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Assessing Vectors of Xylella fastidiosa in California: From Ecology to Transmission Dynamics



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# Assessing Vectors of *Xylella fastidiosa* in California: From Ecology to Transmission Dynamics

By

Dylan Beal

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Committee in charge:

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Abstract

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Although spittlebugs were first confirmed as vectors of *Xylella fastidiosa* in the 1950s, their role in epidemics of Pierce's disease of grapevine in the California North Coast remains unknown. The goal of this dissertation was to assess the biology and ecology of spittlebug vectors, *Philaenus spumarius* and *Aphrophora* nr. *permutata*, in California North Coast vineyards in relation to Pierce's disease and to characterize differences in *X. fastidiosa* transmission following acquisition by spittlebug and sharpshooter leafhopper vectors.

In chapter 1, I provide a brief background on the plant pathogenic bacterium, *X. fastidiosa*, its associated diseases, and its relationship with plant hosts and insect vectors. I also contextualize and summarize the subsequent chapters of my dissertation. Chapter 2 explores the seasonal abundance and infectivity of *P. spumarius* in California North Coast vineyards. Analysis of sweep and trap data revealed significant effects of survey month, vineyard site, and year on adult abundance in sweep and trap surveys. Analysis of *X. fastidiosa* natural infectivity in groups of field-collected spittlebug adults showed significant difference in transmission rates among months. I propose hypotheses on how to contextualize *P. spumarius* infectivity within the established framework of Pierce's disease winter recovery.

In chapter 3, I present results from a four-year field study analyzing the effects of site characteristics and host plant community on the abundance and development of *A.* nr. *permutata* nymphs. Woodland and riparian sites hosted larger nymphal populations than sites surrounded by vineyards. Among plant cover taxa surveyed in 2020, *A.* nr. *permutata* nymph abundance was positively associated with *Helminthotheca echoides*, *Vicia sativa*, and *Daucus carota* cover and negatively associated with *Taraxacum officinale* cover. Analysis of the development surveys found a significant effect of site, year, and plant taxa on first detection of nymphs as well as a significant effect of site and year on the estimated development time between 1$^{st}$ and 5$^{th}$ instars.

In chapter 4, I assess differences in *X. fastidiosa* transmission efficiency over time between *P. spumarius* and the blue green sharpshooter, *Graphocephala atropunctata*. There was a significant effect of days post-acquisition on the proportion of plants infected by *P. spumarius* but the effect of days post-acquisition on *G. atropunctata* transmission efficiency was not significant. I consider species specific feeding behaviors and mouthpart fluid dynamics to explain the observed transmission patterns.

Dedication

I dedicate this dissertation to my family, whose countless years of support and love have proven foundational in my personal and professional life. To my mother, Joanne, who encouraged me to pursue my dreams and inspired me to take chances and apply my interests in new and creative ways. To my father, Greg, for actively listening in our daily conversations and providing sage advice that kept me going during those particularly tough times. To my sister, Lily, for challenging me to think beyond my personal experiences/biases, and for persevering and thriving amidst the many challenges our world can present.

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# Chapter 1: Introduction

Given their capacity to transmit pathogenic microbes, insect vectors have major impacts on plants, animals, humans, and ecosystems. Vector-mediated transmission of pathogenic microbes affects the management costs and productivity of agricultural animals (Mukhebi et al. 1995) and crop plants (Tumber et al. 2014), and has consequences for human health (Enayati and Hemingway 2010). The successful transmission of a pathogen by its associated insect vectors is the result of a multitude of complex interactions between the vector, the pathogen, and their hosts (Eigenbrode et al. 2018). By elucidating the biology and ecology of insect vectors, the field of vector biology informs the development of potential management strategies that aim to reduce the negative impacts of insect vectors and the pathogens they transmit. *Xylella fastidiosa* is a xylem-limited bacterium (Wells et al. 1987) that requires xylem sap sucking insects for its dispersal, and whose management can be informed by an understanding of vector biology and ecology.

*Xylella fastidiosa* causes numerous plant diseases such as olive quick decline syndrome (OQDS), Citrus Variegated Chlorosis (CVC), and Pierce's disease (PD) of grapevines (Sicard et al. 2018). PD was first described by Newton Pierce in Southern California in the 1890s, during an epidemic in which *X. fastidiosa* devastated vineyards of the region (Pierce 1892). *Xylella fastidiosa* was first associated with insect vectors after a series of PD epidemics in California's San Joaquin Valley and North Coast in the 1930s and 1940s (Severin 1949). Previously considered a virus (Pierce 1892), *X. fastidiosa* was confirmed to be a bacterium in the 1970s when it was cultured *in vitro* (Davis et al. 1978). The association of *X. fastidiosa* with CVC in Brazil in the 1980s (Chang et al. 1993), the introduction of *Homalodisca vitripennis* into Southern California in the 1990s, and subsequent PD outbreaks (Sorensen and Gill 1996, Blua et al. 1999) in the late 1990s and early 2000s fueled an intensive period of research on *X. fastidiosa*. These studies inform much of our current understanding of this pathogen within the Americas.

Likewise, the association of *X. fastidiosa* with epidemics of OQDS in Southern Italy in 2013 have expanded and encouraged further research efforts to better understand this pathogen not only within Europe, but also on a global scale (Saponari et al. 2019). While there have been over 100 years of research dedicated to the study of *X. fastidiosa,* its insect vectors, and its associated diseases, PD remains a disease of major agronomic and economic importance. In California, *X. fastidiosa* costs the state approximately $104 million in yield losses as well as in management and regulatory costs (Tumber et al. 2014). These conservative estimates reflect the year-to-year costs that the state must incur in managing this chronic plant disease and do not reflect the additional losses to growers during epidemic years of high disease incidence.

*Xylella fastidiosa* has a broad host range (>500 plant taxa), colonizing plants of agricultural, ornamental, and non-agricultural significance (Purcell 1997, Hopkins and Purcell 2002, EFSA Panel on Plant Health 2018). However, the subspecies and individual strains of *X. fastidiosa* can have much more limited host ranges (Sicard et al 2018). One of the most susceptible hosts of *X. fastidiosa* is the European grapevine *Vitis vinifera*, in which *X. fastidiosa* causes PD (Davis et al. 1978). Plant colonization by *X. fastidiosa* leads to the blockage of xylem vessels (Newman et al. 2003). Plant responses (such as tylose production) and subsequent movement of bacteria into new vessels via degraded pit membranes leads to a reduction in sap

flow (Baccari and Lindow 2011). In susceptible hosts such as grapevines, these plant-pathogen interactions result in symptoms such as leaf scorching, matchstick petioles (leaf blade drop while petioles remain attached to the stem, raisining/desiccation of the fruit, reduced shoot growth, and overall plant dieback (Hopkins and Purcell 2002). However, in most of its hosts, *X. fastidiosa* does not cause symptoms and these asymptomatic hosts may serve as epidemiologically relevant reservoirs of the pathogen (Sicard et al. 2018).

The vectors of *X. fastidiosa* are xylem sap-feeding insects such as sharpshooter leafhoppers (Hemiptera: Cicadellidae, predominantly subfamily Cicadellinae), spittlebugs (Hemiptera: Cercopoidea), and presumably cicadas (Hemiptera: Cicadoidea) (Redak et al. 2004). All xylem sap-feeding species within these groups are assumed to be vectors capable of acquiring and transmitting *X. fastidiosa* (Frazier 1965). The precibarium and cibarium of vector insect mouthparts serve as sites for attachment and colonization of *X. fastidiosa* (Purcell et al. 1979). Vector insects are capable of transmitting *X. fastidiosa* immediately after acquisition, with no latent period (Purcell and Finlay 1979). After acquisition, *X. fastidiosa* multiplies within the mouthparts of the insect vectors (Hill and Purcell 1995). Transmission by *X. fastidosa* vectors is persistent and noncirculative (Purcell and Finlay 1979, Almeida and Purcell 2003) as *X. fastidiosa* colonization is limited to insect mouthparts. Although nymphs (juvenile stages) of vector species transmit *X. fastidiosa*, infectivity is lost after each molt so freshly molted nymphs or adults must reacquire the pathogen (Purcell and Finlay 1979). There is no evidence that *X. fastidiosa* can be transmitted transovarially, as nymphs descended from *X. fastidiosa* colonized adults are notably free of the bacteria (Freitag 1951). Although the potential vectors of *X. fastidiosa* are globally distributed (Redak et al. 2004, EFSA Panel on Plant Health 2015), vectors may vary in their capacity to contribute to *X. fastidiosa* epidemics given numerous ecological and biological factors that can influence their transmission efficiency and seasonal infectivity.

A number of factors influence the transmission of *X. fastidiosa* from insect vectors to plant hosts. For instance, transmission efficiency varies among species (Lopes et al. 2010). Increases in the length of plant acquisition and inoculation access periods increase the likelihood that vector transmission of *X. fastidiosa* will occur (Daugherty and Almeida 2009). The likelihood of insect vector transmission is strongly associated with bacterial population size within host plants (Hill and Purcell 1997). The interaction of preferred feeding sites of the vector insect species with localization of *X. fastidiosa* colonized areas within different tissues of a host plant can affect transmission efficiency (Daugherty et al. 2010). Also, as vectors visually discriminate against symptomatic plants, high disease symptoms can lead to lower transmission efficiency (Daugherty et al. 2017). Increases in vector number are also associated with increased likelihoods of transmitting *X. fastidiosa* (Daugherty and Almeida 2009). Finally, temperature can both insect vector behavior and bacterial multiplication within plants and thus also influence transmission (Daugherty et al. 2009, Daugherty et al. 2017).

