Addressing knowledge gaps in Pierce's disease epidemiology: Underappreciated vectors, genotypes, and patterns of spread

Principal Investigator
Rodrigo P.P. Almeida, Professor
Dept. Environmental Science, Policy and Management
University of California, Berkeley
Berkeley, CA 94720
Phone: (510) 642-1603, e-mail: rodrigoalmeida@berkeley.edu

Co-Principal Investigators
Monica Cooper, Viticulture Farm Advisor
University of California, Cooperative Extension, Napa County
1710 Soscol Ave, Suite 4, Napa 94559
Phone: (707) 253-4221, e-mail: mlycooper@ucanr.edu

Matthew Daugherty, Associate Extension Specialist
Department of Entomology
University of California, Riverside
Phone: (951) 827-2246, e-mail: matt.daugherty@ucr.edu

Rhonda Smith, Viticulture Farm Advisor
University of California, Cooperative Extension, Sonoma County
133 Aviation Blvd., Ste 109, Santa Rosa, CA 95403
Phone: (707) 565-2621, e-mail: rhsmith@ucanr.edu

Reporting Period
The results reported here are from work conducted February 2019 to July 2019.

Abstract
In this report we focus on work done on spittlebug biology (objective 1), and *X. fastidiosa* infection dynamics of commercially grown grapevines (objective 3). Results are preliminary.

Layperson Summary
Recent research by our group is aimed at understanding why PD has recently reached historically high levels of prevalence in the North Coast. It is evident that traditional spatial patterns of PD distribution in vineyards continue to occur. However, there are also disease distribution patterns that do not follow expectations. Furthermore, data suggest that there are key components of PD epidemiology that may have changed over time, leading to the large losses due to PD in recent years. The goal of this project is to target three specific topics we have identified as the most urgent current knowledge gaps in PD epidemiology.

List of Objectives
This research project has three objectives, which were identified as pressing issues that need to be addressed to improve our understanding of PD epidemiology.

Objective 1. Role of spittlebugs in Pierce's disease epidemiology

Objective 2. Mathematical modeling of Pierce's disease spread

Objective 3. Xylella fastidiosa population genomics

Description of Activities

Objective 1. Insect vector phenology

Aphrophora sp.spittlebug nymphs were followed for biological development on up to 30 flagged plants at 4 sites in each Napa and Sonoma counties. (Note: Some flagged plants were lost and changed due to vineyard pests, weather, and cultural practices). Twelve or more nymphs were collected every 2 weeks when possible from non-flagged plants at these sites for head capsule measurements to determine instar stages. The nymphs hatched in late January 2019, and the adults emerged by mid-May 2019. First through fourth instar nymphs were red in body with a black head. Upon molting to fifth instars, the nymphs became entirely brown in color. Bristly oxtongue was the predominant host plant species in Napa County where *Aphrophora* sp. nymphs were found, and poison-hemlock was the predominant host plant species in Sonoma County.

Table 1. Percent *Aphrophora* sp. nymphs at each instar stage by date from 8 vineyards in Napa and Sonoma counties.

		Percent *Aphrophora* sp. nymphs at each instar stage:				
County	Date 2019	First	Second	Third	Fourth	Fifth
Napa	14-Feb	100				
	21-Feb	56	44			
	5-Mar	15	85			
	8-Mar	13	88			
	15-Mar		50	50		
	23-Mar		16	81	3	
	1-Apr		13	52	35	
	11-Apr				60	40
	17-Apr				35	65
	18-Apr				6	94
	2-May				3	97
Sonoma	31-Jan	94	11			
	6-Feb	100				
	7-Feb	90	19			
	18-Feb	100				
	21-Feb	3	86	11		
	22-Feb	86	14			
	23-Feb	58	42			
	8-Mar	41	49	13		
	11-Mar	36	59	5		
	21-Mar	39	57	37	50	
	29-Mar	6	49	34	43	19
	9-Apr		5	43	45	33
	18-Apr				10	90
	19-Apr			5	59	39
	1-May				6	95
	2-May				7	93
	9-May					100
	10-May					100

Table 2. Minimum and maximum measurements (mm) of *Aphrophora* sp. nymphs at each instar stage from 8 vineyards in Napa and Sonoma counties.

		Aphrophora sp. instar stage				
County	Measurement (mm)	First	Second	Third	Fourth	Fifth
Napa	Minimum	0.50	0.75	1.08	1.75	2.47
	Maximum	0.70	0.98	1.38	2.17	3.13
Sonoma	Minimum	0.57	0.75	1.05	1.65	2.40
	Maximum	0.66	0.93	1.32	1.95	2.85
Both	Range	<0.75	<1	<1.5	<2	<3.15

