

UC Berkeley

UC Berkeley Previously Published Works

Title

Progression of Xylella fastidiosa Infection in Grapevines Under Field Conditions.

Permalink

<https://escholarship.org/uc/item/4ms2w7ww>

Journal

Phytopathology, 113(8)

ISSN

0031-949X

Authors

Kahn, Alexandra K

Sicard, Anne

Cooper, Monica L

et al.

Publication Date

2023-08-01

DOI

10.1094/phyto-01-23-0008-r

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial License, available at <https://creativecommons.org/licenses/by-nc/4.0/>

Peer reviewed

1 Progression of *Xylella fastidiosa* infection in grapevines under field conditions

2 Alexandra K. Kahn¹, Anne Sicard^{1*}, Monica L. Cooper², Matt Daugherty³, Monica A. Donegan ¹,
3 and Rodrigo P.P. Almeida¹

4
5 ¹ Department of Environmental Science, Policy, and Management, University of California
6 Berkeley, Berkeley, CA 94720

7 * INRAE, Université de Strasbourg, SVQV UMR-1131, Colmar, France

8 ² Division of Agriculture and Natural Resources, University of California, Cooperative Extension,
9 Napa, CA 94559

10 ³ Department of Entomology, Univ. of California, Riverside, 900 University Ave., CA 92501

11 Corresponding author: rodrigoalmeida@berkeley.edu

12

13

14

Abstract

The pathogen *Xylella fastidiosa* subspecies *fastidiosa* has circulated through California's vineyards since its introduction from Central America in the 1800s. This pathogen is responsible for a bacterial disease called Pierce's disease of grapevine (PD). With no known cure, PD has had devastating effects on some vineyards. Important factors that impact disease severity and persistence include: the presence of insect vectors, grapevine cultivar, management, ecology, and winter temperatures. Removal of infected vines is critical for reducing pathogen spread but relies on accurate and rapid pathogen detection. In this study, we foster a greater understanding of disease symptom emergence by way of a three-year field inoculation project in Napa Valley. Although PD emergence and symptom progression have been studied in greenhouse and experimental plots, there is a large knowledge gap in quantifying disease progression under commercial conditions. After inoculating 80 mature *Vitis vinifera* vines in April 2017, bacterial populations and six symptom types were measured at four locations within each plant, throughout the subsequent 3 growing seasons. The main foci of the project were understanding *X. fastidiosa* movement through the plants, infection, overwinter curing, and symptom development. We observed greater winter recovery than expected, and shriveled grape clusters proved to be a more reliable early indication of infection than other more commonly used symptoms. Although there were differences among wine grape cultivars, this work suggests that disease progression in the field may not fit the paradigm of predominant leaf scorch and low recovery rates as neatly as has been previously believed.

36 **Introduction**

37 The results of in-situ experiments can differ greatly from those conducted in controlled
38 laboratory conditions, especially in applied biological systems. In pathology specifically, there
39 are often ethical and regulatory hurdles that prevent the introduction of disease-causing
40 pathogens into naïve systems, whether it is for human health, food security, or in natural
41 systems. Researchers are therefore typically constrained to using controlled or model systems
42 that are ethically, financially, and logistically feasible. For example, mice are often used to
43 understand human disease, and quarantine greenhouses are used to study plant disease
44 (Perlman, 2016). These proxy systems are imperfect, as we intuitively know that there are
45 differences between a mouse and a human, so there are differences between the potted plant
46 on your windowsill and the towering redwoods of California. These differences are intuitive, but
47 also biological as the effects of interactions between organisms and their complex
48 environments are impossible to recreate, and these environmental and physiological
49 differences directly affect the outcomes of research in plant pathology (Velásquez *et al.*, 2018).
50 The chances to experimentally infect plants in accurate conditions are few and far between, so
51 rare opportunities provide critical research windows.

52 One such system that suffers from a lack of realistic experimental control is Pierce’s
53 disease of grapevines (PD), a persistent burden to the vineyards of California since the
54 introduction of the etiological agent (*Xylella fastidiosa* subsp. *fastidiosa*) into North America in
55 the late 1800s (Nunney *et al.*, 2010; Vanhove *et al.*, 2019). The pathogenic bacterial strains that
56 colonized *Vitis vinifera* and cause PD have since spread to Taiwan, Europe, and the Eastern
57 United States (Landa *et al.*, 2020; Castillo *et al.*, 2021). Although the relationship between

Xylella fastidiosa and PD has been understood since 1978 (Davis *et al.*, 1978), this study is the first to document the progression of symptoms in mature commercial grapevines under field conditions over the course of several years with a known inoculation time and location on the plant. Despite the challenges of conducting this work in-situ, predominantly concern from the proprietors about infection spreading, we have made insights that counter much of the classic understanding of this disease system and deepen our understanding of symptom development and bacterial multiplication and movement in PD.

Several studies have documented PD, which collectively create expectations for the progression of this disease. The pathogen is known to move through mature grapevines at least fast enough to enter the cordon from the shoot within the first year of infection (Feil *et al.*, 2003). However, given that removal of the cordon does not reduce disease severity in subsequent years (Daugherty *et al.*, 2018), the pathogen must be moving further into the plant, presumably into the trunk during the first year. It is also well established that there are differences in response to *X. fastidiosa* infection based on *Vitis vinifera* cultivar, with some cultivars more susceptible than others (Raju & Goheen, 1981; Krivanek *et al.*, 2005; Lieth *et al.*, 2011). Symptoms are presumed to first appear late in the season that infection occurred, except in especially susceptible cultivars, such as Chardonnay, in which they can arise sooner. Characteristic symptoms have been described as leaf scorch, uneven lignification of the shoots, shriveled berries, and “matchstick” petioles, a situation in which the leaf blade becomes detached from the petiole. In this system, disease symptoms and pathogen infection do not always persist through the winter, a phenomenon called overwinter curing. However, the mechanism for recovery has not yet been determined, and current explanations range from

pathogen temperature susceptibility to plant defensive responses. Expectations for overwinter curing are both temperature and cultivar dependent, and in Napa Valley, the expected recovery rate for early-season inoculations is around 30% (Feil *et al.*, 2003; Burbank, 2018).

Progression of PD symptoms has been monitored under controlled conditions (Stevenson *et al.*, 2004; Krivanek *et al.*, 2005). However, such data may not translate to plants grown in the vineyard for various biotic and abiotic reasons. Greenhouse conditions do not account for the complex nutritional, pest, and other biotic (such as the microbiome) (Deyett & Rolshausen, 2019) and abiotic pressures of the field (Poorter *et al.*, 2016), and greenhouse assays of grapevines are typically conducted with an excised shoot, rather than a mature plant. Observational studies are similarly limited because they often miss aspects such as recovery, asymptomatic infections, or unexpected symptoms by not relying on controls, but instead seeking out expected symptoms. These studies have given us a view on characteristics of PD and its progression that we were able to experimentally test. Based on the available observational and greenhouse data, we set out to test the following predictions:

1. Pathogen movement and multiplication post inoculation: By early fall of the inoculation year, symptoms were expected throughout the infected shoot, as well as one or two adjacent shoots, indicating likely spread of the pathogen to those areas (Feil *et al.*, 2003).
2. Symptom expression over time: Leaf scorch as early as 3 months post inoculation has been reported to be the first observable symptom (Krivanek *et al.*, 2005). Based on this we expected leaf scorch beginning mid-late July 2017 as the earliest detectable and most prevalent symptom in year 1.

3. Overwinter curing rates: The paradigm assumes variation in overwinter curing rates based on grapevine cultivar and winter temperatures, and necessitates early-season inoculation to avoid curing (Feil *et al.*, 2003; Burbank, 2018). According to these papers, in Napa County we should expect approximately a 30% recovery rate for Chardonnay, with variability between cultivars shown through cold-treatment greenhouse experiments.
4. Cultivar differences: *V. vinifera* cv. Cabernet Sauvignon is described as less susceptible than Chardonnay and Merlot, which are predicted to have lower recovery rates associated with their susceptibility (Purcell, 1981; Feil & Purcell, 2001; Burbank, 2018).