Since 2015, there has been a rise in PD outbreaks in the California North Coast grape-growing region, particularly in the counties of Napa and Sonoma. Historically, *Graphocephala atropunctata* was considered the main *X. fastidiosa* vector within the California North Coast PD pathosystem (Purcell 1974, Purcell 1975). Disease patterns associated with *G. atropunctata* spread follow a strong edge effect, with a greater incidence of infected grapevines bordering riparian habitats where *G. atropunctata* adults overwinter. However, the current outbreaks also

include infected grapevines far from riparian borders or at sites not bordering riparian areas (Almeida et al. 2019). These divergent spatial patterns led to speculation that vectors other than *G. atropunctata*, such as spittlebugs, might be playing a substantial role in PD ecology. One aim of the present dissertation was to assess the biology and ecology of *X. fastidiosa* vectors, *Philaenus spumarius* and *Aphrophora* nr. *permutata*, in vineyards in the California North Coast and to elucidate the relationship between transmission efficiency and the multiplication of *X. fastidiosa* within the mouthparts of these potential insect vectors. To address these issues, I conducted a series of field studies and greenhouse experiments; the results of which are presented in the following chapters.

In chapter 2, I explore the seasonal abundance and natural infestation of meadow spittlebug, *P. spumarius*, with *X. fastidiosa* in California North Coast vineyards over a three-year field period. This chapter was recently published in Environmental Entomology (Beal et al. 2021). Chapter 3 covers the results of a four-year field study analyzing the effects of site characteristics and host plant community on the abundance and development of *A.* nr. *permutata* nymphs. This chapter also assesses the grapevine-to-grapevine transmission efficiency of *X. fastidiosa* by adult *A.* nr. *permutata* under greenhouse conditions. Chapter 3 was submitted to Environmental Entomology and is under peer review. Finally, in Chapter 4, I assess differences in *X. fastidiosa* transmission efficiency between *P. spumarius* and *G. atropunctata* during the period following acquisition. I consider past studies of species-level feeding behaviors, mouthpart fluid dynamics, and predicted bacterial population within the mouthparts to explain the observed transmission dynamics.

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Chapter 2: Seasonal abundance and infectivity of *Philaenus spumarius* (Hemiptera: Aphrophoridae), a vector of *Xylella fastidiosa* in California vineyards

**Abstract**

The meadow spittlebug, *Philaenus spumarius* (Linnaeus) (Hemiptera, Aphrophoridae), is a vector of the plant pathogen *Xylella fastidiosa* (Wells) (Xanthomonadales: Xanthomonadaceae); however, its role in recent outbreaks of Pierce's disease of grapevine (PD) in California is unclear. While the phenology and ecology of *P. spumarius* can help determine its contributions to PD epidemics, both remain poorly described in the North Coast vineyards of California. We assessed the phenology of *P. spumarius* in the region. Spittlemasses were first observed in February or March, while the emergence of adult spittlebugs did not occur until April or May depending on the year. Analysis of sweep and trap data from 2016-2018 revealed significant effects of survey month, vineyard site, and year on adult abundance in sweep and trap surveys. Spittlebug adults were present in the vineyards from April until December, with the greatest number of adults by sweep net in May or June, whereas adults on traps peaked between July and November. Analysis of natural infectivity in groups of field-collected spittlebug adults showed significant difference in transmission rates among months. Spittlebugs successfully transmitted *X. fastidiosa* to potted grapevines between July and December. The greatest risk of *X. fastidiosa* transmission by *P. spumarius* was in December (60%) followed by October (30%). However, the infectivity patterns of the meadow spittlebug did not align with the historical paradigm of California North Coast PD. We discuss alternative hypotheses in which *P. spumarius* could play a role in the epidemiology of this disease.

## Introduction

The study of trends in the seasonal development and abundance of agricultural insect pests is a valuable component of insect pest management. Biological, landscape, and climatic factors influence the phenology and abundance of any given species in agricultural systems. Understanding these factors and how they influence the relative seasonality of an insect pest can inform and influence management strategies. Degree-day models, which approximate and relate insect and plant phenology to observed or predicted site temperatures, aim to capture the seasonal dynamics guiding insect outbreaks and inform the timing and implementation of management (Higley et al. 1986). When these insect pests are vectors of pathogenic microbes, seasonality has potential implications not only for insect ecology but also for disease dynamics (Purcell 1981, Feil et al. 2003, Gruber and Daugherty 2013, Daugherty and Almeida 2019, Sisterson et al 2020).

The agricultural pest status of a given insect species is subject to change, as shifts in biological, environmental, and social factors can elevate the status of a formerly minor species or diminish the role of a major pest in crop damage. For example, sublethal insecticide use allowed the brown planthopper to become a major pest of rice through the removal of key natural enemies and the stimulation of female reproductive activity (Heinrichs and Mochida 1984). In another instance, the adoption of *Bt* cotton was linked with the emergence of non-target mirid and stinkbug pests that have benefited from potential cases of natural enemy release, insecticide release, or competitive release (Bergé and Ricroch 2010, Catarino et al. 2015, Zeilinger et al. 2016). Additionally, the establishment of introduced plant pathogens in naive agricultural systems and emergence of novel disease outbreaks have necessitated the reassessment of insect vectors associated with these microbes (Cornara et al. 2018, Preto et al. 2019). Understanding the seasonal abundance and ecology of an insect vector species as well as their transmission efficiency are key components in assessing the role of a known vector in disease outbreaks.

Studies of the meadow spittlebug, *Philaenus spumarius* (Linnaeus) (Hemiptera, Aphrophoridae), historically focused on the environmental and genetic factors influencing its color polymorphism or its role as a pest of wheat, oats, and strawberries in North America (Edwards 1935, Weaver and King 1954, Lavigne 1959, Thompson and Halkka 1973). *P. spumarius* is highly polyphagous with over 1,000 reported host plant species (Delong and Severin 1950, Weaver and King 1954, Ossiannilsson 1981, Thompson 1994). *P. spumarius* is widely distributed across the globe (EFSA 2015) and is univoltine: overwintering as an egg with a single adult generation each year (Weaver and King 1954, Cornara et al. 2018). *Philaenus spumarius* is considered an introduced species in North America, and is hypothesized to have been introduced via eggs deposited in plant and straw debris (Whittaker 1969, Whittaker 1973). An introduction from Western Europe is considered most likely, as genetic barcoding of North American *P. spumarius* suggest that these populations are most similar to those inhabiting Great Britain and Iberia (Seabra et al. 2010, Maryańska-Nadachowska et al. 2011). Historically, active meadow spittlebug adults have been collected in some regions as late as February, suggesting that adults can survive winter conditions in some regions (Edwards 1935).

Recent studies on the biology and ecology of *P. spumarius* as a pest in Italian olive agroecosystems have focused on its role as a vector of the bacterial plant pathogen, *Xylella fastidiosa* (Wells) (Xanthomonadales: Xanthomonadaceae) (Saponari et al. 2014, Cornara et al. 2016a, Cornara et al. 2017, Bodino et al. 2019, Dongiovanni et al. 2019). *X. fastidiosa* is a xylem-limited bacterial pathogen associated with numerous plant diseases including olive quick

decline syndrome (OQDS), Citrus Variegated Chlorosis (CVC), and Pierce's disease (PD) of grapevine (Sicard et al. 2018). The vectors of *X. fastidiosa* are xylem sap-feeding insects such as sharpshooter leafhoppers (Hemiptera: Cicadellidae, predominantly subfamily Cicadellinae), spittlebugs (Hemiptera: Cercopoidea), and potentially cicadas (Hemiptera: Cicadoidea) (Redak et al 2004).

Although the ability of *P. spumarius* to transmit *X. fastidiosa* to grapevine is well established (Severin 1950), its ecology in California vineyards is not well characterized (Cornara et al. 2016b). *P. spumarius* was historically prevalent in non-agricultural sites along the coast of northern California (Karban and Strauss 2004). Studies related to almond leaf scorch, another disease caused by *X. fastidiosa*, noted the presence of *P. spumarius* in central California almond orchards, albeit in low abundance (Purcell 1980, Daane et al. 2011). Larger populations of *P. spumarius* were noted in almond orchards that were closer to the San Joaquin Delta (Purcell 1980). A rise in PD outbreaks in vineyards in the counties of Napa and Sonoma circa 2015 led to speculation that *P. spumarius* might be playing a larger role in PD ecology. The recent PD outbreaks differ from historic spatial patterns of disease driven by the vector species *Graphocephala atropunctata* (Signoret) (blue-green sharpshooter, Hemiptera: Cicadellidae) (Purcell 1974, Purcell 1975). Specifically, disease patterns characteristic of *G. atropunctata* spread exhibit greater incidence of infected grapevines bordering riparian habitats where *G. atropunctata* overwinters. In contrast, the current outbreaks also included infected grapevines far from the riparian border or at sites not bordering riparian areas (Almeida et al. 2019).

