Final Report for VA Wine Board

PROJECT TITLE:
Preliminary data collection to understand Pierce's Disease ecosystem in VA. (14-1693-02)

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OBJECTIVES:

1. Obtain preliminary data on vectors, identification of species and presence/absence of *Xylella fastidiosa (Xf)* in vector insects
2. Follow-up on a trial of PD-resistant varieties at an experimental vineyard in SPAREC to evaluate their performance in VA
3. Continue monitoring of *Xf* titer changes within an infected vine over the course of a season

I. Activities Performed

Objective 1) Obtain preliminary data on vectors, identification of species and presence/absence of Xf in vector insects

We selected three vineyard locations 1) AHS AREC at Winchester, VA, 2) Rosemont vineyard, LaCross, VA, and 2) Rees vineyard North Garden, VA. At these locations, rows are running north-south, the wooded area is located to the east side of the vineyard, and distance from the woods to the vineyard is approximately 30-70 ft. We have placed five yellow sticky cards at: 1) near the edge of vineyard, closer to the wooded area; 2) at the end of the row; 3) in the middle of the vineyard (70-100 ft from the #2); 4) at the highest elevation within the vineyard, and 5) at the end of the row, far from the wooded area (150-200 ft from the #2). We collected these traps every two weeks starting May to the end of October 2014. The cards were shipped overnight to the Plant Disease Clinic for 1) Identification of vectors by Dr. Pfeiffer's group and 2) Detection of Xf by the clinic. We have used a quantitative real-time PCR (qPCR) method

adapted from Harper et al., (Harper et al., 2010), which is more sensitive than the ELISA-assay method that was used in the previous survey (Wallingford et al., 2007). In 2013, we found out that the method worked very well with grapes, and we were confirming whether the method will be applicable with insect vectors.

In 2014 season, we have collected 145 total traps (some location were not able to have regular collection). Potential insect vectors (leafhopper sharpshooters) were identified. We are still in a process of identification; however, it looks like there is a big spike of *Oncometopia orbona* and *Graphocephala versuta*, both of which are suspected *Xf* vector for VA, in early July. Since *Xf* requires some time to distribute within the vine, development of insect population late in the season may not be significant in terms of disease development. Thus, this identification of peak period may be useful for development of insecticide application timing; however, we do need more information.

Among those 145 traps, 50 samples have been processed for detection of *Xf* from insect body. Although the quality of the sample was not idea (e.g., traps were taken every two weeks, so, potentially, some of insects were on the trap for more than two weeks), we were able to detect *Xf* from insect body in 8 samples. Thus, we confirmed that we are able to use our *Xf* detection method for plant for insect samples.

Objective 2) Follow-up on a trial of PD-resistant varieties at an experimental vineyard in SPAREC to evaluate their performance in VA

Unfortunately, SPAREC has decided to discontinue their experimental vineyard in 2014. Since this part of the project needs multiple years, we have omitted this part from the new proposal submitted in 2015.

Objective 3) Continue monitoring of *Xf* titer changes within an infected vine over the course of a season

As with the objective 2, the loss of SPAREC vineyard put a holt on this objective too. We have identified a location in central VA with *Xf* positive vines, and samples were obtained in the summer 2015.

Additional information obtained from our effort

At the end of the last fiscal year, we received additional funds to conduct a muti-locus sequence typing (MLST), which enable for us to identify subspecies of *Xylella fastidiosa*. The MLST is used to differentiate subspecies of *X. fastidiosa* (Yuan et al., 2010, Nunney et al., 2012, Maiden et al., 1998). MLST uses sequence variation among several housekeeping loci to characterize isolates of bacteria (Nunney et al., 2014b, Yuan et al., 2010). Alleles found at each gene loci are assigned a unique number, and a combination of specific alleles is called a sequence type (ST) (Nunney et al., 2012, Maiden et al., 1998, Scally et al., 2005). A ST number based on the gene-specific allele numbers represents a strain. Results from recent MLST studies have grouped *X. fastidiosa* into five subspecies: *X. fastidiosa* subsp. *fastidiosa, sandyi, multiplex, pauca, and tashke* (Almeida et al., 2008, Scally et al., 2005, Nunney et al., 2010b, Schuenzel et al., 2005, Yuan et al., 2010, Randall et al., 2009).