For a system that has been studied for so long, knowledge gaps persist regarding how the pathogen and host interact to cause disease. This study adds some clarity to gaps surrounding early symptom development and overwinter curing rates. Using a strain of *X. fastidiosa* isolated from Napa County, we tested the effect of this pathogen on symptom development, described symptom expression over time, and followed pathogen movement and multiplication within the vine.

Materials and Methods

Study sites and Vitis vinifera

Three commercial vineyard blocks were selected as trial sites at two locations in Napa Valley. Plots were set up with permission from vineyard managers; however, the identity and specific locations of sites must be kept confidential. Each block was planted with one of three wine grape cultivars, *Vitis vinifera* cv. Cabernet Sauvignon, Chardonnay or Merlot. The Merlot and Chardonnay blocks were in the Oak Knoll District and the Cabernet Sauvignon block in Calistoga. Given the arrangement of three cultivars at two sites, cultivar and site effects are confounded in this design. Due to the close location of the two sites, these effects are analyzed as cultivar effects with the assumption that the site effects are not large. The Chardonnay and Merlot blocks were planted in 2008, on 101-14 rootstock, and trained to a bilateral system. The Cabernet Sauvignon block was planted in 2006, also on 101-14 rootstock and trained to a quadrilateral system. All blocks were cane-pruned (See Table 1 for summary of study sites).

Inoculation of pathogen X. fastidiosa

Each site included equal numbers of treatment vines (inoculated with sterile succinate-citrate-phosphate buffer (SCP) (Hopkins, 1984) & *X. fastidiosa* (Napa isolate strain “Napa1”) and control vines (mock inoculated with SCP only) (See Table 1). Napa1 was isolated in 2015 in Napa Valley, and was sequenced and published as BioSample SAMN09941406 (Vanhove *et al.*, 2020).

Vines were inoculated on April 14, 2017, (Chardonnay and Merlot) and April 28, 2017 (Cabernet Sauvignon). Cells were grown on PWG solid medium (Hill and Purcell 1995) for one week and suspended in succinate-citrate-phosphate buffer (SCP) in the field just prior to inoculation. The inoculations were at the terminal shoot of the west cane, where 10 µl droplets

were needle inoculated into 2 inoculation points, basal and middle of the shoot, by pin pricking through the bead of cell suspension until absorbed into the plant xylem. Timing of infection was selected to match natural spring infections by the local vector *Graphocephala atropunctata* (Hemiptera, Cicadellidae) (Purcell, 1975; Feil *et al.*, 2003).

Monitoring and sampling

Following inoculation, the vines were monitored at 14-day intervals for the remainder of the 2017 growing season. Subsequently, in the 2018 and 2019 growing seasons, the vines were monitored from bud break through leaf fall. On each monitoring date, the vines were visually assessed for common symptoms of PD: stunted growth, chlorotic leaves, shriveled berries, leaf scorch, incomplete lignification, and matchstick petioles. Each symptom was recorded as present or absent on each of 5 areas of the vine: (1) west and (2) east, corresponding to the terminal ends of each cane; (3) mid-west and (4) mid-east, corresponding to the middle of each cane; and (5) center corresponding to the head of the vine, where the canes originate. Shriveled berries could not be evaluated after the harvest date for each block. For Cabernet Sauvignon, the harvest date for 2017 was October 27th, 2017 and for 2018 – 2019 the harvest was after the completion of our data collection. For Merlot, the harvest dates were September 13th, 2017, September 25th, 2018, and October 8th, 2019. For Chardonnay, the harvest dates were September 13th, 2017, September 25th, 2018, and September 9, 2019.

Three times annually (May, July, and September), petioles were collected from all vines. The basal petiole from the terminal shoots (west and east) as well as from the shoots adjacent to the trunk (west-center and east-center) were collected at each time point (four samples per plant). An additional sampling point was also measured in only July 2017 for finer resolution on

the first year's movement: shoot 2 was measured, the next shoot over from the inoculation site. For the quadrilaterally trained vines, eight samples were taken per plant instead of four, with additional designation as originating from either the south or north side of the vine.

DNA Extraction and qPCR

Petiole samples were analyzed via DNA extraction and quantitative polymerase chain reaction (qPCR) to quantify *X. fastidiosa* population size (in colony forming units (CFU) per gram of petiole tissue) and population distribution across the vines. DNA was extracted from petioles using the DNeasy plant mini kit (Qiagen). All extractions were 5-fold diluted with RNase free water before being quantified by qPCR wherein they were run using a primer pair targeting the gene encoding *RecF*, *RecF1_F+R* (Sicard et al., 2020); for qPCR protocol see Sicard et al. 2020. Following analyses with LinRegPCR (Ramakers et al., 2003), bacterial populations were interpolated using a standard curve. To produce the standard curve, we grew Napa1 cells on PWG media in a series of 10x dilutions and plated D0-D8 while also keeping the same volumes for qPCR. CFU/g were counted per streak of 20 μ l and compared to the C_t values from qPCR to generate a slope to calculate CFU/g based on C_t values. qPCR was run in duplicate with positive and negative controls on each plate; any plates that had duplicates of more than 1 cycle apart were re-run. All samples with C_t values over 36 were considered "undetected".

Statistical analysis

All analyses were performed using R statistical software version 4.1.2 (R Core Team, 2021). We used generalized linear models (GLM) with binomial error to build several models. (1) First we tested grapevine cultivar as a factor predictor for inoculation success (measured as the count of positive plants from July, 2017, 1 for positive, 0 for negative). The only predictor

181 value used was cultivar which included three levels (Merlot, Chardonnay, and Cabernet
 182 Sauvignon). (2) We also tested the effect of pathogen presence (1/0) on symptom detection
 183 (1/0) with a GLMM for each symptom with a random effect of plant identity. We then
 184 computed significance using an analysis of deviance with a chi-squared test statistic. (3)
 185 Additionally, we built GLMMs with binomial error to test if the CFU/g in a dataset of only
 186 positive plants was predictive of each symptom's presence or absence again with a random
 187 effect of plant identity. Most symptoms were tested across all three seasons except for stunting
 188 and leaf chlorosis, early-season symptoms for which the 2017 year was not included due to
 189 spring inoculation precluding year 1 symptoms. For both sets of symptom models (models #2
 190 and #3), the response variable (presence / absence of symptoms) was clustered by symptom
 191 monitoring dates around the three annual qPCR detection dates. The May qPCRs were paired
 192 with symptoms from spring (from May – June 15th), July qPCRs were paired with summer
 193 symptoms (from June 16th – August 15th), and September qPCRs were paired with fall
 194 symptoms (from August 16th – October). If symptoms were not detected during these
 195 windows, then the response variable was 0. If symptoms were detected at any point, the
 196 response variable was 1. (4) Winter recovery was analyzed as a binomial GLM, testing the effect
 197 of cultivar on plant recovery between 2017 – 2018. The response, plant recovery, was a binary
 198 variable in which plants that had been positive during the 2017 year were marked as either
 199 recovered or still infected. (5) We then built a generalized linear mixed effect model (GLMM)
 200 with binomial error to test the effects of cultivar and year on binary infection status, with plant
 201 identity included as a random effect. (6) Finally, we built a set of annual vine position models
 202 (separate models for 2017, 2018, and 2019) to test the effect of sampling location and cultivar

203 on detection of the pathogen, with plant identity as a random effect. For all GLM models we
204 used the glm function in the stats package in R, and for all GLMM models we used the
205 glmmTMB package (v. 1.1.5)(Magnusson *et al.*, 2017). A survival analysis for symptom onset in
206 2017 (the year of inoculation) was conducted using the Surv function in the survival package in
207 R (v. 3.4.0) wherein symptom onset times were compared (Therneau, 2022).

208 ***Plotting***

209 All data were plotted in R using the package ggplot2 (Wickham, 2016) and color sets
210 were picked using the package RColorBrewer (Neuwirth, 2022).

