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

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Reporting Period
The results reported here are from work conducted October 2018 to January 2019.

Abstract
In this report we focus on initial work done on spittlebug biology (objective 1), disease spatial patterns (objective 2), and *X. fastidiosa* infection dynamics of mature grapevines grown under commercial conditions (objective 3). Results are preliminary and such be treated as such.

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

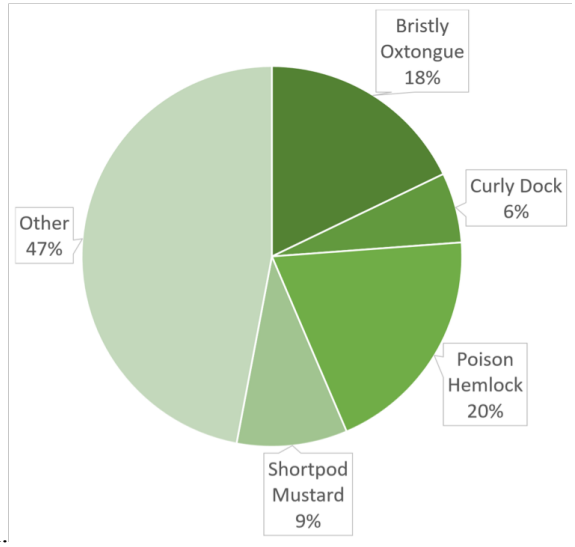
Objective 3. Xylella fastidiosa population genomics

Description of Activities

Objective 1. Insect vector phenology

To elucidate the life cycle of *Aphrophora* spittlebugs, we have focused on identifying the oviposition and nymphal development hosts during late winter and early spring. We identified four field sites in each county (Napa and Sonoma) and soon after egg hatch, marked up to 40 host plants at each monitoring site. Host plants were identified by searching for the presence of spittle masses (created by nymphs as they are feeding). On a weekly basis, we are characterizing the number of nymphs per host plant and the life stage of each nymph. This will allow us to describe not only which plants are potential hosts, but also give us a measure of host preference by species (Fig 1.1-1).

A.



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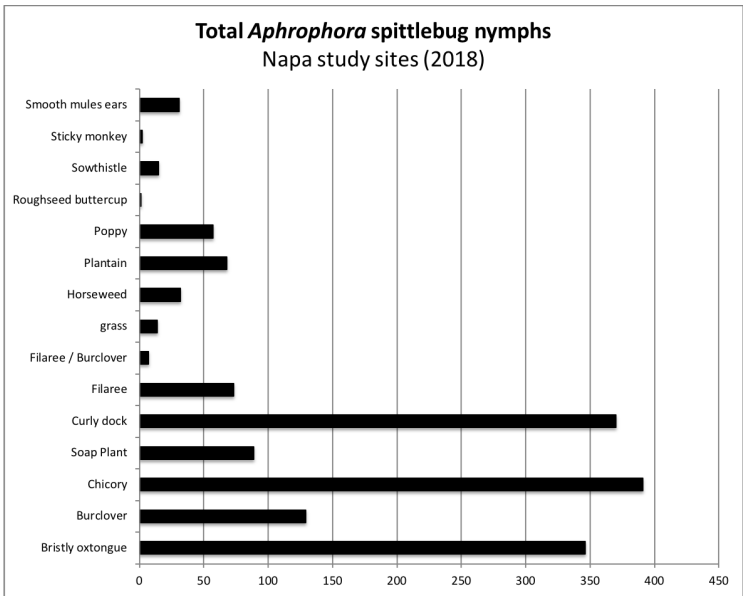


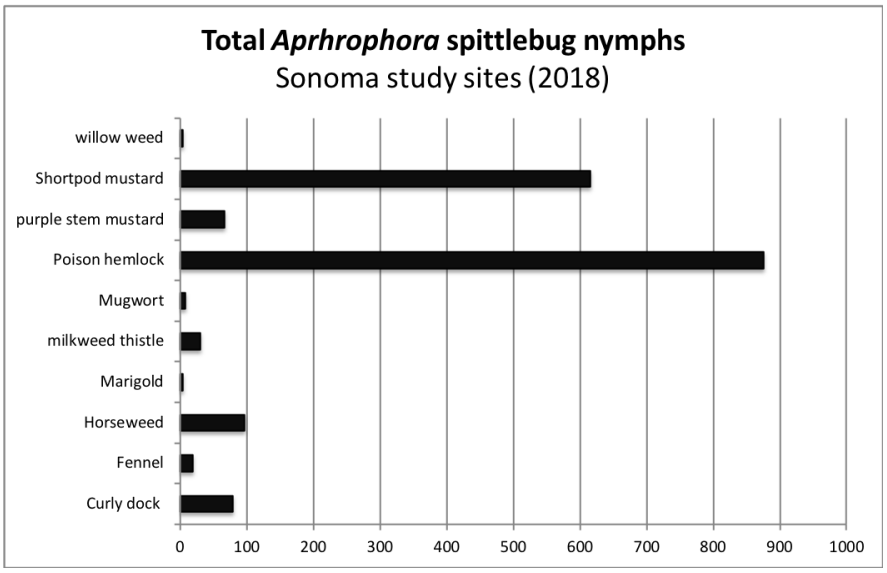
C. 