We hypothesize that *P. spumarius* may be playing a role in the current PD epidemics along the California North Coast grape-growing region. To begin to address this hypothesis, we must understand the current phenology and infectivity of *P. spumarius* in California in relation to historical and global perspectives. Seasonal shifts in abundance and plant preference of *P. spumarius* in Mediterranean olive agroecosystems (Bodino et al. 2019, Bodino et al 2020) appear to explain the periods of infectivity and secondary pathogen spread associated with another disease caused by *X. fastidiosa*, OQDS (Cornara et al. 2017). If *P. spumarius* undergoes similar periods of abundance and infectivity in California North Coast vineyards, it would also contribute to secondary (vine-to-vine) spread of *X. fastidiosa*. Yet the contribution of *P. spumarius* to PD incidence along the California North Coast remains unresolved. As a first step towards assessing the potential contribution of *P. spumarius* to the current PD outbreaks, we characterized the seasonal development, abundance, and infectivity of *P. spumarius* in California North Coast vineyards through a series of field and greenhouse studies.

## Materials and Methods

### Field Sites

Two sites in the California North Coast, in Marin and Napa Counties, chosen for their history of relatively high populations of *P. spumarius* were surveyed for nymphs and adults. These sites were also chosen as they either lacked the blue-green sharpshooter (Napa) or hosted comparatively lower numbers of blue green sharpshooter to *P. spumarius* (Marin) based on past pest surveys by farmers. At the Marin County site, two vineyards were surveyed separately (Marin-1 and Marin-2) while at the Napa County site, one vineyard was surveyed (Napa-1). The Marin site lacked a history of PD and had past reports of very small populations of *G. atropunctata* and green sharpshooter, *Draeculacephala minerva* (Ball) (Hemiptera: Cicadellidae), near irrigation ponds (personal communication with Marin site vineyard

managers). The Napa site had a history of PD and possessed a pattern of disease consistent with the recent outbreaks (a concentration of confirmed *X. fastidiosa* positive vines in the middle of the vineyard rows; personal communication with Napa site vineyard manager). The landscape surrounding the Marin vineyards primarily consisted of rolling hills of pastoral rangeland at an elevation of ~130 m. The Napa vineyard was located in a mountain valley at an elevation of 461 m surrounded by forested areas comprised mainly of conifers, deciduous trees, and shrubs. Marin-1 contained eight rows of 14-year-old *Vitis vinifera* (Linnaeus) (Vitales: Vitaceae) cv. Syrah and 10 rows of *V. vinifera* cv. Montepulciano grapevines interspersed with Montepulciano replants (< 16% of the vineyard) over a total of 3.4 acres (1.38 hectares). Marin-2 contained two rows of *V. vinifera* cv. Refosco and 23 rows of *V. vinifera* cv. Syrah that were grafted onto 14-year-old rootstocks in 2016 over a total of 1.5 acres (0.61 hectares). The vineyard rows of Marin-2 were interplanted with rows of 23-year-old olive trees (*Olea europaea* cv. Frantoio, Leccino, Pendolino, Maurino, Maraiolo, Coratina, and Leccio del Corno). Napa-1 contained rows of mature 16-20-year-old *V. vinifera* cv. Cabernet Sauvignon and 20-year-old *V. vinifera* cv. Merlot grapevines over a total of 11.34 acres (4.59 hectares) interspersed with replants (< 5% of the vineyard). The vineyards at both sites were drip irrigated along the vine or tree line and managed organically.

**Nymph Surveys**

In 2016, 2017, and 2018, nymphal populations of *P. spumarius* were surveyed biweekly within the three vineyards, except in cases of extreme weather (e.g. rain delays). During each survey period, 10 plots in each vineyard were randomly selected for nymphal sampling. For Marin-1 and Napa-1, each plot consisted of two vine-rows and one inter-row. For Marin-2, each plot consisted of one vine-row, one olive-row, and one inter-row. Each survey plot was approximately 2.1 m wide and 4.6 m long (9.66 $m^2$). To sample for nymphs in each plot, a 0.6 by 0.6 m quadrat (0.36 $m^2$) was tossed six times into the plot's inter-row and all nymphal spittlemasses within each quadrat were counted and collected from the vegetation. Placement of the sampling quadrat was semi-random as care was taken to avoid overlap of subsequent samplings in the same plot. Nymphs from each spittlemass within a plot were placed with vegetation in plastic bags organized by site. These bags of live nymphs were brought back to the lab and insects individually counted before being stored in 70% ethanol. The head width of the collected nymphs were measured using a digital microscope (Leica Microsystems) to assess the nymphal instar of each specimen based on the methods of Weaver and King (1954). Nymphal instar development and identification was assessed for each site rather than for each separate vineyard. Nymphs were binned by head width to the nearest 0.1 mm and the distribution of nymphal head widths among the surveyed populations was visualized by site and year as histograms (Supplementary Figure 1A). The nymphal surveys continued through the growing season until no spittlemasses were found in two consecutive survey periods. We analyzed spittlemass abundance with a zero-inflated negative-binomial generalized linear mixed-effects model (GLMM) with survey month, year, and vineyard as fixed effects and plot as a random effect (glmmTMB, Brooks et al. 2017) (Figure 1A). 2016 was omitted from the analysis given that the survey start date in 2016 missed initial nymph emergence. All analyses were conducted in the R programming language, ver. 4.02.

**Adult Surveys**

In late March of 2016, surveys for adult *P. spumarius* began at the three vineyards in conjunction with observations of phenological development. In 2016, 10 plots were randomly selected for each vineyard and were sampled from March 2016 until December 2018. The plot composition and area at each vineyard were consistent with the plots selected for nymphal sampling. In each of 10 plots per vineyard, the two rows and the inter-row vegetation were each subjected to 25 sweeps with a sweep net (35 cm in diameter). This amounted to 75 total sweeps per plot (25 sweeps each for the left and right row and 25 sweeps for the inter-row vegetation). Sweep net surveys were conducted biweekly except during the harvest period for each vineyard (September-October), or in cases of extreme weather such as forest fires that occurred in the region in the fall of 2017 and 2018. In each plot, a yellow sticky trap (10.2 by 35.6 cm; Seabright Laboratories) was hung and replaced monthly at the middle of the lowest trellis wire of each vine-row (~0.5 m above the ground). This amounted to two traps per plot for Napa-1 and Mari-1 and one trap per plot for Marin-2. Adult abundance by sweep net and sticky trap were analyzed with separate zero-inflated negative binomial mixed-effects models with year and vineyard as fixed effects and plot as a random effect (Figure 1B). To understand the relationship between sticky trap adult abundance and survey month, we used a zero inflated negative binomial model with survey month, year, and vineyard as fixed effects and plot as a random effect (Figure 1C). The 2017 and 2018 data were truncated to align with the survey period in 2016, in which sweep and trap surveys were not recorded until April or May, respectively.

**Degree-day Calculations**

To characterize the phenology of *P. spumarius* in terms of temperature-dependent development, we calculated degree-days (DD) with the following formula:

$$DD = \sum_{n=1}^{n} \max\left[0, (T_i - T_0)\right]$$

in which $n$ is the number of days in one year, from 1st January to 31st December; $T_i$ is the mean temperature of day $i$ and $T_0$ is the lower developmental threshold for *P. spumarius*. This formula was adapted from Bodino et al. (2019) and utilizes the same developmental threshold temperature of 8°C. For each vineyard, daily mean temperature data was collected for the years of 2016-2018 from 4 km spatial resolution datasets compiled by the PRISM Climate Group (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 20 Dec 2019). Monthly Maximum, Mean, and Minimum temperatures were calculated from the same PRISM datasets and visualized (Figure 3A). Like Bodino et al. (2019), we used the degree data (Figure 3B) to create a descriptive visualization of the development of *P. spumarius* instars (represented as the proportion of individuals of that instar collected on a given date or degree day) by site and year (Supplementary Figure 1C). We do not provide a quantitative degree day phenology model given the limitations of our data and survey design. Our study's coverage of three years of phenological data at three sites is insufficient for reliable quantitative degree-day phenology models. The Weibull functions on which these kinds of quantitative degree-day models rely (Wagner et al. 1984), are more reliable and provide more comprehensive assessments and predictions when using datasets with more years (>10) or geographic sites (>10) (Emery and Mills 2019).

**Natural Infectivity Assay**

For 2016, 2017, and 2018, the seasonal *X. fastidiosa* natural infectivity of the *P. spumarius* population at the Napa site was assessed. We selected this site as it was experiencing increased PD incidence during the study period. *P. spumarius* adults caught via sweep net during the Napa surveys were brought back to the Oxford Tract Greenhouses at UC Berkeley. Groups of five *P. spumarius* adults were caged on healthy, *X. fastidiosa*-free seedlings of *Vitis vinifera* cv. Cabernet Sauvignon for a four-day inoculation access period. Over the 3 years of surveys at Napa-1, we tested a total 82 groups over 23 total survey dates (N = 410 individuals tested). The test plants were kept for two months at the Oxford Tract Greenhouses to allow for symptom development and for potential *X. fastidiosa* populations within the plants to reach levels detectable by bacterial culturing following Hill and Purcell (1995). The natural infectivity on a given test date was defined as the proportion of *P. spumarius* exposed test plants that were confirmed positive for *X. fastidiosa* by culture. Given the lack of consistent testing and data availability for each combination of month and year, the results of these natural infectivity assays were pooled by month and year to test the degree to which natural infectivity of *P. spumarius* was contingent on differences in seasonality (Figure 2). When pooled by month, five tests were conducted in May, 25 tests were conducted in June, 17 tests were conducted in July, 16 tests were conducted in August, 10 tests were conducted in October, and 10 tests were conducted in December. When pooled by year, 35 tests were conducted in 2016, 15 tests were conducted in 2017, and 33 tests were conducted in 2018. To assess the effect of seasonality on natural infectivity, we ran a binomial GLM with year and month as continuous variables (Crawley 2007). Months in which no surveys were conducted were omitted from the analyses.