211

212

Results

Inoculations were detectable by qPCR across the three years

The inoculation success ranged between 73-100% per cultivar (Table 2), measured via positive qPCR in either July or September, 2017. At the first sampling post inoculation, in July 2017, between 74-90% of total vines were positive for *X. fastidiosa* presence, showing a successful inoculation (see Figure 1). That percentage dropped by autumn of year 1, then increased again in July and September of year 2. By the second year, most Chardonnay plants had been removed by the vineyard (rogued), so our proportion increased drastically for that cultivar, while Merlot and Cabernet Sauvignon were not as frequently infected as in either previous year due to most of the positive plants recovering from the *X. fastidiosa* infection. The effect of grape cultivar on the inoculation success in year 1 was not significant when analyzed by a GLM with binomial error ($\text{Chi}^2 = 3.5808$, $p = 0.1669$, $\text{df} = 2$). Using the 3-year dataset, we also tested the effect of cultivar and year, with plant identity as a random effect. Cultivar was overall not significant in the effect on infection status throughout the experiment ($\text{Chi}^2 = 4.624$, $p = 0.0991$, $\text{df} = 2$); however, year was significant ($\text{Chi}^2 = 65.317$, $p = 6.555\text{e-}15$, $\text{df} = 2$). One mock inoculated plant tested positive by qPCR in Fall, 2019 (see supplemental Figure 1).

Symptoms were detectable early, and in an unexpected order

In the Merlot and Chardonnay blocks, symptoms were first noted on July 21, 2017 (3 months post inoculation), and included uneven lignification, leaf scorch and shriveled berries (see Figures 2 and 3). Of these, the shriveled berries (Figure 4E) were the most consistent symptom across vines and within single vines. In the Cabernet Sauvignon block, the first

symptoms were also noted on July 21, 2017. The only symptom noted on that date, and the following evaluation date, was shriveled berries. Leaf scorch and uneven lignification were not noted until August 28, 2017, for the Cabernet block. Stunted growth was noted on the first evaluation dates of 2018 and 2019 (Figure 4A), coinciding with bud break and early shoot growth on Apr 10, 2018, and Apr 23, 2019 (Merlot and Chardonnay) and Apr 25, 2018, and May 6, 2019 (Cabernet Sauvignon).

In 2017, shriveled berries were the most common symptom; however, all other symptoms were also observable in the first year. The only symptom that was detectable in the control inoculated vines was stunting, which, in 2018 and 2019 was still much less common in the SCP-controls compared to the pathogen-inoculated plants (see Figure 2, and Supplemental 2). These symptoms were analyzed with survival curves based on their times of emergence in 2017 and the cox proportional hazards models did not converge to compare symptoms to each other. A comparison of symptom emergence in controls and inoculated plants can be seen in the supplemental materials (See supplemental Figure 3).

Differences in symptom progression among cultivars were substantial

The three cultivars showed different trends of symptom development over the three years of the study. Overall, Chardonnay exhibited the highest counts of symptoms including leaf chlorosis, leaf scorch, matchstick petioles, stunting, and shriveled berries. In Cabernet Sauvignon year 1, the prominent symptoms were shriveled berries and uneven lignification in the fall. In years 2-3, the few continuously infected plants exhibited all measured symptoms starting in July or August through the end of the growing season (see Figure 3). The controls

and experimental vines exhibited stunting in Cabernet Sauvignon in May and June (see Figure 2). In Chardonnay, year 1 began with shriveled berries in July that peaked in September (the last observation time before berry harvest) and exhibited subsequent leaf scorch and matchstick petioles in the fall. The following years showed high stunting, scorch, and chlorosis, all beginning in May when symptoms were first measured. There was uneven lignification detected in the summer and fall. Matchstick petioles increased throughout the fall in both years. For Merlot, July of year 1 showed shriveled berries and leaf scorch/chlorosis, followed by fall matchstick petioles. The following years showed stunting beginning in May similar to Chardonnay along with some leaf scorch, matchstick petioles, uneven lignification, and shriveled berries in the fall (See Figure 3).

With the data from three cultivars combined, annual trends emerged. Shriveled berries were the most informative symptom during the late season of the inoculation year. Although less abundant than shriveled berries, other symptoms in the first year included leaf scorch, matchstick petioles, uneven lignification, and stunting. In the second year, there was widespread stunting immediately observable after bud break, as well as leaf scorch which increased in August and September. Although many plants in this dataset had recovered by the third year, during 2019 there was predominantly early-season stunting along with some leaf chlorosis, followed by late season leaf scorch and matchstick petioles.

Number of positive plants drops in all three cultivars due to recovery and roguing

Although between 73-100% of plants per cultivar tested positive for *X. fastidiosa* infections during the first year (See Table 2), the number of positive plants dropped in all three

cultivars throughout the experiment. For Chardonnay, upon symptom development in year 1, 10 of the 15 experimental plants were rogued by the caretakers of the vineyard (see Figure 1). However, in Merlot and Cabernet Sauvignon, there was substantial recovery during the first winter, and between both seasons, respectively (see Figure 1). For Cabernet Sauvignon, the recovery is immediate, with all but one plant recovering during the first winter, and that one plant remaining both positive and highly symptomatic throughout the course of the experiment. In Merlot, there is a more gradual decline, with some plants recovering each winter. The differences between cultivars in recovery in the winter of 2017-2018 were not significant according to a binomial GLM ($\chi^2 = 3.5297$, $p = 0.1712$, $df = 2$).

Bacterial populations of positive samples remained relatively consistent

Bacterial populations for each positive sample were calculated; however, most positive samples were within several orders of magnitude in terms of their CFU/g tissue (see Figure 5). Bacterial populations ranged from 4.1×10^4 ($C_t = 35.8$) to 4×10^6 ($C_t = 19.6$). The mean CFU/g for all positive samples across the three years were respectively 2.3×10^5 , 1.4×10^5 , and 4.9×10^5 . While the number of total plants testing positive decreased over the course of the three years (see Figure 1), the population sizes remained fairly stable (see Figure 5). The only year where the expected seasonal change in population sizes was observed was in 2018, where no samples test positive in May, then population sizes for all three cultivars in July average 10^4 CFU/g, followed by the September sampling in which the average population sizes for the three cultivars increase to averages in the 10^5 CFU/g range.

Movement was faster than previously documented, and inoculated site only relevant in year 1

The inoculations were intentionally conducted on the terminal western shoot in order to standardize the inoculation point for tracking of visual symptoms and population sizes by vine position. During the first year, both symptoms and qPCR-positive locations were concentrated near the inoculation point in the west, mid-west, and west-center sampling positions. In year 1, leaf scorch, matchstick petioles, uneven lignification, and shriveled berries were predominantly detected on the western part of the vine. By year 2, vine position was no longer relevant, and both symptom development and bacterial populations appeared evenly throughout the plant (see Figures 6, 7 and supplemental Figure 4). Vine location was significant in 2017, with vine locations other than the western shoot having negative coefficients. This indicates that there was the greatest detection on the western (inoculated) shoot ($\text{Chi}^2 = 13.971$, $p = 0.00184$, $df = 3$). In 2018, vine location was also significant; however, the highest coefficient was for the mid-west sampling location ($\text{Chi}^2 = 11.793$, $p = 0.00813$, $df = 3$). In 2019, the effect of vine position was no longer significant ($\text{Chi}^2 = 0.722$, $p = 0.868$, $df = 3$).

Pathogen positivity was predictive of symptom presence, but pathogen population size was not

Several GLMMs with binomial error were constructed to test if the presence or absence of each symptom was dependent on the CFU/g value in the plant at that point. This model only included positive plants ($\text{CFU/g} > 0$) to examine if increasing bacterial populations resulted in increased symptom prevalence. These relationships were not significant for any symptom. Next, the presence of symptoms over the course of the year was evaluated as a response to the

323 plant being infected. Those models were set up with the symptoms as the response variable
324 and with one predictor variable: infection status. Infection status is a significant factor
325 influencing all symptoms aside from uneven lignification (See Table 3).