Fig. 1.1-1. Summary of insect vector phenology monitoring for *Aphrophora* spittlebugs. (A) most common host plants across all 8 monitoring sites in Napa and Sonoma counties; *Aphrophora* spittlebug nymph population levels on host plants at Napa (B) and Sonoma (C) study sites.

Objective 2. Statistical modeling of disease incidence.

We are leveraging a dataset collected by the project team at 32 vineyards in Napa and Sonoma counties between 2016 and 2018. This dataset includes regular sticky-trap monitoring for BGSS, sweep-net monitoring for BGSS and other vectors in the spring, and PD mapping each fall. The data show substantial seasonal and spatial variability in BGSS abundance at each site, along with the abundance of other sharpshooters, spittlebugs, and other potential vectors (Almeida 2016, 2017). We conducted a hotspot analysis using ArcGIS software; this analysis uses the Getis-Ord Gi* statistic and identifies statistically significant hot and cold spots, given a set of weighted features. Study blocks fell into one of two groups, based on this analysis. One group of blocks showed an expected disease incidence and BGSS relationship (Fig. 2.1-1). These blocks have hotspots of disease incidence at the edges of the block, where BGSS detections also tended to be higher. Another group of blocks had more unexpected hot and cold spot patterns, where hotspots did not appear to be associated with higher counts of BGSS in monitoring traps (Fig 2.1-2). Subsequent analyses will explore potential relationships between populations of other vectors and incidence of disease (hot and cold spots).

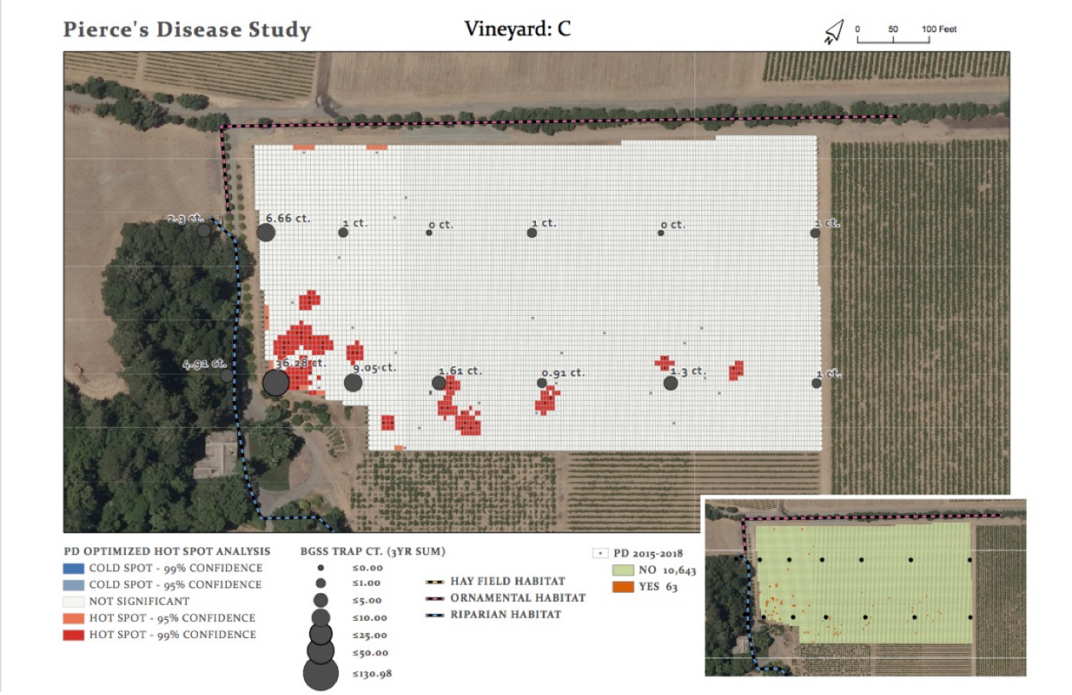


Fig. 2.1-1. Study site in Napa County, demonstrating an expected pattern where disease hotspots occur at the edge of the vineyard, where blue-green sharpshooter (BGSS) trap counts are higher.