**Results**

**Spittlebug Nymph Phenology**

Across all vineyards each year, *P. spumarius* spittlemasses were first observed and collected in March, except for Marin-2 in 2018, in which spittlemasses were first observed in February (Figure 1A). In Marin, the physiological time of first instar appearance ranged from 176.8-246.7 DD depending on the year (Supplementary Figure 1C). First instars were first observed at 287.2 DD in Napa in 2016 (Supplementary Figure 1C). First instars were not found in the 2017 or 2018 Napa surveys. Spittlemasses reached peak levels in late April to early May (Figure 1A). In Marin, the seasonal nymphal population peak occurred at 338.1-484.3 DD (Figure 1A, Supplementary Figure 1B). In Napa, the seasonal nymphal population peak occurred at 306.7-521.1 DD (Figure 1A, Supplementary Figure 1B). The overall nymphal population peaks were primarily made up of $4^{th}$ and $5^{th}$ instars for Napa and Marin across the three years (Supplementary Figure 1B, 1C). In Marin, the last nymphal instars were collected at 509.4-582.8 DD in mid-May (Figure 1A, Supplementary Figure 1C). In Napa, the final nymphal instars were collected at a minimum of 441.6 DD on May 11, 2017 and a maximum of 657.6 DD on May 29, 2018 (Figure 1A, Supplementary Figure 1C). A majority of the sampled populations did not reach the $5^{th}$ instar until the end of April to early May (~300-500 DD) (Figure 1A, Supplementary Figure 1). The timespan between initial observations of nymphs and initial observations of adults at Marin ranged from 46-62 days (250.9-274.8 DD) (Figure 1, Supplementary Figure 1). At Napa, the period between the first observations of nymphs and the first observations of adults ranged from 12-62 days (82.7-411.3 DD) (Figure 1, Supplementary Figure 1). The presence of later instars at the first observation of nymphs in Napa (2016: $2^{nd}$-$5^{th}$, 2017: $2^{nd}$-$5^{th}$, 2018: $2^{nd}$-$3^{rd}$) and Marin (2016: $2^{nd}$, 2017: $2^{nd}$-$3^{rd}$, 2018: $2^{nd}$) (Supplementary

Figure 1B, 1C), indicates that emergence of nymphs occurred before initial detection in the field. The zero-inflated negative binomial GLMM found that there were significant effects of month ($\chi^2$= 121.008, df=4, $P$< 0.0001), year ($\chi^2$= 7.8308, df=1, $P$= 0.0051) and vineyard ($\chi^2$= 27.344, df=2, $P$<0.0001) on spittlemass abundance (Figure 1A). For all vineyards, April and May were the months with the greatest spittlemass abundance. Spittlemass counts in 2018 were greater than in 2017 or 2016. At Napa-1, spittlemasses were observed at greater levels than Marin-1 and Marin-2, except in 2018 when Marin-2 had the greatest spittlemass counts.

**Spittlebug Adult Abundance**

Newly emerged adult *P. spumarius* were first observed in sweep net surveys from April to May (Figure 1B). Adults first appeared in sweep catches from 369.9-657.6 DD depending on site and year (Figure 1B, Supp. Table S1). At Napa-1, *P. spumarius* adults collected by sweep were abundant in May and June (up to 17.10 ± 3.89 individuals/plot) and were found at low populations until October to November (up to 2.00 ± 0.70 individuals/plot) (Figure 1B). Forest fires during these months in Napa and Sonoma in 2017 and 2018 impacted the ability to access and survey these sites therefore delaying and reducing the number of survey dates. Peak adult abundance by sweep at Napa-1 occurred from 561.3-657.6 DD (Figure 1B, Supp. Table S1). At both Marin vineyards, *P. spumarius* adults were rarely caught by sweep net, even for years in which surveyed nymphal abundance within the vineyard was comparable to the Napa site. *P. spumarius* adults were present at low populations (up to 1.55 ± 0.41 individuals/plot) at the Marin vineyards from April until December of each survey year (Figure 1B). Peak adult abundance by sweep occurred at 584.3-928.8 DD for Marin-1 and at 532.9-2252.3 DD for Marin-2 (Figure 1B, Supp. Table S1). In 2018, a single adult female *P. spumarius* was found by sweep net in February at Marin-2 (Figure 1B). This adult was likely a survivor of the previous 2017 adult generation, given that nymphs had only just emerged at this vineyard in 2018. The zero-inflated negative binomial GLMM found a significant relationship between survey month and adult abundance by sweep ($\chi^2$ = 210.597, df = 8, $P$ < 0.0001) with significant effects of year ($\chi^2$ = 41.394, df = 2, $P$ <0.0001) and vineyard ($\chi^2$ = 139.676, df = 2, $P$ < 0.0001). Napa-1 had greater sweep catches of spittlebugs by sweep than Marin-1 or Marin-2. For Napa-1, 2017 was the year with the greatest number of catches by sweep while for Marin-1 and Marin-2, 2018 was the year with the greatest number collected by sweep. For Napa-1, May or June were the months with the greatest catch by sweep while for Marin-1 and Marin-2, the greatest catch by sweep occurred in May, June, or July depending on the year.

Adult *P. spumarius* were first collected by yellow sticky trap in May to June (Figure 1C). Trap catches of adults first occurred at 561.3-657.6 DD in Napa and at 465.2-1284.2 DD in Marin depending on year (Figure 1C, Supp. Table S2). Peak adult abundance by trap occurred at 1276.4-2467.4 DD depending on the site and year (Figure 1C, Supp. Table S2). For the Marin vineyards across all 3 years, adult *P. spumarius* were collected by yellow sticky trap at <5 individuals per plot that peaked between July and November depending on the year (Marin-1: up to 1.90 ± 0.35 individuals/plot, Marin-2: up to 2.30 ± 0.56 individuals/plot) (Figure 1C). At Napa-1 in 2016, adult *P. spumarius* were collected by yellow sticky trap at <5 individuals per plot from May until December with a small peak in August (2.50 ± 0.95 individuals/plot) (Figure 1C). For 2017 and 2018 at Napa-1, trap collections of *P. spumarius* reached peak levels (up to 10.40 ± 2.26 individuals/plot) from July to August with occasional pulses in the fall months (up to 4.20 ± 1.01 individuals/plot) (Figure 1C). The zero-inflated negative binomial GLMM found

that there was a significant relationship between survey month and adult abundance by trap ($\chi^2$ = 61.29, df = 7, $P$ <0.0001) with significant effects of year ($\chi^2$ = 120.85, df = 2, $P$ < 0.0001) and vineyard ($\chi^2$ = 155.02, df = 2, $P$ < 0.0001). Compared to 2017 and 2018, 2016 had fewer catches of spittlebug adults by trap. Napa-1 generally had greater trap catches than either Marin-1 or Marin-2. For Napa-1, peak trap catches occurred between June and September. At Marin-1 and Marin-2, peak trap catches occurred between August and December depending on the year.

**Natural Infectivity**

In 2016-2018, *P. spumarius* collected from the Napa site transmitted *X. fastidiosa* to greenhouse test plants between the months of July and December (Figure 2). Analyzing the binomial GLM of natural infectivity, year and the interaction of year and month were not significant although there was a nearly 4-fold greater proportion of positive test plants in 2016 versus in 2017 or 2018 (Figure 2). Dropping the interaction and year effect through model simplification left a significant effect of month ($\chi^2$ = 21.643, df = 11, $P$ < 0.0001), with no *X. fastidiosa* transmission occurring in May or June, increasing to approximately 60% of vector groups transmitting in December, the month with the highest transmission rate (Figure 2). Re-running the model without December (for which data was only available from 2016), there was still a significant effect of month on risk of transmission ($\chi^2$ = 8.1719, df = 10, $P$ < 0.01), predominantly driven by October, in which 30% of tested vector groups transmitted (Figure 2).