Discussion

This study emphasizes the necessity of in-situ experimentation with grapevine pathogens. The established paradigm for PD of low recovery and leaf scorch in Napa Valley was challenged with this study. Our results indicate shriveled grape berries as the predominant early symptom, rapid spread of the bacterial pathogen through the grapevine, and high overwinter curing.

Our findings suggest that previously overlooked initial symptoms are significantly associated with bacterial presence at the inoculation site. Leaf scorch is widely considered the most prominent and first visible symptom of PD. However, we showed that the most prominent symptoms in year 1 were shriveled berries and uneven lignification. While the symptom of shriveled berries has been previously observed (Feil *et al.*, 2003), it has been largely ignored since then. These symptoms became visible in the year the vines were inoculated, near the point of veraison (July 21st), as vines were starting to ripen fruit. In addition, shriveled berries were the only symptoms that occurred in all three cultivars at that time. Given the large amount of variability between cultivars, having a symptom that is consistent across cultivars may be essential for management. On the other hand, leaf scorch was observable in Chardonnay and Merlot, but was much less frequent in Cabernet Sauvignon. Uneven lignification was a prominent early symptom in Chardonnay, but not in the two other cultivars. Shriveled berries appeared more reliably and sooner after inoculation than foliar symptoms in all cultivars. Foliar symptoms tended to appear more consistently in grapevines that had been infected for over a year and may already be serving as sources of inoculum. Given the consistency of shriveled berries across the three wine grape cultivars, this appears to be the key

348 symptom to visually detect early infections of *X. fastidiosa*, whereas other symptoms such as
349 stunting, leaf scorch and uneven lignification arise later in the infection cycle.

350 Symptom and pathogen movement through the plants were tracked throughout the
351 project. In the first year, neither symptoms nor detectable bacterial populations extended past
352 the trunk to the non-inoculated side of the plant (these vines are trained horizontally similar to
353 a candelabra). However, in years 2-3, symptoms and populations were evenly distributed on
354 both sides of the trunk, even concentrated near the trunk, supporting that the infection favors
355 the side of initial inoculation only in the first year. Although not explored in the current study,
356 symptom distribution and pathogen movement may be affected by the manner of pruning.
357 These plants were annually subjected to cane pruning, whereby the fruiting wood is retained as
358 8 to 15 node fruiting units (canes). Annual growth is from buds on these canes, and the
359 permanent vine structure consists of trunk and arms. In contrast, spur pruning retains shorter
360 fruiting units (1 to 3 nodes) distributed along the length of a cordon, another aboveground
361 permanent structure. Differences in the way in which fruiting wood is selected and retained
362 between cane and spur pruned vines, as well as the overall proportion of permanent to annual
363 vine structure may impact recovery rates and should be investigated further.

364 Overwinter curing varied substantially between the three wine grape cultivars, but
365 overall, it was greater than previously hypothesized, with two of the cultivars experiencing
366 nearly 75% recovery of plants. Based on early-season inoculation and high bacterial population
367 levels, these plants were expected to become chronically infected. The high recovery rates
368 suggest that there might be substantially more plants exposed to *X. fastidiosa* infections than
369 has been previously presumed. If many more plants are infected, but avoid chronic infection,

we may be underestimating the amount of vector spread of this pathogen. These commercial vines were inoculated early in the growing season, which has been shown to be most effective inoculation timing to result in chronic infection in Napa Valley (Purcell, 1981). Therefore, the diseased plants that we observe under commercial growing conditions may represent a fraction of plants that are exposed to *X. fastidiosa*. Overwinter curing is still poorly understood, but temperature adaptation in PD strains across California might contribute to the pathogen's tolerance of colder winters (Vanhove *et al.*, 2020).

A strong association was present between symptom detection and bacterial presence in the plant. Typical infections averaged 10^5 CFU/g of plant petiole tissue; however, population size was not found to impact symptoms. Only binary infection status significantly influenced symptoms. Plants that tested positive were above the threshold of population size needed for transmission by the local vector, *Graphocephala atropunctata*, measured as populations above 10^4 CFU/g of stem tissue (Hill & Purcell, 1997). In May, qPCR detection was rare, even for plants that tested positive later in the growing season. Given the complications with detection via qPCR that vary seasonally and throughout a plant, this study supports symptom identification as the best way to detect infected grapevines in Napa Valley early in the growing season. These data also indicate that even in plants that are chronically infected and those with severe PD symptoms, populations will still be undetectable if measured even a few months too early.

This project provides new insight into the characteristics of early PD infection, which should improve disease detection in vineyards. The long-term goal of elucidating the development of this infectious disease in a high-value agricultural system is vital to the California agricultural economy. As PD has recently expanded into global grape growing regions

in Europe and Asia (Castillo et al., 2021), there has never been a more urgent time to understand the infection process of *X. fastidiosa* in *Vitis vinifera*.

Acknowledgements

We acknowledge and thank Michael Voeltz, Amielia Adams, Karina Gadkari, Emily Donaldson, Clarisse Dantas Lima, Juliet Pavy, Akila Ganpathi, Andreina Castillo Siri, Kirk Pearson, Sheri Katz, and Dylan Beal for contributions to the field, lab, and editing work for this project. Thanks to the anonymous vineyards for allowing us an opportunity to conduct this research. This work was supported by the California Department of Food and Agriculture PD/GWSS Research Program and the European Union’s Horizon 2020 research program (XF-ACTORS). A.K. received support from the UC Berkeley William Carroll Smith Plant Pathology Fellowship. A.S. received support from a Marie Skłodowska-Curie Fellowship (European Union’s Horizon 2020 Research and Innovation Program, grant agreement 707013).

Works Cited

- Burbank L, 2018. Winter climate and cultivar effects on severity of Pierce's Disease in table grapes. *Proceedings of the 2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management of Key Crops Winter*, 159–166.
- Castillo AI, Tsai CW, Su CC *et al.*, 2021. Genetic differentiation of *Xylella fastidiosa* following the introduction into Taiwan. *Microbial Genomics* **7**.
- Daugherty MP, Almeida RPP, Smith RJ, Weber EA, Purcell AH, 2018. Severe pruning of infected grapevines has limited efficacy for managing pierce's disease. *American Journal of Enology and Viticulture* **69**, 289–294.
- Davis MJ, Purcell AH, Thomson S V., 1978. Pierce's disease of grapevines: Isolation of the causal bacterium. *Science*.
- Deyett E, Rolshausen PE, 2019. Temporal Dynamics of the Sap Microbiome of Grapevine Under High Pierce's Disease Pressure. *Frontiers in Plant Science* **10**, 1–15.
- Feil H, Feil WS, Purcell AH, 2003. Effects of date of inoculation on the within-plant movement of *Xylella fastidiosa* and persistence of Pierce's disease within field grapevines. *Phytopathology* **93**, 244–251.
- Feil H, Purcell AH, 2001. Temperature-Dependent Growth and Survival of *Xylella fastidiosa* in Vitro and in Potted Grapevines. *Plant Disease* **85**, 1230–1234.
- Hill BL, Purcell AM, 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. *Phytopathology* **87**, 1197–1201.
- Hopkins DL, 1981. Seasonal Concentration of the Pierce's Disease Bacterium in Grapevine Stems, Petioles, and Leaf Veins. *Phytopathology* **71**, 415.
- Hopkins DL, 1984. Variability of virulence in grapevine among isolates of the Pierce's disease bacterium. *Phytopathology* **74**, 1395–1398.
- Krivanek AF, Stevenson JF, Walker MA, 2005. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. *Phytopathology* **95**, 36–43.
- Landa BB, Castillo AI, Giampetruzzi A *et al.*, 2020. Emergence of a plant pathogen in europe associated with multiple intercontinental introductions. *Applied and Environmental Microbiology* **86**, 1–15.
- Lieth JH, Meyer MM, Yeo KH, Kirkpatrick BC, 2011. Modeling cold curing of Pierce's disease in *Vitis vinifera* "Pinot Noir" and "Cabernet Sauvignon" grapevines in California. *Phytopathology* **101**, 1492–1500.
- Magnusson A, Skaug HJ, Nielsen A *et al.*, 2017. *Generalized Linear Mixed Models using Template Model Builder*.
- Nunney L, Yuan X, Bromley R *et al.*, 2010. Population genomic analysis of a bacterial plant pathogen: Novel insight into the origin of pierce's disease of grapevine in the U.S. *PLoS ONE* **5**.
- Perlman RL, 2016. Mouse Models of Human Disease: An Evolutionary Perspective. *Evolution, Medicine, and Public Health*, eow014.
- Poorter H, Fiorani F, Pieruschka R *et al.*, 2016. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist* **212**, 838–855.

