Final report for The Virginia Wine Board 2016
"Preliminary data collection to understand Pierce's Disease ecosystem in VA"

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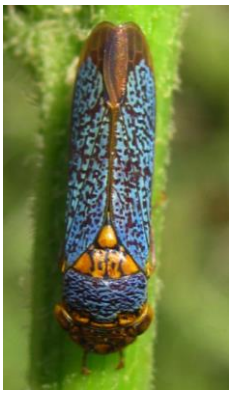
Figure 1. Typical PD symptoms.

Introduction/Justification

Pierce's Disease (PD) is a vascular disease of grapes caused by the bacterium *Xylella fastidiosa* Wells et al. (*Xf*). *Xf* is vectored by xylem-feeding sharpshooters, especially *Oncometopia orbona, O. nigricans, Graphcephala versuta,* and *Homalodisca coagulata,* in the Southern and Eastern United States [1]. After infection the bacteria proliferate in the xylem tissues, and that leads to the development of symptoms that include interveinal chlorosis, marginal necrosis (often with yellow or red lines), green islands on shoots, and leaf abscission that leaves characteristic "matchstick petioles" with necrotic tips (Fig. 1). The infected vines will suffer vine decline, yield loss, and even death. With severe infection on a susceptible variety, it only takes two to three years for the vine to be killed. *Xf* can cause diseases on a number of crops, including peach, citrus, and almond, and is known to have a wide range of other host species [2].

In general, areas with daily minimum temperatures below -9.5C for two or more days are considered to be at low risk for Pierce's disease [3] because the low temperature can kill the bacterium [4]. However, due to recent climate trends [5, 6] and the expansion of grape growing regions in VA, there are several reasons to be concerned about PD in the near future. A study by Anas, et al., showed that almost the entire state of VA is now considered a high risk area based on recent 8-year average data [3]. The same study presented results of a 2008 survey in NC and GA, where 82% and 75% of surveyed vineyards, respectively, were positive for PD. Moreover, a small survey that took place in 2006 revealed that PD-positive vines were found in more than 70% of surveyed vines in VA [7]. Furthermore, our recent survey revealed that even in areas where more than two days of -9.5C were observed, there was high incidence of PD.

Figure 2. Blue sharpshooter



The number of vineyards has been increasing rapidly in VA: from 105 in 2005 to over 230 in 2013. On a 2013 VA winery map, there are 64 wineries located in the lower half of VA where PD has historically been considered an issue. The lower half of VA includes the Blue Ridge Highlands, Southern, Central (not including the Monticello American Viticulture Area), Northern Neck, Hampton Roads, and Eastern Shore regions. Moreover, based on the aforementioned study [3], even Monticello AVA and Northern VA, where the majority of vineyards are located, are considered high risk.

A frequent question from growers is when to apply insecticides to control PD

vectors. Because previous studies done in CA [2, 8] showed that *Xf* is more likely to become systemic in the plant when a grapevine acquires *Xf* in early spring, we often recommend applying an insecticide during pre-bloom. On the other hand, another study showed that the vector was not able to acquire *Xf* from grapevines until bacterial titer became high in the vine [8]. Thus, it is important for us to understand when the *Xf* population is high within a grapevine under VA conditions in order to identify the high-risk time period(s) for within-vineyard spread of PD.

The other uncertainty growers face is how to deal with the infected vines. Due to the lack of easy (and economical) detection techniques, our recommendation is to rogue out entire infected vines. However, many grapevines grown in VA are trained to have two or more trunks. We do not know whether we should assume that the whole vine is infected if only one trunk is infected.

With support from the VA Wine Board and VT's College of Agriculture internal competitive grant, we conducted a VA PD survey in 2013. The results indicated that 1) the *Xf*-detection we used is far more sensitive than methods used in previous PD surveys [3, 7], 2) PD is widespread in VA, 3) symptom expression was not a good indicator for the disease, and 4) there are two subspecies of *Xf* associated with VA wine grapes.