**Discussion**

The seasonal variability in insect vector development, abundance, or activity may have important consequences for disease spread and potential management (Purcell 1979, Gruber and Daugherty 2013). In this study, we characterized the seasonal development and abundance of *P. spumarius* at two vineyard sites along the California North Coast (Marin and Napa Counties). Our observation of 4$^{th}$ and 5$^{th}$ instars during some initial surveys indicates that the emergence of *P. spumarius* occurred before we could reliably detect nymphs. Given a nymphal instar development time of approximately 8-10 days (Weaver and King 1954), we hypothesize that *P. spumarius* may have emerged as nymphs as early as the first half of February at the Napa and Sonoma sites. Our observed developmental period suggests a slightly prolonged development for *P. spumarius* in the California North Coast compared with the March to May development of *P. spumarius* reported from the Apulia and Liguria regions of Italy (Bodino et al. 2019, Dongiovanni et al. 2019). In the Ligurian study (Bodino et al. 2019), climatic data was obtained directly from dataloggers placed at the level of the herbaceous cover, while in our study, we used datasets of modeled temperatures based off weather station data. Differences in the phenological estimation of degree-day between our studies could be related to this distinction. The collection of *P. spumarius* adults from April until November to December is comparable to surveys of meadow spittlebug adults in Ligurian olive orchards (Bodino et al. 2019). For the Napa site's sweep-net surveys, we observed a single peak of adults in late spring. This peak coincided with the development of late-instar nymphs during these months. Our results align with a three-year survey of olive groves in Spain, in which a single peak of *P. spumarius* adults also occurred in late spring, following the eclosion of a majority of nymphs to adults (Morente et al. 2018). These results contrast with sweep-net surveys of *P. spumarius* in olive orchards in Apulia (Cornara et al. 2017) and Liguria (Bodino et al. 2019), where two seasonal peaks in *P. spumarius* adult abundance were observed. The authors of the Italian studies suggested that the observed seasonal movement of *P. spumarius* adults in olive orchards were linked to changes in the

management or water status of olive trees and weed species. Meadow spittlebug abundance decreased during the summer due to the desiccation of herbaceous cover during summer months, especially in South Italy, and then increased in the late summer and fall months following rains that encouraged the production of new shoots (Cornara et al. 2017, Bodino et al. 2019). In a study that tested the effect of supplemental summer rainfall on Auchenorrhynchan population abundance and structure in calcareous grasslands, overall Auchenorrhynchan abundance per $m^2$ (including *P. spumarius*) was greater in water supplemented plots compared to control and water excluded plots (Masters et al. 1998). While weeds and cover crops in our survey plots were tilled in late spring or desiccated during the summer months, the grapevines at these sites were irrigated. This observation might explain the rise in *P. spumarius* caught by trap at the Napa site in 2017 and 2018 from June to August.

Even at low populations, *P. spumarius* may pose a threat to vineyards in California as a vector of *X. fastidiosa*. With an experimental transmission efficiency of *X. fastidiosa* to grapevine of ~15% per individual per day, this insect species is a competent vector that may play a role in ongoing PD epidemics in California (Cornara et al. 2016b). Xylem sap-feeding insects with an experimental transmission efficiency of 10% or greater are considered competent vectors, although vectors with transmission efficiencies <10% may still contribute to the disease ecology of certain *X. fastidiosa* pathosystems, such as CVC in Brazil (Krugner et al, 1998, Redak et al. 2004). The grapevine to grapevine transmission efficiency of the glassy winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is also ~15% per individual per day (Almeida and Purcell 2003), while the transmission efficiency of *G. atropunctata* can be as high as 70-90% per individual per day (Purcell and Finlay 1979, Hill and Purcell 1995). Even though the transmission efficiency of *H. vitripennis* is lower than *G. atropunctata*, the ability of *H. vitripennis* to reach high population levels in citrus orchards bordering vineyards is considered central to its large impact on PD epidemiology in southern California (Blua et al. 1999, Daugherty and Almeida 2019).

There may be certain landscape specific conditions in which *P. spumarius* reaches high population densities. Historically, alfalfa fields have harbored peak densities of 1280 nymphs/$m^2$ and 466 adults/$m^2$ (Wiegert 1964a). It is possible that sites or regions with a high proportion of leguminous cover crops or weeds as ground cover might encourage larger *P. spumarius* populations, although this hypothesis requires further analysis. Alternatively, *P. spumarius* may be capable of feeding on and transmitting *X. fastidiosa* to woody or dormant grapevine tissue, as reported for *H. vitripennis* (Almeida and Purcell 2003). If present, this feeding behavior would allow infective adults that survive into the following year to transmit *X. fastidiosa* to grapevine during a period conducive for chronic PD infections (Feil et al. 2003). While it is more difficult for *P. spumarius* nymphs to feed on older, woodier plant tissues (Hoffman and McEvoy 1986), studies of adult spittlebug feeding, survival, and transmission to woody or dormant grapevine are lacking. *P. spumarius* was the only reported vector species of *X. fastidiosa* to be consistently collected at our Napa survey site in the midst of the PD outbreaks (*D. Beal, unpublished data*). Although nymphs of another spittlebug species, *Aphrophora permutate* (Uhler) (Hemiptera: Aphrophoridae), were collected during the spring surveys at the Napa site, our trap and sweep net surveys only collected one adult specimen in December 2017 (*D. Beal, unpublished data*). During our surveys at Napa, *G. atropunctata* was never found by sweep or trap (*D. Beal, unpublished data)*. The only sharpshooter leafhopper collected at Napa, a *Cuerna* sp., consisted

of <5 individuals collected by trap each year in 2018 and 2019 (*D. Beal*, unpublished data). In the three years of surveys at Marin-2, only one to two *G. atropunctata* total were collected off traps each year in 2018 and 2019, and PD was never observed or reported (*D. Beal*, unpublished data). These observations support our hypothesis that at sites that lack large numbers of sharpshooter leafhoppers, *P. spumarius* may be contributing to PD.

While vineyard growers anecdotally note that spittlebugs have become more prevalent in vineyards in recent years, the abundance of *P.spumarius* in California's natural areas has reportedly declined. Over the last 30 years, meadow spittlebug populations in natural areas along the California Coast appear to have declined or shifted northward due to shifts in temperature and humidity (Karban and Huntzinger 2018, Karban and Strauss 2004). We hypothesize that irrigated agricultural sites may serve as a refuge for *P. spumarius*. Irrigated agricultural systems such as vineyards may be able to support and maintain populations of *P. spumarius* by providing a more humid microclimate and host plants under lower water stress. Studies of Auchenorrhynchan ecology in vineyards have suggested that vineyard treatments or conditions, such as irrigation, are conducive to support insect populations. For example, the seasonal abundance of the grape leafhopper, *Empoasca vitis*, was greater in irrigated vineyards vs non-irrigated vineyards (Fornasiero et al. 2012). In a survey of xylem sap-feeding Auchenorrhyncha in Texas vineyards, populations of *Clastoptera* sp. spittlebugs were present throughout the growing season, although the environmental requirements necessary for the development of these species may differ greatly from *P. spumarius* (Lauzière et al. 2008). During spring surveys of potential vineyard study sites, we only found *P. spumarius* in drip-irrigated, organically managed vineyards (*D. Beal and A. Purcell, personal observation*). As our study did not include surveys of *P. spumarius* abundance in non-cultivated sites, we cannot document whether cultivated or non-cultivated sites are more suitable for *P. spumarius*. In addition, differences in spittlebug occurrence and abundance at our sites may be influenced by landscape composition. Previous studies have shown that elevation is positively associated with *P. spumarius* occurrence while grassland cover is not associated with larger spittlebug populations in Abruzzo olive orchards (Santoiemma et al. 2019). Compared to the Marin site, the Napa site is at a higher elevation and is surrounded by mixed hardwood coniferous forests rather than pastoral grasslands. Site specific differences in minimum, mean, and maximum temperatures across the season and differences in the degree day accumulation at these sites might impact *P. spumarius* occurrence and abundance (Figure 3). These factors, among others, might explain why Napa supported larger populations of *P. spumarius* than Marin. The species richness of non-cultivated areas and degree of agronomic intensity surrounding agricultural sites are also speculated to influence the population abundance of *P. spumarius* (Bodino et al. 2019, Santoiemma et al. 2019). Studying *P. spumarius* occurrence and abundance at a larger scale across a wider sample of vineyard and non-cultivated sites along the California North Coast would elucidate the potential influence of landscape composition.

By providing data on the development and ecology of *P. spumarius* in California North Coast vineyards, our study may provide insight on the potential management of this insect vector. Initial studies of *P. spumarius* as a vector of *X. fastidiosa* provided an extensive list of associated host plants present in California (Delong and Severin 1950). However, the degree that these plant species are present in vineyards and the relative association of these plants with *P. spumarius* development and abundance remains to be clarified. Among associated groundcover

species in Italian olive orchards, *P. spumarius* preferentially selected plant genera (such as *Picris*, *Medicago*, and *Vicia*) (Dongiovanni et al. 2019) that are also present in California vineyards. This study suggested that agricultural management favoring grasses over herbaceous dicotyledon plants would assist in suppressing *P. spumarius* populations. Surveys of Aphrophoridae nymphs among groundcover species in Portuguese olive orchards found that *P. spumarius* mainly fed on species belonging to the Asteraceae and Fabaceae while *Neophilaenus* sp. mainly fed on *Poaceae* (Villa et al. 2020). Of the groundcover plants surveyed in Portuguese olive orchards, only 3 species on one sampling date (unidentified Asteraceae sp., *Medicago* sp., and *Trifolium glomeratum*) supported more nymphs than expected, while a number of the most common plant species at sites (associated Asteraceae, Fabaceae, Caryophyllaceae, and Brassicaceae) lacked nymphs. The authors of that study noted that the large amount of site and year variations in spittlebug occupancy and host plant preference highlight the need for studies that survey and experimentally assess different community compositions of groundcover species for spittlebug management. The common association of spittlebugs with nitrogen-fixing plants (Thompson 1994) is of potential management concern as nitrogen-fixing legumes are often utilized as cover crops in vineyard systems (Ingels et al. 1998). Nymphal *P. spumarius* association with agricultural groundcover species suggest that mowing, tillage, or pesticide regimes targeting the groundcover would also reduce *P. spumarius* population levels (Cornara et al. 2018). Mandatory mechanical weeding of winter and spring hosts of *P. spumarius* nymphs currently underway in Apulian olive orchards will hopefully provide evidence for the efficacy of these methods (Saponari et al. 2019). To ensure the adequacy and efficiency of cover crop and weed control efforts, timing the management of the less mobile nymphal stages to their periods of peak abundance may be key.

