BACTERIAL POPULATIONS IN GRAPEVINES APPARENTLY RESISTANT TO PIERCE'S DISEASE OF GRAPEVINE

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Reporting Period: The results reported here are from work conducted September 2007 to May 2008.

ABSTRACT
Specific strains of the bacterium *Xylella fastidiosa* are economically important plant pathogens and cause scorch diseases in a variety of plants. One of these strains causes a scorch disease known as Pierce's disease (PD) of grapevine. This disease has caused significant disruption to the wine industry centered in the Temecula, California region; at the height of the most recent PD epidemic in the late 1990's, 25% of the grapevines in this area were lost before emergency quarantine and control measures could be instituted. Under these circumstances, the 2006 discovery of a population of apparently PD-resistant grapevines in the area was of particular interest. The vines were all located in a single vineyard, which had total PD-related losses of approximately 10%, while a neighboring vineyard suffered a nearly 100% loss of the same variety. In addition, a similar phenomenon was observed in a grapevine population located on the Agricultural Operations grounds at the University of California, Riverside. While the cause of this apparent resistance is unknown, one possible explanation for this resistance is that it is being conferred by bacteria present in resistant vines but not in susceptible vines. In order to test this hypothesis, cane samples from both the apparently susceptible populations and the apparently resistant populations were surface sterilized and plated onto standard microbiological media. Any observed bacterial growth was diluted into standard liquid media and then streaked out in order to obtain pure cultures, which were identified using 16S sequencing. Current results show that multiple *Paenibacillus* species are present more often in asymptomatic plants than in symptomatic plants at both locations.

INTRODUCTION
Specific strains of the bacterium *Xylella fastidiosa* (*Xf*) cause disease in almonds, grapevines, and a variety of other economically important plants (Davis 1978, 1980 and Purcell 1999). *Xf* is spread by the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis,* formerly known as *H. coagulata* (Redak et al. 2004 and Takiya et al. 2006).

In grapevines, one strain of this bacterium is the cause of Pierce's disease (PD). Since the preferred host of GWSS is citrus, vineyards close to a citrus grove are at increased risk for the development of PD (Perring et al. 2001). In addition, Chardonnay vines are known to be more susceptible to PD than other varieties (UC IPM). The Weaver vineyard is planted with Chardonnay vines and is immediately across from two citrus groves, meaning that it is at high risk of developing PD. However, while adjacent Chardonnay vineyards suffered catastrophic crop failure, the Weaver vineyard had a PD-related loss of far less, approximately 10%. This observation was of special interest since many of the plants in this vineyard were old enough to have survived the initial PD epidemic that occurred after the GWSS was accidentally introduced into California. The Agricultural Operations vineyard at the University of California, Riverside contains both symptomatic and asymptomatic Chardonnay vines in close proximity. These vines are younger than the ones at the Weaver Vineyard.

One possible explanation for this phenomenon is that it is being conferred by bacterial endophytes that live inside the apparently resistant plants but not in the more susceptible plants. The endophytic bacterium, *Curtobacterium flaccumfaciens,* has already been shown to confer resistance to *Xf* in sweet orange plants (Lacava et al. 2004).

To test this hypothesis, cane samples from asymptomatic and symptomatic grapevines at both locations were surface-sterilized and then plated on microbiological media. The genus of any resulting bacterial growth was then identified using 16S gene sequencing. The 16S gene has been widely used to classify unknown organisms (Turner 1997). Because this gene evolves very slowly, it is most useful for classifying organisms at the genus level, but not at the species or subspecies level (Weisburg et al. 1991). Even so, it is widespread practice to include a species name when identifying bacteria based on this sequence. These designations can be considered putative in nature.

OBJECTIVES
The primary goal of this research was and continues to be to test the initial hypothesis through isolating bacterial endophytes from symptomatic and asymptomatic grapevines at both locations and using 16S analysis to identify them.

RESULTS AND DISCUSSION
A comparison of the endophytes isolated from the vines at both locations showed that members of the genus *Paenibacillus* occurred more frequently in asymptomatic vines than in symptomatic vines (**Table 1**) (Parker 2008). This observation was

of special interest since it is already known that *Paenibacillus polymyxa* can confer resistance to the bacterial plant pathogen *Erwinia carotovora* in gnotobiotic *Arabidopsis thaliana* plants (Timmusk and Wagner 1999). It is possible that *Paenibacillus* could be playing a similar role inside the asymptomatic grapevines, since one of the *Paenibacillus* isolates tested in the laboratory was found to retard the growth of the PD strain of *Xf* in both co-culture and in grapevines (A. Arora, personal communication). In addition, it has recently been shown that other members of *Paenibacillus* can reduce the growth of *Xf* or even clear it altogether on microbiological media (Kirkpatrick and Wilhelm 2007).