Data from the 2013 season also suggests that the bacterial titer is detectable soon after bud break, but the titer became very low in mid-season and remained so until very close to the harvest. This suggests a possibility that transmission of *Xf* among vines in a vineyard may not be the major mean of dissemination, especially if the vector is not arriving to the vineyard very early in the season. If that is the case, control of *Xf*-vector insects coming into the vineyard could be the most effective means of avoiding PD in a vineyard.

Unfortunately, vectors involved in transmission of *Xf* in VA (or the southeast in general) have not been well studied. There are a few potential vectors identified (Table 1); however, *Oncometopia orbona* (common name: Blue sharpshooter or Broadheaded sharpshooter, Fig. 2) seems to be the most important vector based on its feeding habits.

Table 1. Potential *Xf* vectors found in VA.

Name	Common name	Location found
Oncometopia orbona	Blue sharpshooter or Broadheaded sharpshooter	Southeastern US, up to MD
Graphocephala versuta	Versute sharpshooter	TX to VA
Draeculacephala mollipes, and D. minor	Watercress sharpeshooter	Vienna and Blacksburg
Graphocephala coccinea	Candy-striped leafhopper, or red-banded leafhopper	Falls Church, Blacksburg

Therefore, during the 2015 season, we would like to finish our preliminary data collection on insect vectors to help understand the ecosystem of Pierce's Disease. This second year will help us to determine if insect vector data collected in 2014 is representative for VA; moreover, it will give us an opportunity to publish our results in a peer-reviewed journal.

We have three objectives to answer our questions:

1. Obtain preliminary data on vectors: Identification of species listed in Table 1 and presence/absence of *X. fastidiosa* in vector insects.
2. Continue monitoring *X. fastidiosa* titer changes within an infected vine over the course of a season.
3. Expand identification of *X. fastidiosa* subspecies.

A biennial research needs/priority survey of Virginia Vineyards Association members (2008) rated Pierce's Disease 3.59, on a scale of 1 to 5, with 5 being of highest importance and 1 being unimportant. PD decreases both quality and quantity of wine grape yield; thus, this proposal meets Vision 2015 under objective 4, improvement of the profitability of vineyard and wine businesses in Virginia. Also, development of management strategies through better understanding of disease biology will benefit extension education, which is emphasized in objective 4.9 of Vision 2015 and the National Road Map for Integrated Pest Management. Problems associated with Pierce's Disease are certainly one of the major causes of increased cost operation of Virginia vineyards, thus, it will address items listed in the VVA's "Beyond vision 2015" blue print,

"Biological and environmental threats to grape production" which was listed as one of the major threats, and "High capitalization costs of vineyards" which was listed as one of the major weakness of the industry.

Results

Objective 1) Obtain preliminary data on vectors and detection method

We asked managers from three vineyards to collect insects 1) AHS AREC in Winchester, VA, 2) Rosemont vineyard in La Cross, VA, and 3) Rees vineyard near Shipman, VA. At all three vineyards, the wooded area is located within 50-70 ft. We instruct them to place four 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); and 4) at the end of the row, far from the wooded area (150-200 ft from the #2). We asked to time trap placement to target a four-month period from April to July. The cards were in the field for 14 days, and once collected, will have spacers inserted to keep the insects from being crushed, then will be shipped overnight to the Virginia Tech Insect Identification Lab for 1) identification of vectors listed in Table 1 and 2) Detection of *Xf* by the Virginia Tech Plant Disease Clinic. We used a quantitative real-time PCR (qPCR) method adapted from Harper et al. [7]. We found out that the method was extremely sensitive at detection of *Xf* (to 4 copies) in grape petioles, and preliminary results show the method works well with detection of *Xf* in insect vectors.

In 2014 season, we have collected 145 total traps (some location were not able to have regular collection) (Fig. 1 and 2). Two potential insect vectors (leafhopper sharpshooters) were identified. There were consistent catches of both *Oncometopia orbona* and *Graphocephala versuta*, both of which are suspected *Xf* vector for VA. At all three locations, *O. orbona* populations seemed to be high in May to June, while *G. versuta* populations were more prevalent between July and September.