At the Napa site, *P. spumarius* adults emerge and are abundant during a period (April-June) that has historically been considered critical to chronic PD development (Purcell 1981) and reduced winter recovery (Feil et al. 2003) in the California North Coast. However, this period of initial activity does not overlap with our observations of naturally infective *P. spumarius*. The initial detection of naturally infective spittlebugs aligns more closely with peak trap catch in July and August as well as a prior report of *P. spumarius* natural infectivity at the Napa site (Cornara et al. 2016b). That study noted that sampled grapevines had *X. fastidiosa* populations of $10^4$-$10^6$ colony forming units/g in June and $10^6$-$10^8$ CFU/g in July and August (Cornara et al. 2016b). These within-plant *X. fastidiosa* populations are conducive for vector acquisition and transmission (Hill and Purcell 1997). As there is no evidence for vertical transmission of *X. fastidiosa* (Freitag 1951), vector insects like *P. spumarius* can only acquire the pathogen during periods when the bacterial population in host plants have reached sufficient levels. The alignment of the *P. spumarius* infectivity period with the period of transmissible *X. fastidiosa* populations in field grapevines suggests that *P. spumarius*, where present, may play a role in the secondary (i.e. vine-to-vine) spread of *X. fastidiosa* within vineyards. *P. spumarius'* transmission of *X. fastidiosa* in Apulian olive orchards also appears to follow a pattern of secondary spread, although those meadow spittlebugs were found to be infective almost immediately after emergence and movement to olive trees (Cornara et al. 2017). Instead, the apparent lag of 9-11 weeks between the emergence of adult *P. spumarius* and *X. fastidiosa* transmission is similar to the 6-10 week lag in *X. fastidiosa* natural infectivity noted for the sharpshooter *Oncometopia nigricans* (Walker) (Hemiptera: Cicadellidae) in Florida vineyards (Adlerz and Hopkins 1979). These differing infectivity trends are likely driven by the

availability of *X. fastidiosa* host plants that have bacterial populations conducive for vector acquisition and transmission.

Compared to our results on *P. spumarius* natural infectivity, native sharpshooters (*G. atropunctata*, *Draecculacephala minerva*, and *Xyphon fulgida* (Nottingham) (Hemiptera: Cicadellidae)) collected from North Coast grapevine and wild plant hosts were naturally infective year-round with a small increase in the proportion of infective groups of vectors in the fall (Freitag and Frazier 1954). *G. atropunctata* is associated with the primary spread of *X. fastidiosa* from infected wild host plants bordering California North Coast vineyards (Purcell 1975). It is possible that the *P. spumarius* collected for the natural infectivity assays may have acquired *X. fastidiosa* from non-grapevine vegetation within or surrounding the vineyard. As we do not have consistent samples for each combination of month and year, this limits the accuracy and reliability of our estimates of how seasonality impacts natural infectivity. However, even when December 2016 transmission assays were removed from our model, there was a significant effect of month on risk of transmission, supporting our observations of a seasonal natural infectivity concentrated in the late summer and fall months. Assessing early summer infectivity of *P. spumarius* in trials with a larger sample size would be critical given the link between early summer *X. fastidiosa* transmission events and chronic PD (Purcell 1981).

In the historic paradigm of PD winter recovery, >80% of grapevines infected by *X. fastidiosa* after the month of May recover in the following year (Purcell 1981, Feil et al. 2003). That work and studies of *G. atropunctata* ecology and its relationship to *X. fastidiosa* epidemiology in California (Purcell 1974, Purcell 1975) inform our current approach to studying *P. spumarius*. If *P. spumarius* played a similar role to *G. atropunctata* in PD ecology, adult populations in or near vineyards should be abundant and infective between April and June, a period historically associated with the establishment of chronic PD in the California North Coast vineyards (Purcell 1981). The linkage between the early season abundance and movements of *G. atropunctata* into the vineyard with PD incidence along the vineyard edges supported the hypothesis that early season inoculations are critical in establishing chronic PD infections (Purcell 1975, Purcell 1981). Purcell 1975 also noted high densities of infective sharpshooters in the vineyard interior in the late growing season although disease incidence in this section was consistently low. Under this scenario, the infection events initiated by meadow spittlebug at shoot tips in the late summer and fall months would be unlikely to result in persistent, chronic cases of PD along the California North Coast. However, as *P. spumarius* preferentially feeds on actively "growing parts" of host plants (Wiegert 1964b, McEvoy 1986) and is capable of feeding on grapevine stems (personal communication with D. Cornara), feeding events closer to the base of the shoot in July and August may result in transmission events that lead to chronic PD infections. A reduction in vine spacing and changes in canopy management in Napa county vineyards since 1980 (Kliewer 1980, Cooper et al. 2012) has led to an increase in the number and vigor of late summer green lateral shoots that may be conducive for these transmission events. It is possible that the novel incidence patterns of the current outbreaks might result from an interaction between primary spread associated with *G. atropunctata* (Purcell 1974) and secondary spread associated with *P. spumarius* (Cornara et al. 2017). The presence of sites with chronic cases of PD, a lack of sharpshooter leafhoppers, an abundance of spittlebugs, and temporal patterns of late season spittlebug infectivity indicate that there are gaps in the current

paradigm of California North Coast PD and the PD winter recovery hypothesis (Purcell 1981, Feil et al. 2003).

Fully understanding the potential contribution of *P. spumarius* to the current California PD outbreaks may require a reassessment of the PD winter recovery phenomenon. Infective *P. spumarius* adults that reach large populations in the fall and persist through mild winters may be capable of inoculating grapevines with *X. fastidiosa* during the early season of the following year. Although the vines encountered during this period would be dormant, *P. spumarius* may be capable of transmitting *X. fastidiosa* to dormant grapevines like the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae) (Almeida et al. 2005). If infectious adults are able to survive until March and feed on growing grapevines undergoing budbreak, these transmission events could support chronic PD infections in North Coast vineyards. Additionally, milder winters may allow for the persistence of late season *X. fastidiosa* infections initiated by *P. spumarius* due to a reduced induction of winter recovery (Feil et al. 2003). Although the likelihood of these scenarios under current winter conditions may be low, they may become more common as winter temperatures and the frequency of extreme warm patterns have risen over the past century in California (Swain et al. 2016, Wang et al. 2017).

**Conclusions**

In this three year study, we characterized the phenology, population dynamics, and abundance of the meadow spittlebug, *P. spumarius*, at two vineyard sites in Marin and Napa County. *P. spumarius* nymphs were first observed in field surveys in March and completed their development from April-May. Our reports of *P. spumarius* nymphal development in California vineyards are similar to the reported phenology of *P. spumarius* in olive orchards in Italy (Bodino et al. 2019). The current Italian efforts to control the *P. spumarius* nymphs through timed management of common weed and cover crop hosts or the use of less suitable plants hosts for the vector and pathogen are worth testing and implementing in California (Bodino et al. 2020, Saponari et al. 2019). Our descriptions of nymphal development and abundance in terms of chronological and physiological time will inform potential management practices and may allow for further development and testing of predictive degree-day models for the in-field development of *P. spumarius*. Depending on the site and year, spittlebug adults were abundant by sweep from May-July and by trap from July-September. The observed period of *X. fastidiosa* infective meadow spittlebugs from July-December does not align with the spring flights of infective *G. atropunctata* considered critical for chronic PD in the California North Coast (Purcell 1981). Under the established paradigm of PD winter recovery in California, meadow spittlebugs cannot support chronic PD as infections initiated after May or June are likely to recover the following year (Feil et al. 2003). However, *P. spumarius* was the only vector species of *X. fastidiosa* with observable, abundant adult populations at the Napa site during an ongoing PD epidemic. This study posits that *P. spumarius* contributes to PD incidence in some vineyards affected by the current epidemic, particularly those that lack sharpshooter leafhoppers, and that elements of the PD winter recovery hypothesis should be reexamined.