In addition, the presence of certain endophytes within the plants (most notably *Bacillus*) appeared to be dependent on the time of year the plants were sampled (Parker 2008). In the Weaver vineyard, Bacillus was most commonly isolated in May. However, in the Agricultural Operations vineyard, Bacillus was most frequently in May and October (see **Tables 2 and 3**). The reasons for this are not yet clear.

Table 1.

Code	Genus	Bit Score	E-value	Symptomatic Plant	Date Collected
45V16 1F C1	*Achromobacter*	2039	0	No	4-May-06
45V16 2D C1	*Bacillus*	863	0	No	4-May-06
45V16 2E C1	*Bacillus*	2771	0	No	4-May-06
46V16 2D C1	*Bacillus*	500	7.00E-138	No	4-May-06
46V19 1C C7	*Bacillus*	2454	0	No	27-Jul-06
46V19 2D C1	*Bacillus*	2736	0	No	4-May-06
46V19 2F C1	*Bacillus*	2605	0	No	4-May-06
47V1 1A C1	*Paenibacillus*	2789	0	No	4-May-06
47V1 1B C1	*Bacillus*	1844	0	No	4-May-06
47V1 1C C2	*Bacillus*	2389	0	No	18-May-06
47V1 1E C1	*Bacillus*	1162	0	No	4-May-06
47V1 1F C1	*Bacillus*	2365	0	No	4-May-06
47V1 2B C1	*Paenibacillus*	293	1.00E-75	No	4-May-06
47V1 2C C3	*Bacillus*	2692	0	No	31-May-06
47V3 1B C1	*Bacillus*	2351	0	No	4-May-06
47V3 1C C2	*Bacillus*	1015	0	No	18-May-06
47V3 1E C12	*Bacillus*	401	2.00E-108	No	5-Oct-06
47V3 2D C13	*Bacillus*	1009	0	No	19-Oct-06
47V3 O C3	*Planococcus*	2609	0	No	31-May-06
47V8 1A C9	*Bacillus*	2561	0	Yes	24-Aug-06
47V8 R6	*Bacillus*	2627	0	Yes	4-May-06
48V10 1B C1	*Bacillus*	2591	0	No	4-May-06
48V10 1B C2	*Bacillus*	2407	0	No	18-May-06
48V15 1C C3	*Bacillus*	1084	0	No	31-May-06
48V15 1D C2	*Bacillus*	2605	0	No	18-May-06
48V15 2A C7	*Bacillus*	910	0	No	27-Jul-06
48V19 2F C2	*Bacillus*	2379	0	No	18-May-06
49V9 1A C2	*Bacillus*	979	0	No	18-May-06
49V9 1C C2	*Bacillus*	2533	0	No	18-May-06
49V9 1C C3	*Bacillus*	2670	0	No	31-May-06
49V9 1D C3	*Bacterium*	880	0	No	31-May-06
49V9 1F C1	*Bacillus*	1203	0	No	4-May-06
49V9 1F C2	*Bacillus*	2660	0	No	18-May-06
49V9 1F C7	*Bacillus*	1154	0	No	27-Jul-06
49V9 2B C1	*Bacillus*	2577	0	No	4-May-06
49V9 2C C1	*Bacillus*	650	0	No	4-May-06

Table 2. Presence of *Bacillus* in Grapevines in the Weaver Vineyard, by Month.

Plant	May	June	July	August	September	October	November	December
45V16	X							
46V16	X							
46V19	X		X					
47V1	X							
47V3	X					X		
47V8				X				
48V10	X							
48V15	X		X					
48V19	X							
49V9	X							

Table 3. Presence of *Bacillus* in Grapevines in Agricultural Operations, by Month.

Plant	May	June	July	August	September	October	November	December
A-4	X					X		
B-3	X		X					
C-1	X		X			X		
D-7						X		
E-1								
F-15						X		
G-6	X					X		
H-11	X			X				
I-6						X		
J-9	X			X		X		

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-Winged Sharpshooter Board