448 Purcell AH, 1975. Role of the Blue-green Sharpshooter, *Hordnia circellata*, in the Epidemiology
449 of Pierce's Disease of Grapevines. , 745–752.

450 Purcell AH, 1981. Vector Preference and Inoculation Efficiency as Components of Resistance to
451 Pierce's Disease in European Grape Cultivars. *Phytopathology* **71**, 429.

452 Raju BC, Goheen AC, 1981. Relative Sensitivity of Selected Grapevine Cultivars to Pierce's
453 Disease Bacterial Inoculation. **32**, 0–3.

454 Stevenson JF, Matthews MA, Greve LC, Labavitch JM, Rost TL, 2004. Grapevine susceptibility to
455 Pierce's disease II: Progression of anatomical symptoms. *American Journal of Enology and*
456 *Viticulture* **55**, 238–245.

457 Therneau T, 2022. A Package for Survival Analysis in R.

458 Vanhove M, Retchless AC, Sicard A *et al.*, 2019. Genomic diversity and recombination among
459 *Xylella fastidiosa* subspecies. *Applied and Environmental Microbiology* **85**, 1–17.

460 Vanhove M, Sicard A, Ezennia J, Leviten N, Almeida RPP, 2020. Population structure and
461 adaptation of a bacterial pathogen in California grapevines. *Environmental Microbiology*
462 **00**.

463 Velásquez AC, Castroverde CDM, He SY, 2018. Plant–Pathogen Warfare under Changing Climate
464 Conditions. *Current Biology* **28**, R619–R634.

465

Figures and Tables

Table 1. Description of study sites, vines, and inoculation dates.

Vineyard	Cultivar	Number of Vines	Inoculation Date	Vine Training	Pruning
Oak Knoll	Chardonnay	15 Exp 15 Ctrl	April 14, 2017	bilateral canes	Cane pruned
Oak Knoll	Merlot	15 Exp 15 Ctrl	April 14, 2017	bilateral canes	Cane pruned
Calistoga	Cabernet Sauvignon	10 Exp 10 Ctrl	April 28, 2017	quadrilateral canes	Cane pruned

Table 2. Plants that were positive based on 2017 July or September samplings, the year inoculations occurred.

Cultivar	Positive Plants	Percentage
<i>Overall</i>	<i>34/40</i>	<i>85%</i>
Cabernet Sauvignon	8/10	80%
Chardonnay	15/15	100%
Merlot	11/15	73%

Table 3. Results from binomial GLMM showing relationships between all symptoms and infection status of the plant. P-values, Chi-square values, and degrees of freedom are shown for each test.

Symptom	Infection Status (df = 1)	
	p	Chi ²
Chlorosis	0.02367	5.1186
Matchstick petioles	1.4e-07	27.723
Leaf scorch	1.567e-09	36.449
Stunting	0.003282	8.6439
Uneven lignification	0.9987	0
Shriveled berries	4.164e-05	16.795

Figure 1. Count of plants positive by qPCR, divided by cultivar, year, and sampling month. In Chardonnay, 10 plants were removed over the winter of 2017-2018. In Merlot, only one plant was removed, between the sampling dates of July and September 2019. Plants that were removed are not plotted but subsequently might have been either positive or negative if they had not been rogued. In May of all years, all samples were negative so May is not included in the plot.

Figure 2. Total number of symptomatic analyzed shoots per sampling time measured from April 26, 2017 – October 23rd, 2019, at two-week intervals during the growing season. The plants were each measured at 5 locations for symptoms, with a binary presence or absence of each symptom, so the scale is the count of symptoms at an observation point. Axes vary by year as the magnitude of symptoms is highest in year 2. Symptom names are shortened to LC (Leaf Chlorosis), LS (Leaf Scorch), MP (Matchstick Petioles), SB (Shriveled Berries), St (Stunting), and UL (Uneven Lignification).

Figure 3. Total number of symptomatic shoots per sampling time divided by cultivar, only including inoculated plants. Axes vary by year and show total sites per sampling point with each symptom. Symptom names are shortened to LC (Leaf Chlorosis), LS (Leaf Scorch), MP (Matchstick Petioles), SB (Shriveled Berries), St (Stunting), and UL (Uneven Lignification). Asterisks indicate the harvest date for each block, after which, berry shrivel can no longer be evaluated. For Cabernet Sauvignon, the harvest date for 2017 was October 27th, 2017 and for 2018 – 2019 the harvest was after the completion of our data collection. For Merlot, the

harvest dates were September 13th, 2017, September 25th, 2018, and October 8th, 2019. For Chardonnay, the harvest dates were September 13th, 2017, September 25th, 2018, and September 9, 2019.

Figure 4. Visual symptoms of inoculated vines included A. stunting of one vine surrounded by unstunted growth (Chardonnay, June 19th, 2018), B. asymptomatic leaf (Merlot, June 19th, 2018), C. leaf chlorosis (Merlot, June 19th, 2018), D. matchstick petioles circled in red (Merlot, August 14th, 2017), E. shriveled berries adjacent to healthy berries (Cabernet Sauvignon, August 15th, 2018), F. uneven lignification (Merlot, July 21st 2017), G. leaf scorch (Chardonnay, August 12th, 2019), and H. leaf scorch (Merlot, August 12th, 2019).

Figure 5. Boxplot of log transformed CFU per gram of positive samples only, samples with $C_t > 36$ are not included in these calculations. Sampling points were from July 2017 – September 2019. In September, 2019, no Merlot samples tested positive. In May of all years, all samples were negative so May is not included in the plot.

Figure 6. Number of positive plants divided by position on the vine. Y axes vary due to recovery and fewer plants in years 2-3 and show qPCR positive plants per location on the vine. Most sampling was done at the basal petiole from the terminal shoots (west and east) and from the shoots adjacent to the trunk (west-center and east-center), which were collected at each time point (four samples per plant). The only exception is shoot 2, a location only sampled in 2017 – representing the proximal shoot to the terminal shoot on the western cane. In 2017, the

terminal shoot on the eastern cane (east) was not sampled. In 2018-2019, plants were tested in May, July, and September. However, in May, all samples were negative so they are not included in the plots. Grape cultivars are shortened to Ch (Chardonnay), M (Merlot), and CS (Cabernet Sauvignon).

Figure 7. Symptom progression over the three years of the study, broken down by vine position, including 3 symptoms. Sampling was conducted every 2 weeks throughout the growing season. Y axes vary by year. Symptom names are shortened to LS (Leaf Scorch), SB (Shriveled Berries), and St (Stunting). Each symptom was recorded as present or absent on each of 5 areas of the vine: (1) west and (2) east, corresponding to the terminal ends of each shoot; (3) mid-west and (4) mid-east, corresponding to the middle of each cane; and (5) center corresponding to the head of the vine, where the canes originate. Shriveled berries could not be evaluated after the harvest date for each block. For Cabernet Sauvignon, the harvest date for 2017 was October 27th, 2017 and for 2018 – 2019 the harvest was after the completion of our data collection. For Merlot, the harvest dates were September 13th, 2017, September 25th, 2018, and October 8th, 2019. For Chardonnay, the harvest dates were September 13th, 2017, September 25th, 2018, and September 9, 2019.