Among those 145 traps, 50 samples have been processed for detection of *Xf* from insect body. Although the quality of the sample was not ideal (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.

In 2015, unfortunately, one of vineyard location (Rosemont) never collected insects, but at two locations, we have collected insect from May to August. The total number of insect and species found is summarized in the Fig. 3. Although the population of *O. orbona* was low, there were consistent captures of *G. versuta* at both locations. Another 50 samples were processed for *Xf*; however, only two were found to be positive. However, we speculated that this poor detection rate was not true reflection of *Xf* in the insects, rather, it was due to the difficulty of extracting DNA from trapped insects.

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. As speculated in the previous studies, *O. orbona* maybe the most important vectors of *Xf* for VA, since it tends to come early (i.e., higher chance of transmitting *Xf*). Although the high capture number of *G. versuta* was observed, this vector tends to feed on the upper part of the canopy, thus, *Xf* may take a while to translocated to the trunks and other more permanent structures of the vine.

Figure 1. Total number of insect species collected at AHS AREC and Rees vineyards, 2014

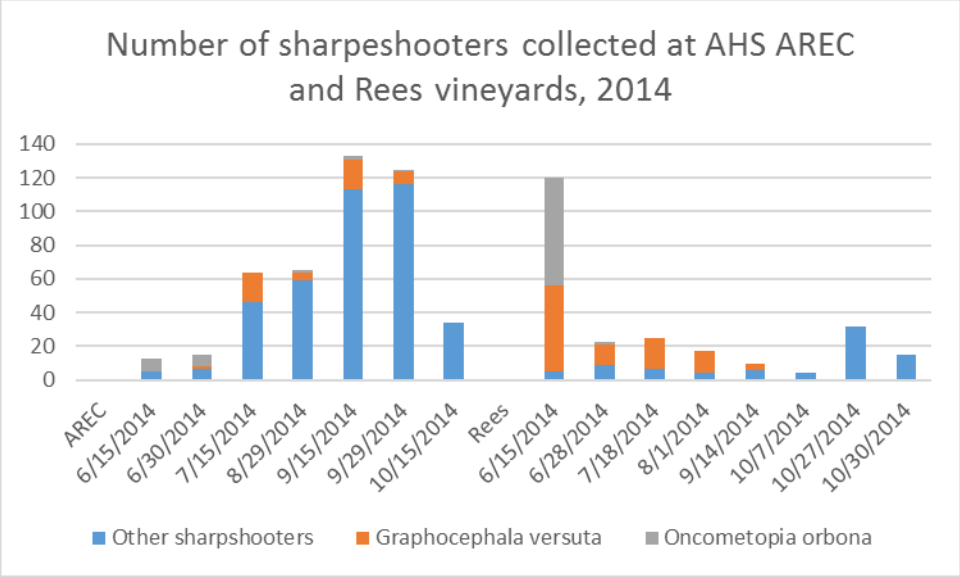


Figure 2. Number of sharpeshooters collected from Rosemont vineyard 2014

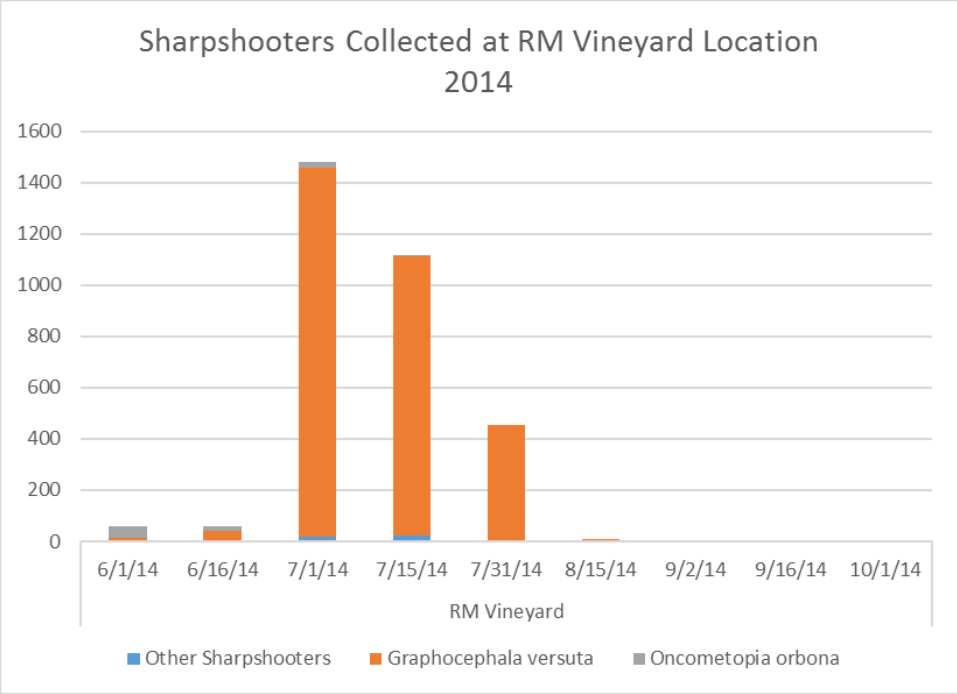
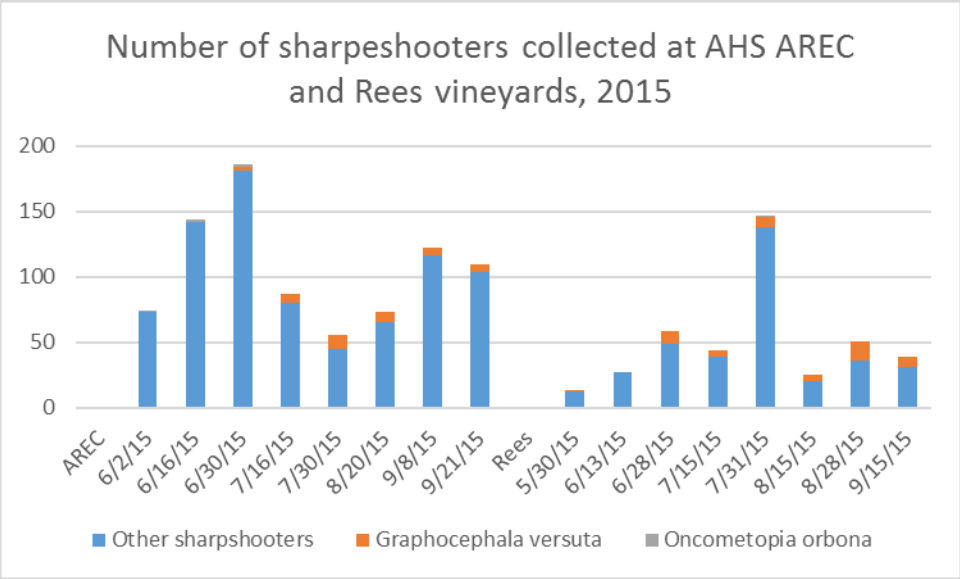


Figure 3. Number of sharpeshooters collected from AHS AREC and Rees vineyards, 2015



Objective 2) Continue monitoring of *Xf* titer changes within an infected vine over the course of a season

In the 2013 study, we used ten varieties at Blackstone vineyard. Unfortunately, the Southern Piedmont AREC discontinued operation of the vineyard in 2014. However, we identified several varieties in Orange, VA with high *Xf* titer. We selected four varieties (Cabernet Franc, Petit Manseng, Vidal Blanc, and Viognier) that are commonly grown in VA, and tested for *Xf* using the qPCR procedure described in objective 1. We selected two vines from each variety, and petiole samples were collected at four stages of grape development: 1) pre-bloom (3-4 weeks after bud break); 2) at fruit set (2-3 weeks after bloom); 3) at veraison; and 4) near harvest (1-2 weeks prior to harvest). These vines were trained with a vertical shoot positioning system with two trunks (cordons), and samples were taken from four locations per cordon (lower center, lower end, upper center, upper end). Each sample consisted of 7 petioles randomly taken from the target area for processing and qPCR-detection.