Among these five subspecies, *X. fastidiosa* subsp. *fastidiosa* is known to cause PD of grape (Nunney et al., 2014b, Scally et al., 2005). Due to lack of genetic variability among *X. fastidiosa* subsp. *fastidiosa* from California, Texas, and Florida (Scally et al., 2005), and wide genetic variation found in Costa Rica samples (Nunney et al., 2010b), it has been suggested that the isolates of *X. fastidiosa* in the US originated from an introduction of *X. fastidiosa*-diseased plant material, most likely coffee plants, from Central America in the late 1850s (Nunney et al., 2010b). On the other hand, high genetic variability among isolates of *X. fastidiosa* subsp. *multiplex* suggests that it is native to North America (Retchless et al., 2014, Nunney et al., 2014b). *X. fastidiosa* subsp. *multiplex* has been associated with several hosts, including oak, blueberry, and almond (Scally et al., 2005, Nunney et al., 2014b, Nunney et al., 2014a). Some *X. fastidiosa* MLST studies have included a few samples from the southeastern US (Scally et al., 2005); however, information relating to subspecies from the eastern US is lacking.

Criteria for sample selection for MLST were Ct value, region, and cultivar. A total of 24 *X. fastidiosa*-positive genomic DNA samples from the survey were selected for MLST analysis from six VA wine regions (Central, Chesapeake, Eastern, Hampton Roads, Northern, and Southern). Since relatively low Ct value (i.e., high DNA concentration) is required for MLST, only one sample from Shenandoah Valley and no samples from Blue Ridge were selected. The number of sample per region varied from three to eight. For our study, five housekeeping genes of *X. fastidiosa* (*cysG*, *gltT*, *holC*, *leuA*, and *nuoL*) were amplified using primers designed by Yuan et.al. 2010 (Yuan et al., 2010), then the products were sent out to sequencing. Sequences were compared to sequences in the *Xylella fastidiosa* MLST Databases (http://pubmlst.org/xfastidiosa/) and NCBI (http://www.ncbi.nlm.nih.gov/nuccore/).

Our results showed that 18 of 24 total isolates sequenced showed 99-100% homology to *X. fastidiosa* subsp. *fastidiosa* based on the draft genomes of GB514 (Schreiber et al., 2010), M23 (Chen et al., 2010), Tulare (Schuenzel et al., 2005) and Temecula-1(Van Sluys et al., 2003) (Table 1). All 18 isolates had the same allelic profiles (ST1 in Yuan (Yuan et al., 2010) and Scally (Scally et al., 2005) among the 5 gene loci. However, 6 isolates showed 99-100% homology to *X. fastidiosa* subsp. *multiplex* based on draft genomes of M12 (Chen et al., 2010), Multiplex Riv 5 (Rogers and Stenger, 2012), and MUL0267 (Nunney, 2011). *X. fastidiosa* subsp. *multiplex* was identified from isolates from five (Central, Chesapeake, Eastern, Hampton Roads, Northern) of the six VA wine regions, but was not identified from five isolates selected for MLST from the Southern VA wine region. As with *X. fastidiosa* subsp. *fastidiosa* samples, five of *X. fastidiosa* subsp. *multiplex* samples shared the same allelic profiles (ST43 in Nunney et al. (Nunney et al., 2013)). Vines associated with isolates of *X. fastidiosa* subsp. *fastidiosa* or *multiplex* displayed symptoms at the time of sampling.

X. fastidiosa subsp. *fastidiosa* is known as the causal agent of PD of grape, and ST1 has been identified in various regions of California (Scally et al., 2005). Some ST differences among CA and southeastern US isolates were reported in previous studies (Lin et al., 2013, Scally et al., 2005, Parker et al., 2012). However, *X. fastidiosa* subsp. *fastidiosa* is known to have very low genetic variability in the US (Nunney et al., 2010a, Yuan et al., 2010), plus, the number of isolates typed using MLST in this study is relatively small. Thus, there is a possibility for more variability among the survey isolates. Moreover, many vineyards in VA purchase their vines from nurseries located in CA, and it would not be surprising if *X. fastidiosa* subsp. *fastidiosa* ST1 was introduced to VA via infected nursery stocks.

To our knowledge, this is the first report of *X. fastidiosa* subsp. *multiplex* isolated from European wine grapes, *Vitis vinifera L.* (cultivar Chardonnay, Merlot, and Petit Verdot), as well as interspecific hybrid grapes (Vidal blanc and Norton). In addition, although it was not severe, the vines that were associated with *X. fastidiosa* subsp. *multiplex* exhibited PD symptoms. Since host specificity among subspecies and among STs within subspecies has been discussed (Parker et al., 2012, Nunney et al., 2014a), detection of *X. fastidiosa* subsp. *multiplex* in *V. vinifera* was not expected. However, in a study by Schaad (Schaad et al., 2004), *X. fastidiosa* subsp. *multiplex* was identified from a pigeon grape (*V. aestivalis Michx.*). One of cultivars associated with *X. fastidiosa* subsp. *multiplex* in our survey was Norton (also known as Cynthiana). Norton was developed in Virginia around 1820 (Ambers, 2012, Ambers and Ambers, 2004), and about 140 acres of Norton are planted in VA according to 2013 data (2014). Although there is still uncertainty about the exact parentage of Norton, it is considered that *V. aestivalis* is one of its parents based on phenotype (Reisch et al., 1993, Ambers, 2012, Ambers and Ambers, 2004).