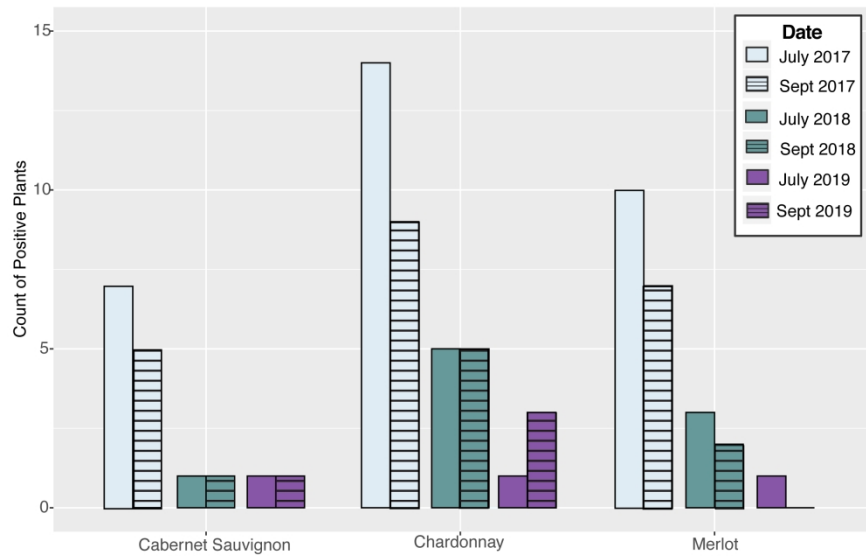


Figure 1. Count of plants positive by qPCR, divided by cultivar, year, and sampling month. In Chardonnay, 10 plants were removed over the winter of 2017-2018. In Merlot, only one plant was removed, between the sampling dates of July and September 2019. Plants that were removed are not plotted but subsequently might have been either positive or negative if they had not been rogued. In May of all years, all samples were negative so May is not included in the plot.

254x152mm (300 x 300 DPI)



Figure 2. Total number of symptomatic analyzed shoots per sampling time measured from April 26, 2017 – October 23rd, 2019, at two-week intervals during the growing season. The plants were each measured at 5 locations for symptoms, with a binary presence or absence of each symptom, so the scale is the count of symptoms at an observation point. Axes vary by year as the magnitude of symptoms is highest in year 2. Symptom names are shortened to LC (Leaf Chlorosis), LS (Leaf Scorch), MP (Matchstick Petioles), SB (Shriveled Berries), St (Stunting), and UL (Uneven Lignification).

271x124mm (300 x 300 DPI)

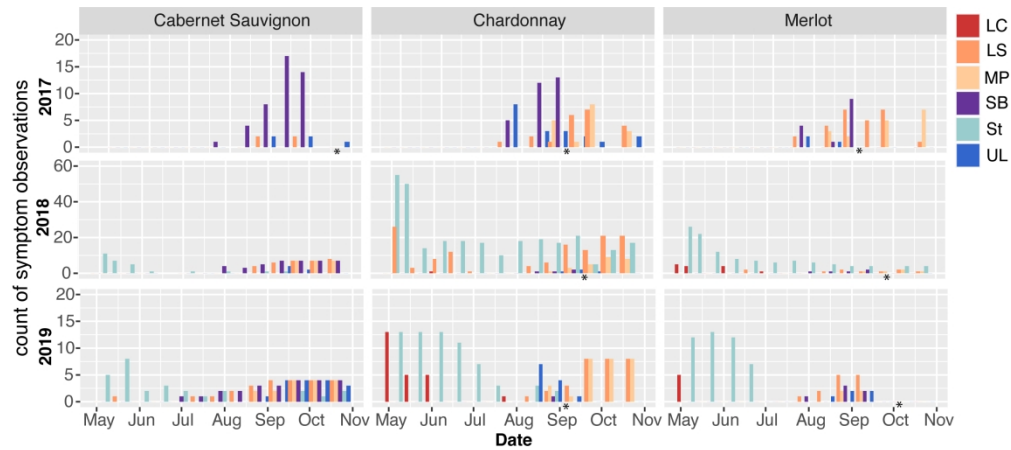


Figure 3. Total number of symptomatic shoots per sampling time divided by cultivar, only including inoculated plants. Axes vary by year and show total sites per sampling point with each symptom. Symptom names are shortened to LC (Leaf Chlorosis), LS (Leaf Scorch), MP (Matchstick Petioles), SB (Shriveled Berries), St (Stunting), and UL (Uneven Lignification). Asterisks indicate the harvest date for each block, after which, berry shrivel can no longer be evaluated. For Cabernet Sauvignon, the harvest date for 2017 was October 27th, 2017 and for 2018 – 2019 the harvest was after the completion of our data collection. For Merlot, the harvest dates were September 13th, 2017, September 25th, 2018, and October 8th, 2019. For Chardonnay, the harvest dates were September 13th, 2017, September 25th, 2018, and September 9, 2019.

247x110mm (300 x 300 DPI)



Figure 4. Visual symptoms of inoculated vines included A. stunting of one vine surrounded by unstunted growth (Chardonnay, June 19th, 2018), B. asymptomatic leaf (Merlot, June 19th, 2018), C. leaf chlorosis (Merlot, June 19th, 2018), D. matchstick petioles circled in red (Merlot, August 14th, 2017), E. shriveled berries adjacent to healthy berries (Cabernet Sauvignon, August 15th, 2018), F. uneven lignification (Merlot, July 21st 2017), G. leaf scorch (Chardonnay, August 12th, 2019), and H. leaf scorch (Merlot, August 12th, 2019).

232x160mm (300 x 300 DPI)

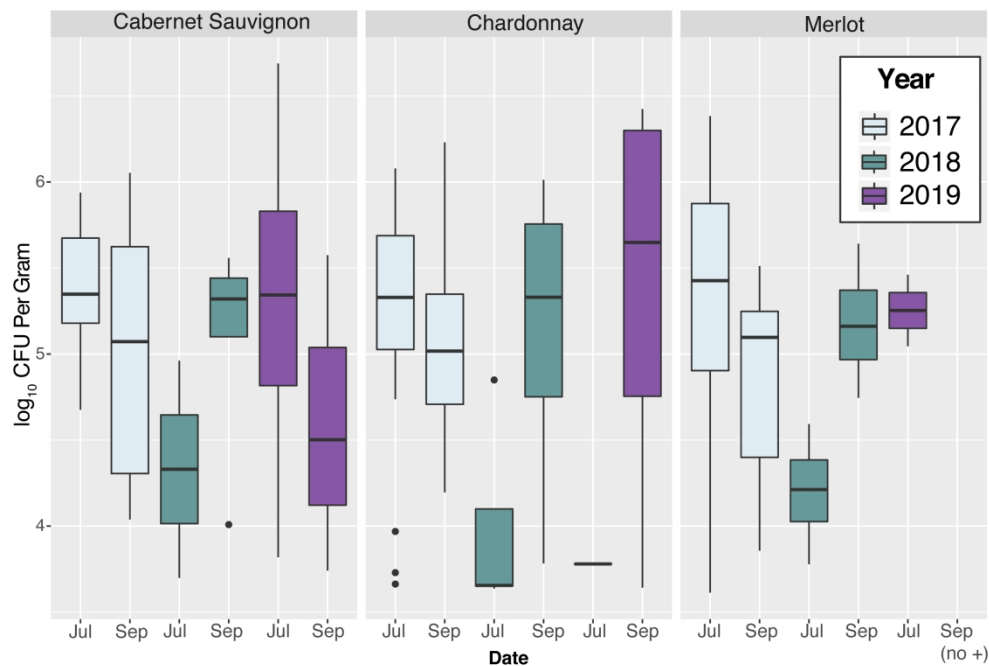


Figure 5. Boxplot of log transformed CFU per gram of positive samples only, samples with Ct > 36 are not included in these calculations. Sampling points were from July 2017 – September 2019. In September, 2019, no Merlot samples tested positive. In May of all years, all samples were negative so May is not included in the plot.

