 2006. At 14 weeks post inoculation these vines will be assessed for *Xf* infection using Immunocapture (IC)-PCR and rated for Pierce's disease symptom severity.

A new biological control experiment was started in the greenhouse this spring. This experiment is comprised of seven endophyte treatments in 15 Thompson seedless vines per treatment. Vines were mechanically inoculated with the endophytes and 6 weeks later they were inoculated with Stagg's Leap *Xf*. *Xf* infection and symptoms will be assessed after 14 weeks. We are testing the protective capabilities of three *Bacillus* sp. strains, three *Pseudomonas* strains, and one treatment co-inoculated with two *Pseudomonas* sp.

INTRODUCTION

The environment inside grape vine xylem vessels is a distinct ecological niche that supports a sparse microbial community. *Xylella fastidiosa* (*Xf*), the causative agent of Pierce's disease (PD), is one possible inhabitant. But our research, as well as work done in Nova Scotia reveals a diversity of other bacterial species capable of surviving in grape xylem (Bell et al., 1994). Endophytes are microbial organisms that do not visibly harm the host plant but can be extracted from surface sterilized tissue (Hallman et al., 1997). Some bacterial endophytes have been proven beneficial to plant health and are used to promote growth or as biological control treatments for fungal and bacterial pathogens. Previous researchers in our lab isolated an extensive library of endophytes collected from healthy grapevines, PD-infected vines, and asymptomatic vines in areas of high PD incidence (escape vines). We hypothesize that some of these bacterial endophytes may be antagonistic or compete with *Xf* for nutrients. Our library includes endophytes, such as *Pantoea agglomerans* and *Pseudomonas* sp., already tested as biological control agents in other crop systems (Stockwell et al., 2002, Barka et al., 2002). At this time our assays have yielded three additional endophytes that are antagonistic to *Xf in vitro* and are also capable of moving systemically within the grapevine. In this reporting period we have confirmed previous results and streamlined our *in vitro* antagonism procedure. Current PD management practices primarily involve keeping vector numbers low and removing infected vines. Biological control utilizing a systemic bacterial endophyte would be an implementable and environmentally desirable solution to this problem.

OBJECTIVES

1. Finish screening our existing library and recently acquired grape endophytic bacteria to identify potential antagonists of *Xf*.
2. Determine if *Xf*-antagonistic endophytes can systemically move in grapevines.
3. Evaluate the biocontrol abilities of endophytes against *Xf* including
   i) prevention of infection
   ii) suppression of Pierce's disease symptoms in greenhouse and field studies
   iii) long term health and survival of infected vines in the field
4. Isolate additional endophytes from escape vines and characterize these for antagonistic traits.