X. fastidiosa subsp. *multiplex* is considered native in North America due to its high genomic variability and wide host range reported in the US (Nunney et al., 2010a, Nunney et al., 2013), as "multiplex" implies. Known hosts include some *Prunus* species, peach (*P. persea L. Batsch*), plum (*P. domestica L.*), and almond (*P. dulcis Webb L.*). Red oak (*Quercus rubra*), pin oak (*Q. palustris*), American elm (*Ulmus americana*), and sycamore (*Platanus occidentalis*) have also been identified as hosts in Georgia and Washington DC (Harris et al., 2014, Chen et al., 2013, Schaad et al., 2004). In recent studies, *X. fastidiosa* subsp. *multiplex* has been characterized using different MLST approaches, and one finding is that *X. fastidiosa* subsp. *multiplex* isolates (STs) can be grouped into two

phylogenetically different groups: one with evidence of intersubspecific homologous recombination (IHR) and without evidence of IHR (Parker et al., 2012, Nunney et al., 2014b, Scally et al., 2005).

All six *X. fastidiosa* subsp. *multiplex* isolates we identified were identical to ST43 from Georgia and Florida, which is known to cause infection on blueberry (*Vaccinium corymbosum* and *V. corymbosum* X *V. angustifolium* hybrid) (Nunney et al., 2013, Parker et al., 2012). ST43 belongs to "clade A" and "IHR *multiplex*" in Parker (2012) (Parker et al., 2012) and Nunney et al. (2014) (Nunney et al., 2014a), respectively. IHR is considered to be associated with host specificity of *X. fastidiosa* subsp. *multiplex* (Nunney et al., 2014a), and six different hosts in different genera (almond, purple leaf plum, giant ragweed, blackberry, blueberry, and American elm) were associated with the IHR *multiplex* group. Thus it is not surprising to find our isolates in the IHR *multiplex* group. We used five loci in for MLST, and there were some SNPs observed; therefore, a further MLST study with genes with environmentally mediated genes as suggested in Parker (2012) and Nunney et al. (2014) (Parker et al., 2012, Nunney et al., 2014a) would provide more resolution of the *X. f.* subsp. *multiplex* isolates from the VA survey.

Table 1. Allelic profiles identified in the multilocus sequence typing of 24 *Xylella fastidiosa* isolates collected from seven geographic regions in Virginia in 2013.

			Locus type					
Region	**Cultivar**	**Sample type[z]**	***cysG***	***gltT***	***holC***	***leuA***	***nuoL***	**Clonal complex**
Central VA	Norton	Random	18	7	4	3	3	*X.f.* subsp. *multiplex*
	Petit Manseng	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Vidal blanc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
Chesapeake bay	Vidal blanc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Merlot	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Chambourcin	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Vidal blanc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Petit Verdot	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Cabernet Franc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Merlot	Suspect	18	7	4	3	3	*X.f.* subsp. *multiplex*
	Chardonnay	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
Eastern VA	Chardonnay	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Chardonnay	Random	18	7	4	3	3	*X.f.* subsp. *multiplex*
Hampton Roads	Norton	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Cabernet Franc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
Northern VA	Vidal blanc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Vidal blanc	Suspect	18	7	4	3	3	*X.f.* subsp. *multiplex*
	Merlot	Suspect	18	7	4	3	3	*X.f.* subsp. *multiplex*
Shenandoah Valley	Petit Verdot	Suspect	18	7	4	3	3	*X.f.* subsp. *multiplex*
Southern VA	Pinot Grigio	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Vidal blanc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Cabernet Franc	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Chambourcin	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*
	Cabernet Sauvignon	Suspect	1	1	1	1	1	*X.f.* subsp. *fastidiosa*

[z] Random samples did not show visual symptom(s) at the time of sampling; suspect samples were symptomatic for PD

II. Problems and Delays

As noted above, objectives 2 and 3 were not completed because of SPAREC vineyard has been discontinued. We have been identified alternative location for the objective 3.

III. Future Project Plans

We will continue analyzing leafhopper sharpshooter species on the traps, and conduct *X. fastidiosa* detection assays.

IV. Funding Expended To Date

We have utilized 100% of our budget.

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