229x156mm (300 x 300 DPI)

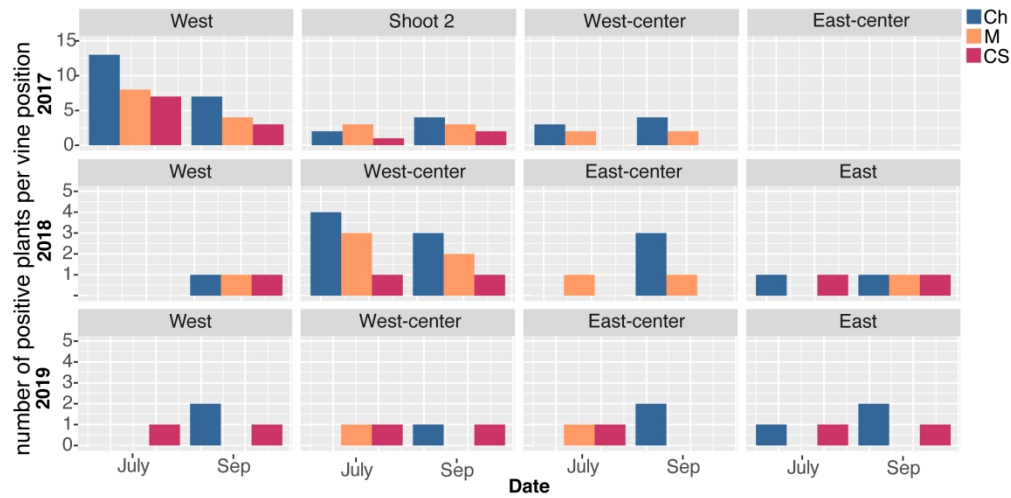


Figure 6. Number of positive plants divided by position on the vine. Y axes vary due to recovery and fewer plants in years 2-3 and show qPCR positive plants per location on the vine. Most sampling was done at the basal petiole from the terminal shoots (west and east) and from the shoots adjacent to the trunk (west-center and east-center), which were collected at each time point (four samples per plant). The only exception is shoot 2, a location only sampled in 2017 – representing the proximal shoot to the terminal shoot on the western cane. In 2017, the terminal shoot on the eastern cane (east) was not sampled. In 2018-2019, plants were tested in May, July, and September. However, in May, all samples were negative so they are not included in the plots. Grape cultivars are shortened to Ch (Chardonnay), M (Merlot), and CS (Cabernet Sauvignon).

222x110mm (300 x 300 DPI)

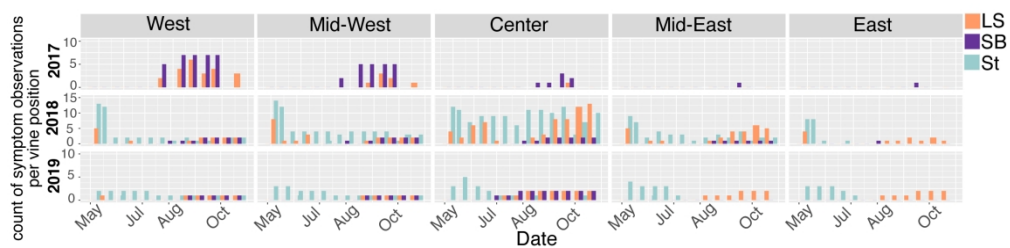
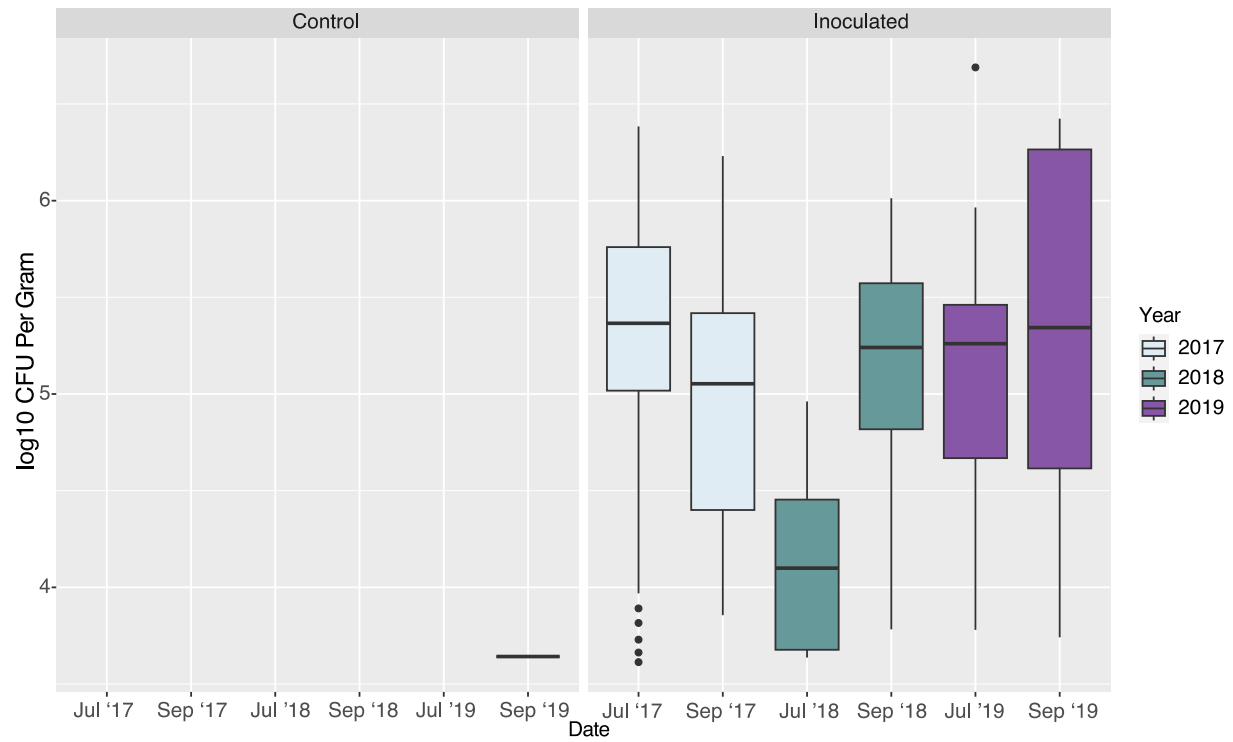


Figure 7. Symptom progression over the three years of the study, broken down by vine position, including 3 symptoms. Sampling was conducted every 2 weeks throughout the growing season. Y axes vary by year. Symptom names are shortened to LS (Leaf Scorch), SB (Shriveled Berries), and St (Stunting). Each symptom was recorded as present or absent on each of 5 areas of the vine: (1) west and (2) east, corresponding to the terminal ends of each shoot; (3) mid-west and (4) mid-east, corresponding to the middle of each cane; and (5) center corresponding to the head of the vine, where the canes originate. Shriveled berries could not be evaluated after the harvest date for each block. For Cabernet Sauvignon, the harvest date for 2017 was October 27th, 2017 and for 2018 – 2019 the harvest was after the completion of our data collection. For Merlot, the harvest dates were September 13th, 2017, September 25th, 2018, and October 8th, 2019. For Chardonnay, the harvest dates were September 13th, 2017, September 25th, 2018, and September 9, 2019.