We are still in the process of detection, as of Aug. 22nd 2016. The delay was due to the limitation of labor. DNA from all samples were extracted, and waiting for detection of target DNA, which should be completed by the end of September 2016. However, preliminary results showed that very low titers were observed from these vines, even at the end of the season.

Objective 3) Expand our search for *X. fastidiosa* subspecies.

In our 2013 study, we screened a subset our survey samples using multi-locus sequence typing (MLST) to determine the *Xf* strain. We sequenced 7 *Xf* genes and compared the sequences against a Xf sequence database to determine the strain [9]. Our results showed that all *X. fastidiosa* subspecies *fastidiosa* (the causal agent of PD) identified are identical to the original CA strain that caused the PD outbreak in Temecula, CA in 1986 [10]. Although different strains of *X.f.* subsp. *fastidiosa* have been identified in TX and FL, it is not surprising to see CA strain in VA because many vineyards purchase their vines from CA nurseries.

However, our study also revealed the association of a different subspecies, *X. fastidiosa* subspecies *multiplex*, associated with VA grapevines [10]. As the name multiplex implies, this subspecies is known to cause disease on a wide variety of hosts; however, this is the first report of this subspecies from *Vitis vinifera*. The current understanding of *Xf* biology suggests that genetic variability among these strains are, in fact, the determining factor of the host range (i.e., a strain causing disease on host species A is less likely to cause disease on host species B). The multiplex strain that we identified is similar to the strain that causes disease on blueberry. Therefore, our multiplex strain appears to be unique. We believe that this discovery warrants more studies. The first obvious question is their ability to cause PD on grape. Based on our observational data, all

vines with the multiplex isolate showed some type of symptoms. Also, it becomes critical to understand how/when this subspecies suddenly gained the ability to infect *V. vinifera*. Another question would be whether the bacterium or the insect vector caused the change of its host range.

In order to start answering these questions, we would like to test more from of samples to identify the proportion of *X.f.* subsp. *multiplex*. So far we found 6 *X. fastidiosa* subspecies *multiplex* samples among the subset of 24 samples that we sequenced, and we found that *X. fastidiosa* subspecies *multiplex* were present in 6 different wine regions of VA. We would like to analyze 25 more samples from 2013 survey, and 25 more from 2015 insect vector samples, which we are currently testing for *Xf*. We will use 9 different genes for the MLST for the most accurate identification of subspecies [11].

Technology transfer plan:

Results (identification of peak time of sharpshooter and *Xf* subspecies associated with them) will be used to develop new recommendations for PD management that are tailored to VA wine grape growers. The results will be presented at grower-oriented meetings (VVA meetings, vineyard meetings, etc), scientific conferences (APS conferences), and published in a peer-reviewed journal, such as Plant Disease. We are ready to submit our manuscript in September 2016.

OUTCOMES AND BENEFITS

Our proposed studies provided guidances to understand important questions relating to the ecosystem of PD in the eastern US. The preliminary results from 2014 have demonstrated that the qPCR method sensitively detects *Xf* associated with insect tissue. We have found both broadheaded and versute sharpshooters in both 2014 and 2015. The key for the management seems the early season management of broadheaded sharpshooters, probably in May.

This was a joint project between Department of Plant Pathology, Physiology, and Weed Science (Nita), VT Plant Disease Clinic (Hansen and Bush), and Department of Entomology (Pfeiffer and Day). We also envision using the results to develop a program in the high value crops emphasis area as a component of the USDA-NIFA Regional IPM program in the future.

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