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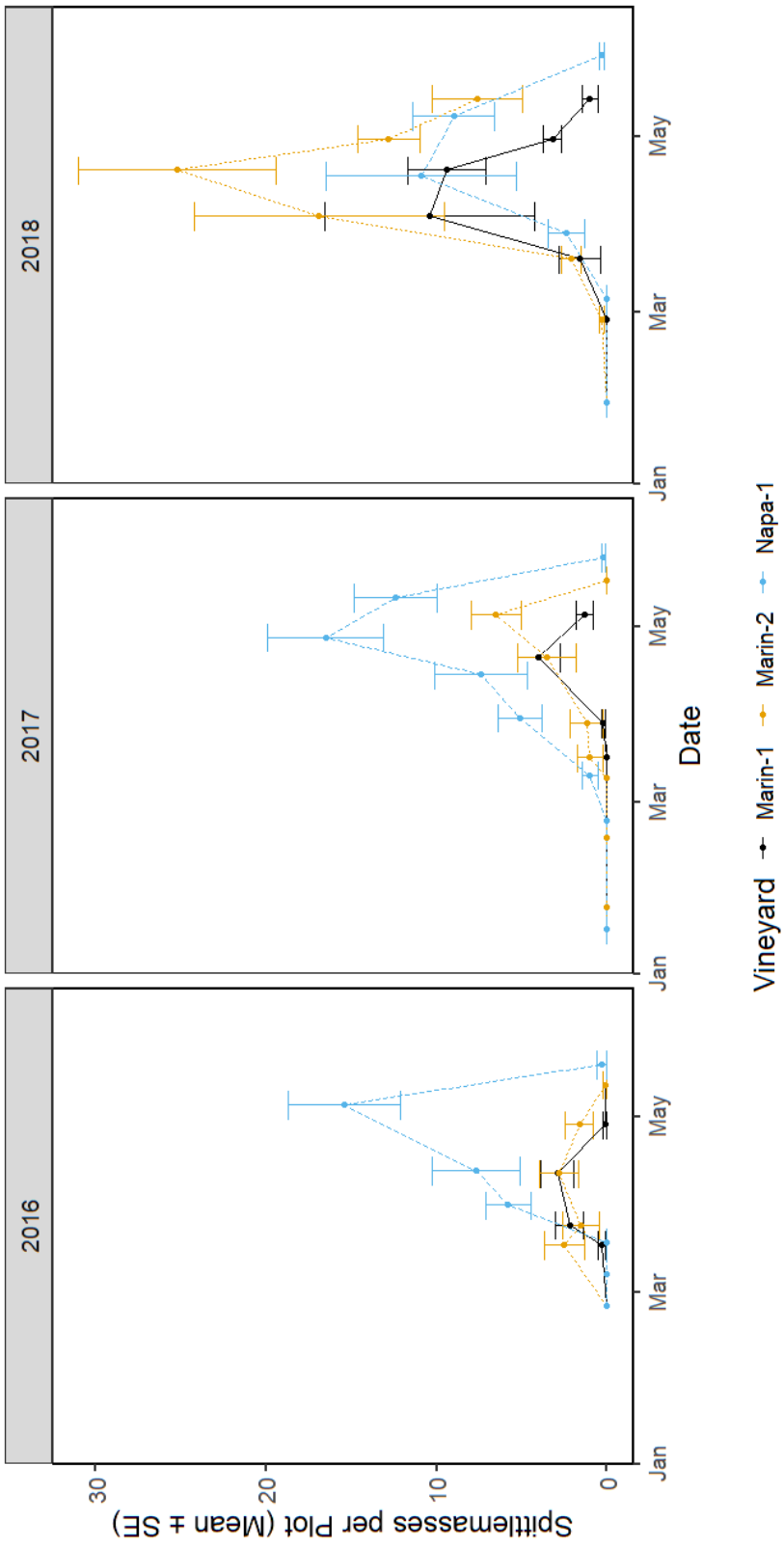
**Figure legends**

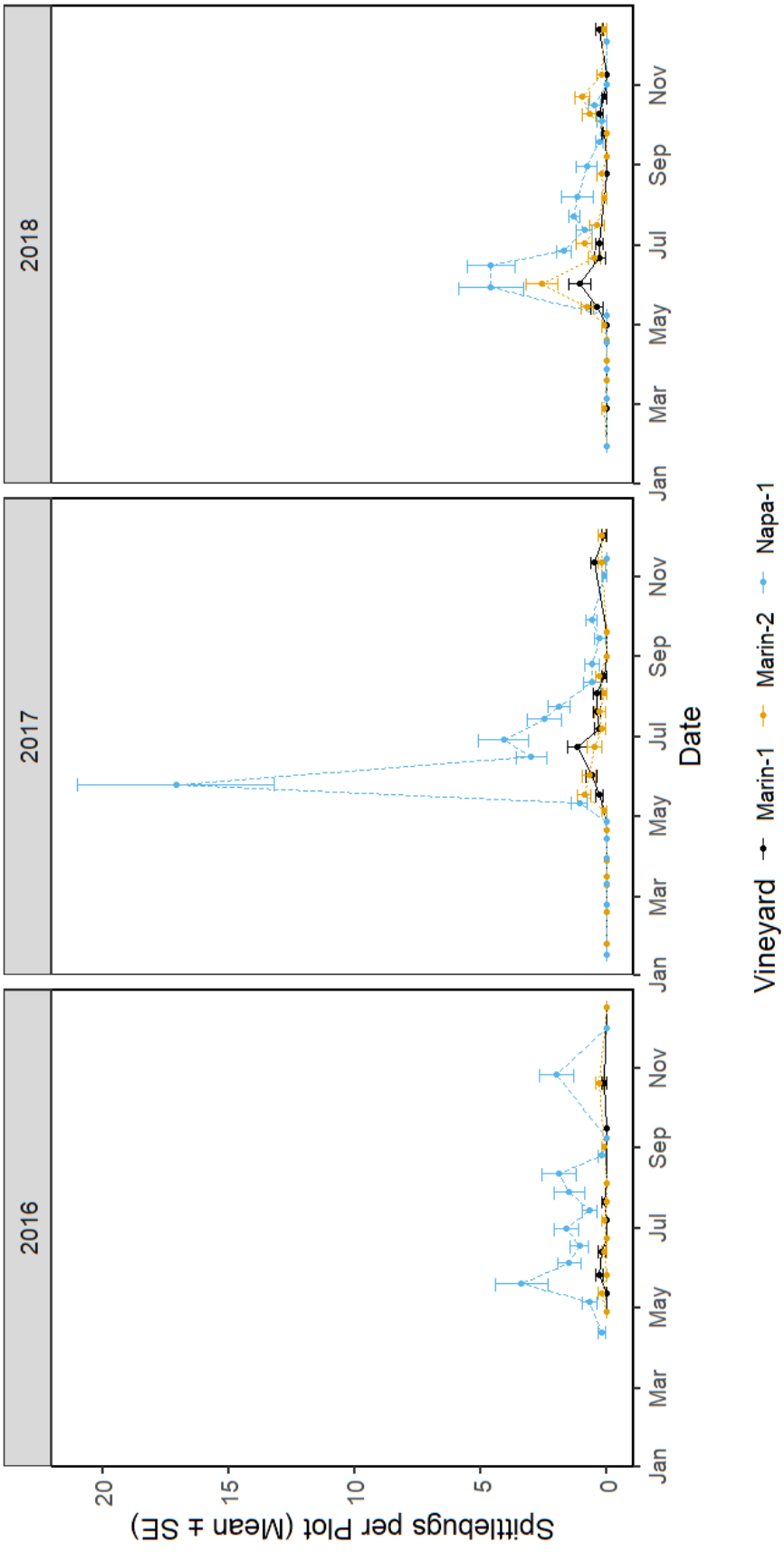
Figure 1: Mean *P. spumarius* abundance per survey plot of spittlemasses (A.), sweep caught adults (B.), and trap caught adults (C.) vs survey date for the years 2016, 2017, and 2018 for Marin-1 (black/dashed line), Marin-2 (goldenrod/dotted line), and Napa-1 (blue/solid line). Although the survey data is presented for all three years, please note that zero-inflated negative binomial GLMM analyzing spittlemass data excluded 2016 and that the 2017 and 2018 sweep and trap data were truncated to the 2016 date range for their respective zero inflated negative binomial GLMMs.

Figure 2: Results of a binomial GLM relating *P. spumarius* natural infectivity at Napa-1 with month and year as continuous variables and natural infectivity reported as the proportion of *X. fastidiosa*-positive test plants. Please note that while this graph is presented to delineate differences among months and years, the final model selected after model simplification dropped the year and interaction effect.

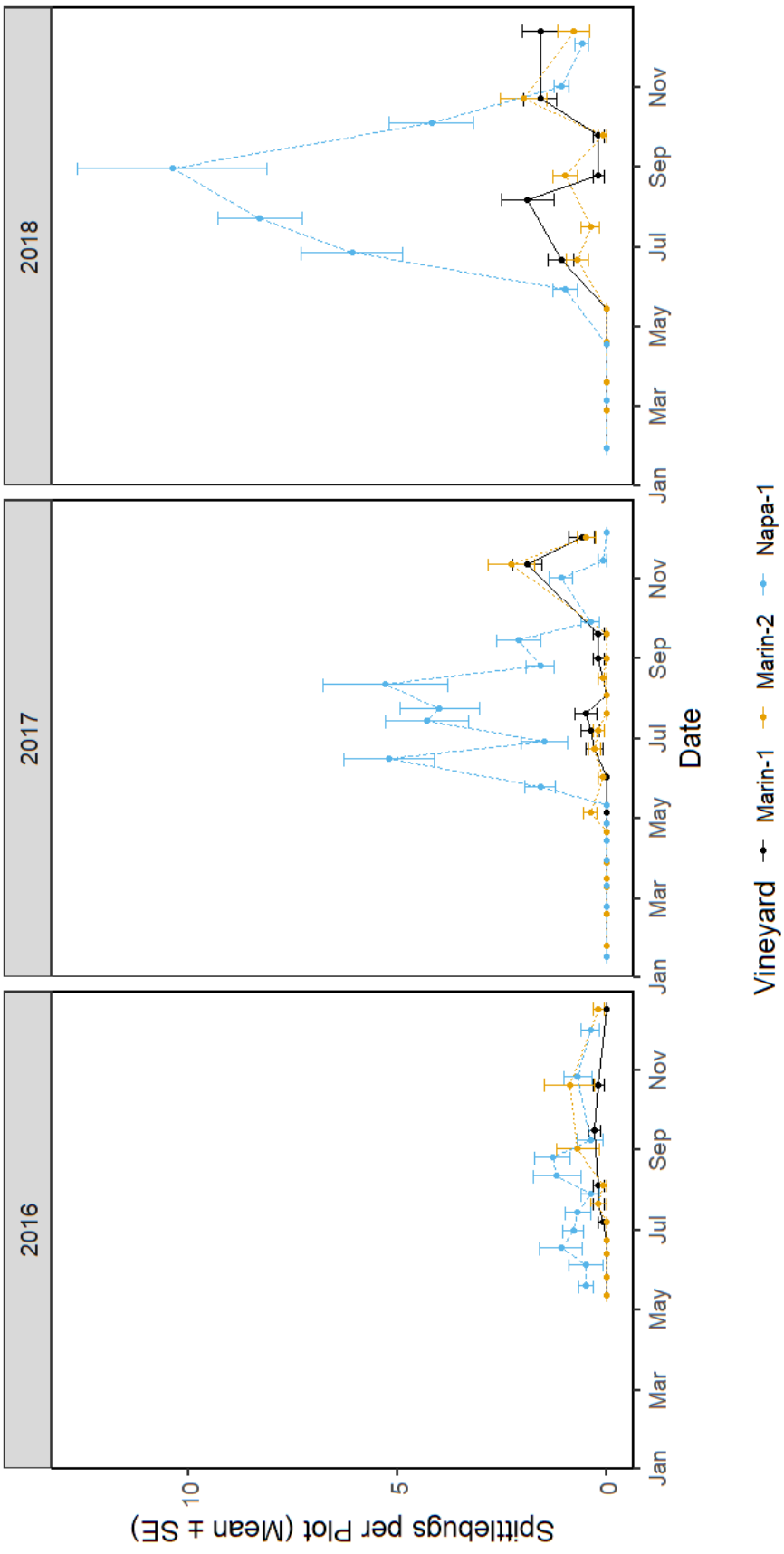
Figure 3: A. Max monthly temperature (red/dashed), mean monthly temperature (black/solid) and minimum monthly temperature (blue/dotted) for Marin-1, Marin-2, and Napa-1 from 2016-2018. Cumulative degree days for Marin-1 (red/dashed), Marin-2 (green/dotted) and Napa-1 (blue/solid) for 2016, 2017, and 2018.