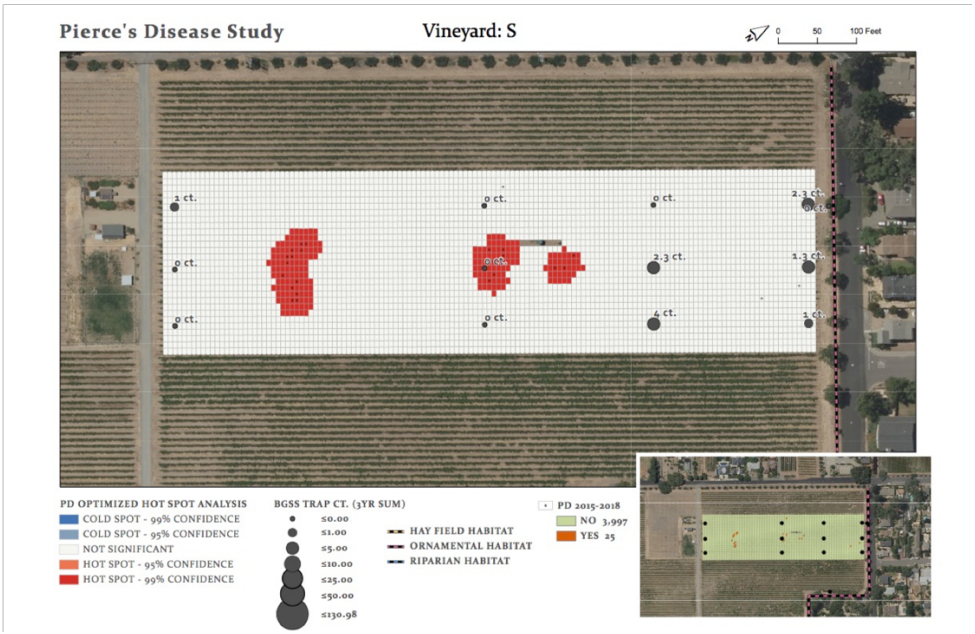


Fig. 2.1-2. Study site in Napa County, demonstrating an unexpected pattern where disease hotspots do not appear to be associated with higher blue-green sharpshooter (BGSS) trap counts, suggesting the possible impotance of another *X. fastidiosa* vector.

Objective 3. Evaluation of infection development in commercial vines

The goal of this objective is to focus on the effect of the unique genomic sequences located in Napa and Sonoma counties on symptom development, pathogen movement and multiplication within the vine and symptom expression over time. Within-vine pathogen colonization patterns may indicate that the different sequences of the genome are biologically distinct and thus affect PD epidemiology. In 2017, three sites, each planted with a unique wine grape cultivar (*Vitis vinifera* cv. Cabernet Sauvignon, Chardonnay and Merlot), were inoculated at 2 vineyard locations (Oak Knoll and Calistoga) in Napa County with a local isolate (Fig 3.2-1). Vines were inoculated on April 14 (Chardonnay and Merlot, Oak Knoll) and April 28 (Cabernet Sauvignon, Calistoga) and symptoms were monitored at bi-weekly intervals from the inoculation date to leaf fall in 2017, and from bud break to leaf fall in 2018. Petioles were collected in May and September 2017 and 2018, from inoculated and control vines. Samples were analyzed to quantify *X. fastidiosa* and its distribution within the vines. In both years, the first noticeable symptom to develop was cluster shrivel (Fig 3.2-2), followed by limited leaf scorch. Uneven lignification was a less consistent symptom, as were matchstick petioles. Stunted growth and delayed bud burst in spring 2018 was common on many vines that were symptomatic and infected (qPCR positive) in 2017.

Figure 3.2-1. (A) Mechanical inoculation (April 14, 2018) of a Merlot vine and (B) subsequent development of shriveled clusters (July 21, 2018) in inoculated Merlot vines in Oak Knoll, Napa, CA; and (C) shriveled clusters in a previously inoculated vine of Cabernet Sauvignon with (D) notably absent canopy symptoms of PD of the vine shown in (C) in Calistoga, Napa CA.

Symptom	Chardonnay	Merlot	Cabernet Sauvignon
Inoculated 2017	Apr 14 @ 8-10 Leaves	Apr 14 @ 6 Leaves	Apr 28 @ 4 Leaves
Cluster Shrivel	Jul 21, 2017 Aug 15, 2018	Jul 21, 2017 Jul 31, 2018	Jul 21, 2017 Jul 31, 2018
Leaf Scorch	Jul 21, 2017 Aug 15, 2018	Jul 21, 2017 Aug 15, 2018	Aug 28, 2017 Aug 28, 2018
Uneven Lignification	Jul 21, 2017 Aug 28, 2018	Jul 21, 2017 None 2018	Aug 28, 2017 Sep 11, 2018
Matchstick Petiole	Aug 28, 2017 Aug 28, 2018	Aug 14, 2017 Sep 11, 2018	None 2017 Sep 25, 2018
Xf(+) by qPCR (Jul)	2017: 15/15 2018: 8/15	2017: 14/15 2018: 5/15	2017: 9/10 2018: 1/10
Stunted Shoots	Apr 10, 2018	Apr 10, 2018	Apr 25, 2018
Leaf Chlorosis	May 9, 2018	May 9, 2018	None 2018

Fig 3.2-2. Description of PD symptoms and timing of their development in mature, commercial vines inoculated with *Xylella fastidiosa* in April 2017 in Napa County.

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.