Objective 3. Evaluation of infection development in commercial vines

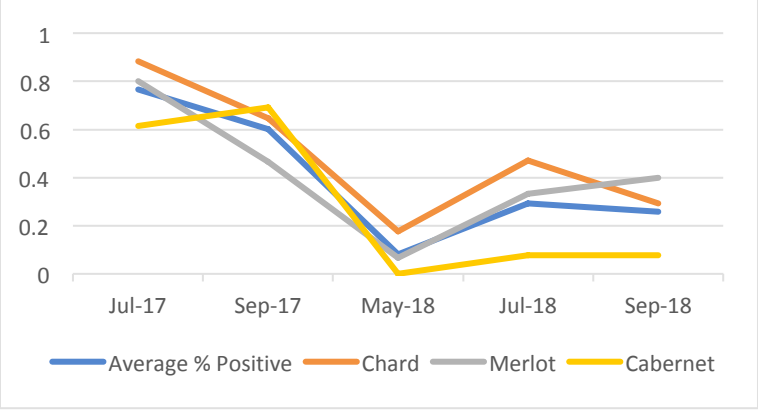
A strain of *X. fastidiosa* subsp. *fastidiosa* was isolated from grapevines in Napa, grown in our lab, and sequenced via both Illumina and Pacbio so as to create a complete genome. In April 2017, 45 *Vitis vinifera* were inoculated in Napa Valley with this strain: 15 Merlot, 17 Chardonnay, and 13 Cabernet Sauvignon, as well as buffer-inoculated negative controls inoculated. Since then, *X. fastidiosa* populations at four or eight locations of each inoculated plant (depending on vine training) have been quantified three times per growing season using quantitative PCR(qPCR). Each year, *X. fastidiosa* from positive plants were also cultured for genome re-sequencing. Throughout the growth season, symptoms of Pierce's disease (PD) have also been quantified biweekly. The symptoms that we focused on as most typical of PD are shriveled clusters, leaf Scorch, uneven lignification, matchsticking petioles, stunted shoots, and leaf chlorosis.

Preliminary Results

Symptoms: In 2017, symptoms correlated with qPCR positives well for all three varietals. Only four inoculated plants that tested positive for *X. fastidiosa* did not display any symptoms in that first year. Those observed symptoms were predominantly shriveled clusters, and leaf scorch. In 2018, the most severe symptom observed in the field was stunting of shoots early season for all three varietals, with some leaf scorch later in the season as the plants begin to experience more water stress.

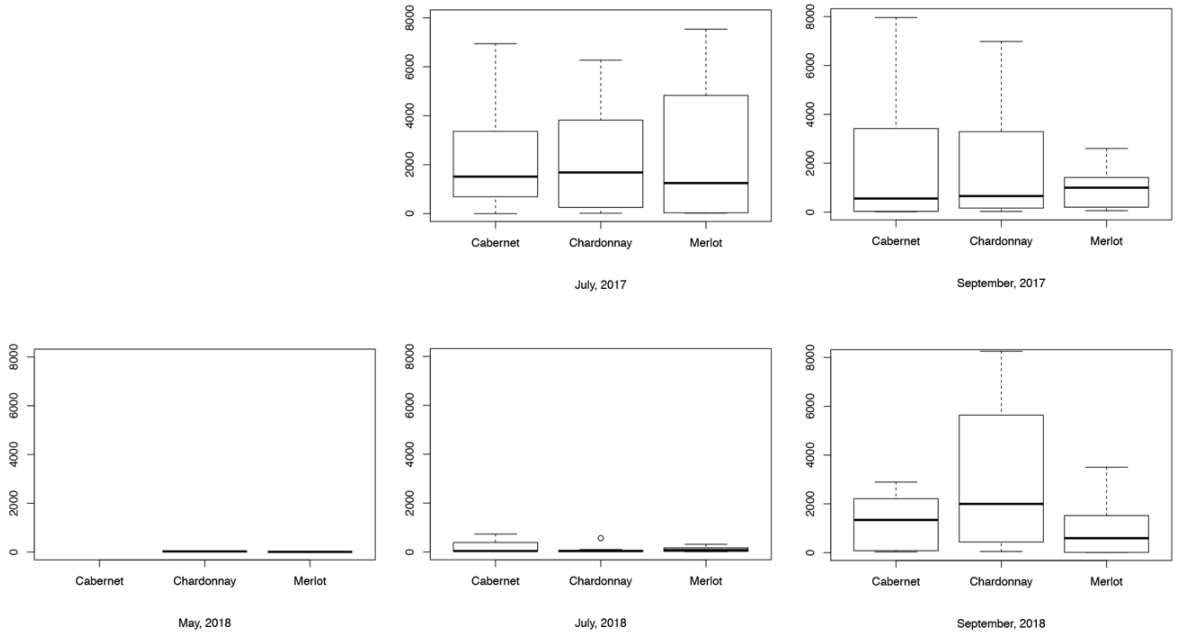
qPCR:

While initial inoculation success was high (88% for Chardonnay, 80% for Merlot, and 62% for Cabernet Sauvignon), many of the plants recovered from infection after the first winter. During subsequent seasons, there was also trimming done at the vineyards on some of the more severely symptomatic plants (by vineyard managers), which influenced our ability to properly monitor both symptom progression and population data.



Fraction of inoculated grapevines that tested positive for *X. fastidiosa* via qPCR from July 2017 through September 2018.

Population sizes in grapevines that tested positive for *X. fastidiosa* via qPCR from July 2017 through September 2018. Measured in Colony forming units per gram of grape petiole.



In terms of both percentage of infected plants and population (CFU/gram) within plants that tested positive for *X. fastidiosa* at each time point, the two years exhibit different patterns. In 2017, post inoculation, the percentage of infected plants as well as the average populations slightly decrease throughout the season. While in 2018, there is an increase from May to July in the number of plants that test positive, which stays consistent in September. However, *X. fastidiosa* populations in plants increase by one order of magnitude between the July and September samplings. We have no data available for 2019.

Sequencing: Petioles from three time points have been used to culture *X. fastidiosa*. Those cultured cells have had their DNA extracted and frozen, and will all be Illumina sequenced and assembled after this final year of the project (2019). At that point, we will be able to compare all genetic changes from the original inoculum to each sample.

Status of Funds
Funds are being used as originally proposed.

Summary of IP
Not applicable.

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