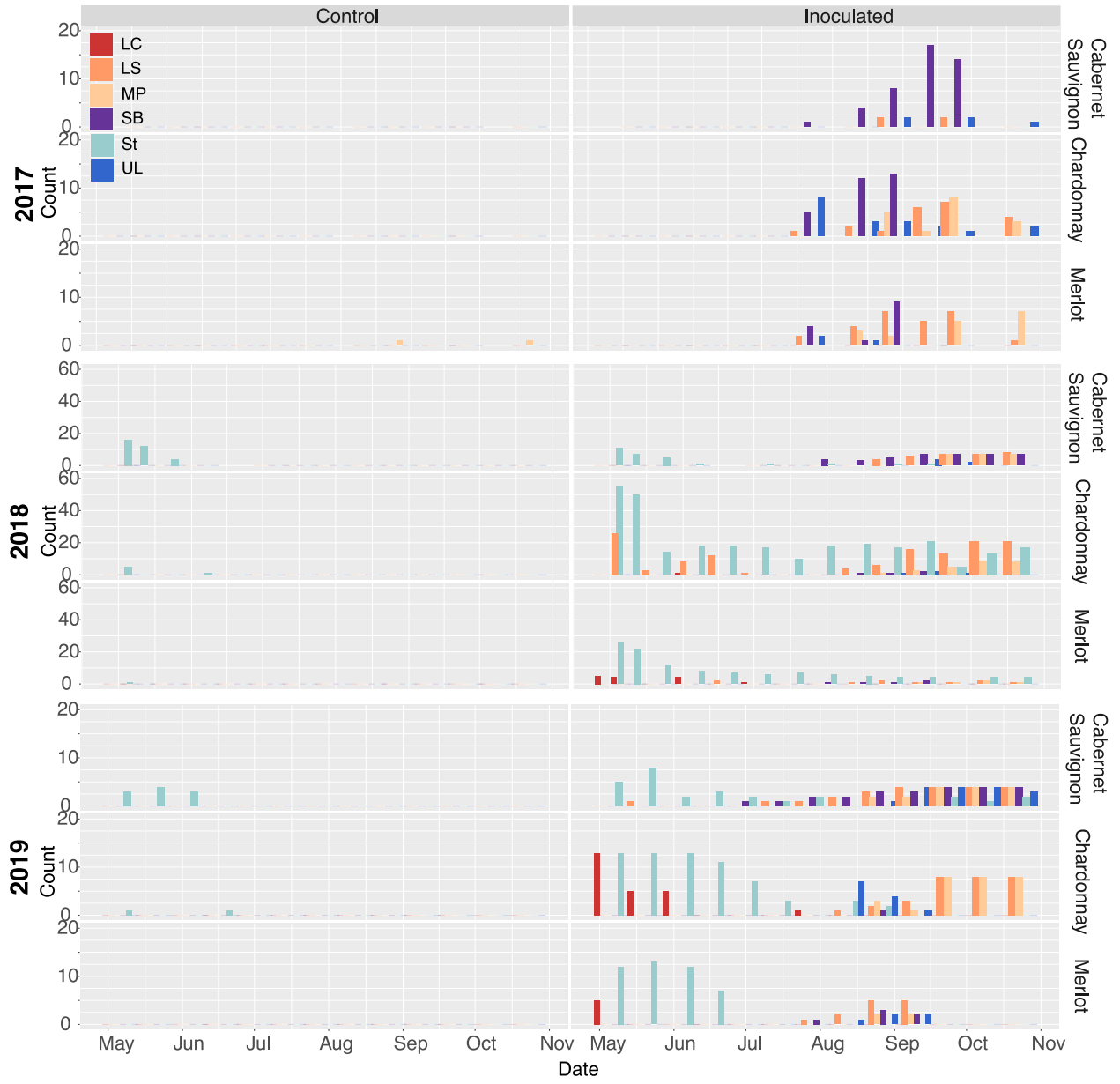
217x54mm (300 x 300 DPI)

Supplementals

S1 Bacterial population sizes by sampling date and treatment: Data are split between inoculated and control samples so as to see if any control plants tested positive.

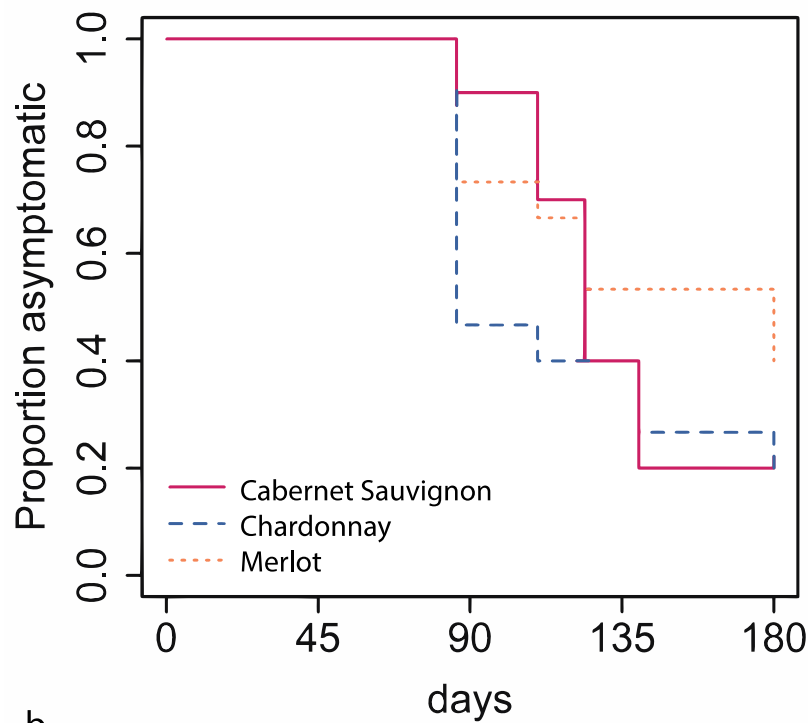


S2 Symptoms split by treatment and variety: All symptoms are displayed, broken up by variety so that controls where symptoms were detected can be identified by variety. Symptom names are shortened to LC (Leaf Chlorosis), LS (Leaf Scorch), MP (Matchstick Petioles), SB (Shriveled Berries), St (Stunting), and UL (Uneven Lignification).

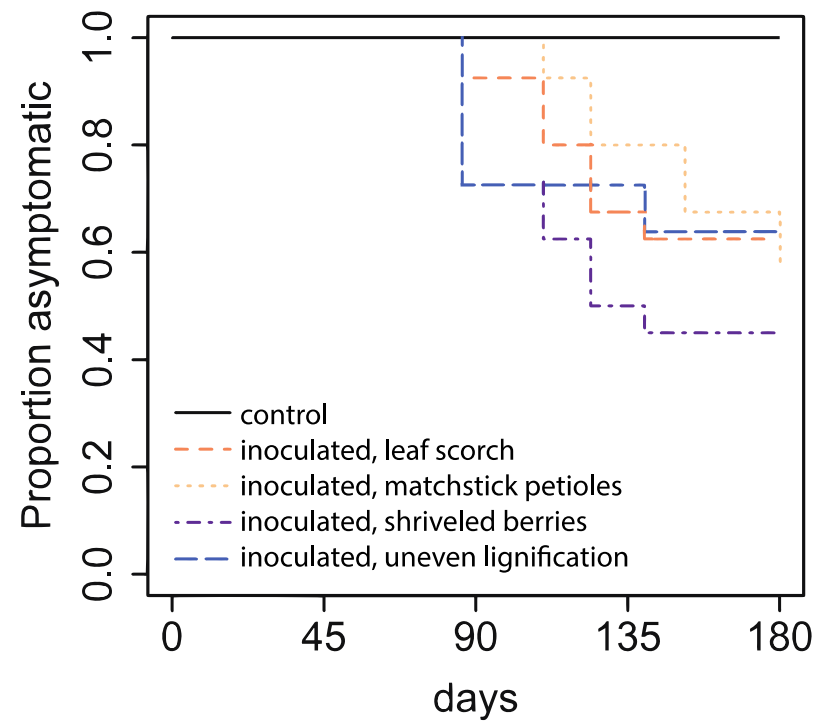


S3 Survival curves: Visualization of survival analysis of symptom onset in 2017 between (a) grape cultivars and (b) the four symptoms present during the inoculation year.

a



b



S4 Bacterial populations split by vine location and year: A boxplot is shown displaying the range of bacterial population sizes per location on the vine over the three years.