2016
2017
2018
Jan
Mar
May
0
10
20
30
Spittlemasses per Plot (Mean ± SE)
Date
Vineyard
Marin-1
Marin-2
Napa-1

**A.**


2016
2017
2018
Jan
Mar
May
Jul
Sep
Nov
0
5
10
15
20
Spittlebugs per Plot (Mean ± SE)
Date
Vineyard
Marin-1
Marin-2
Napa-1

**B.**


2016
2017
2018
Jan
Mar
May
Jul
Sep
Nov
0
5
10
Spittlebugs per Plot (Mean ± SE)
Date
Vineyard
Marin-1
Marin-2
Napa-1

**C. Figure 1.**

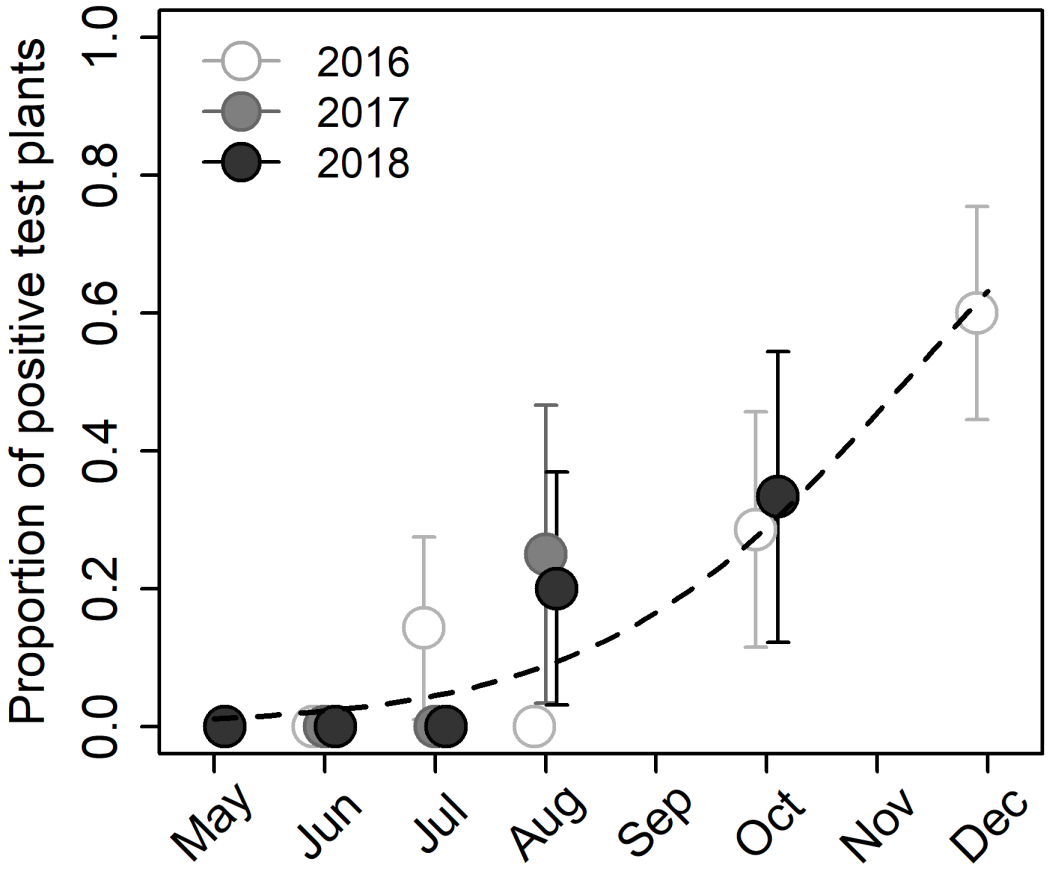
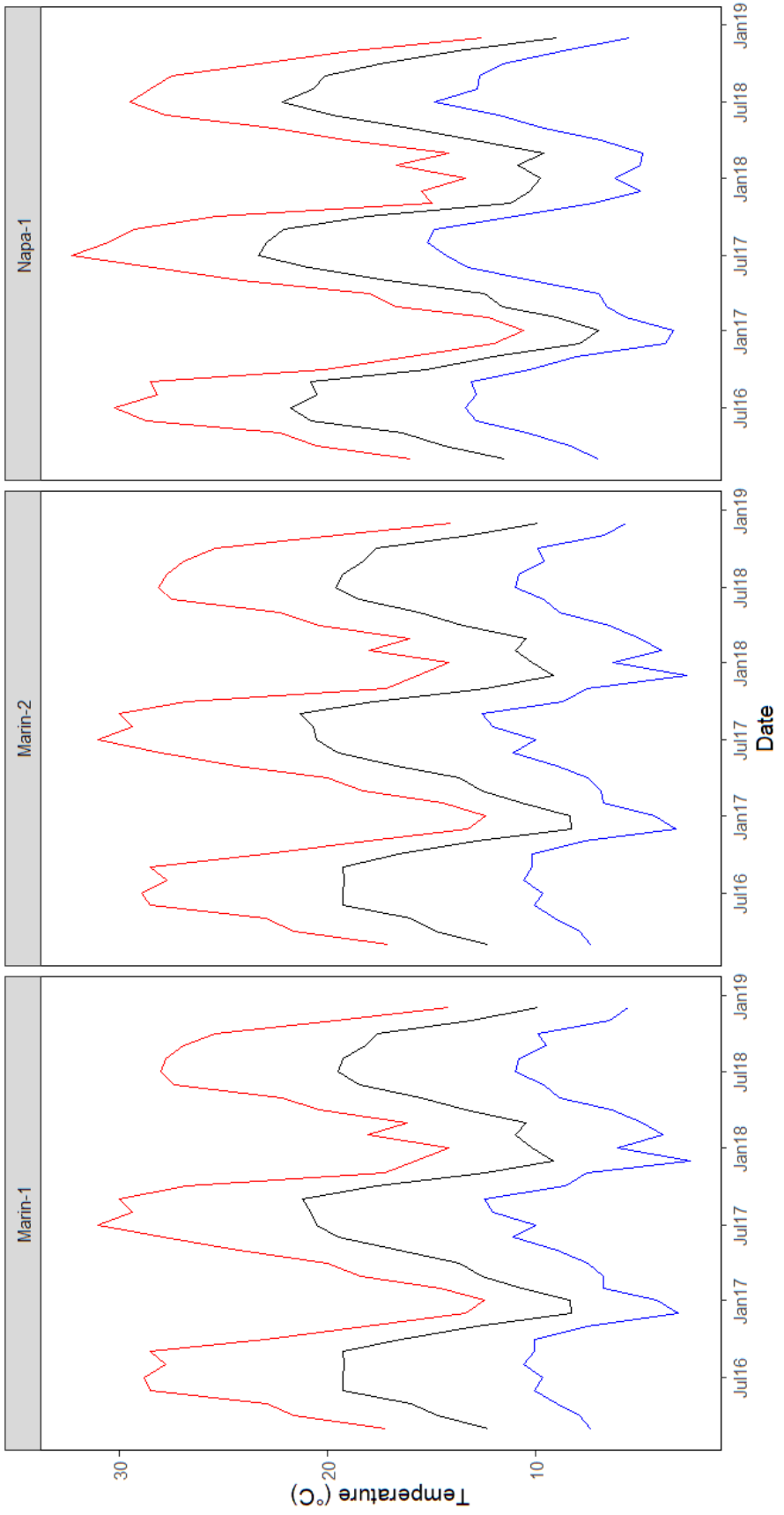
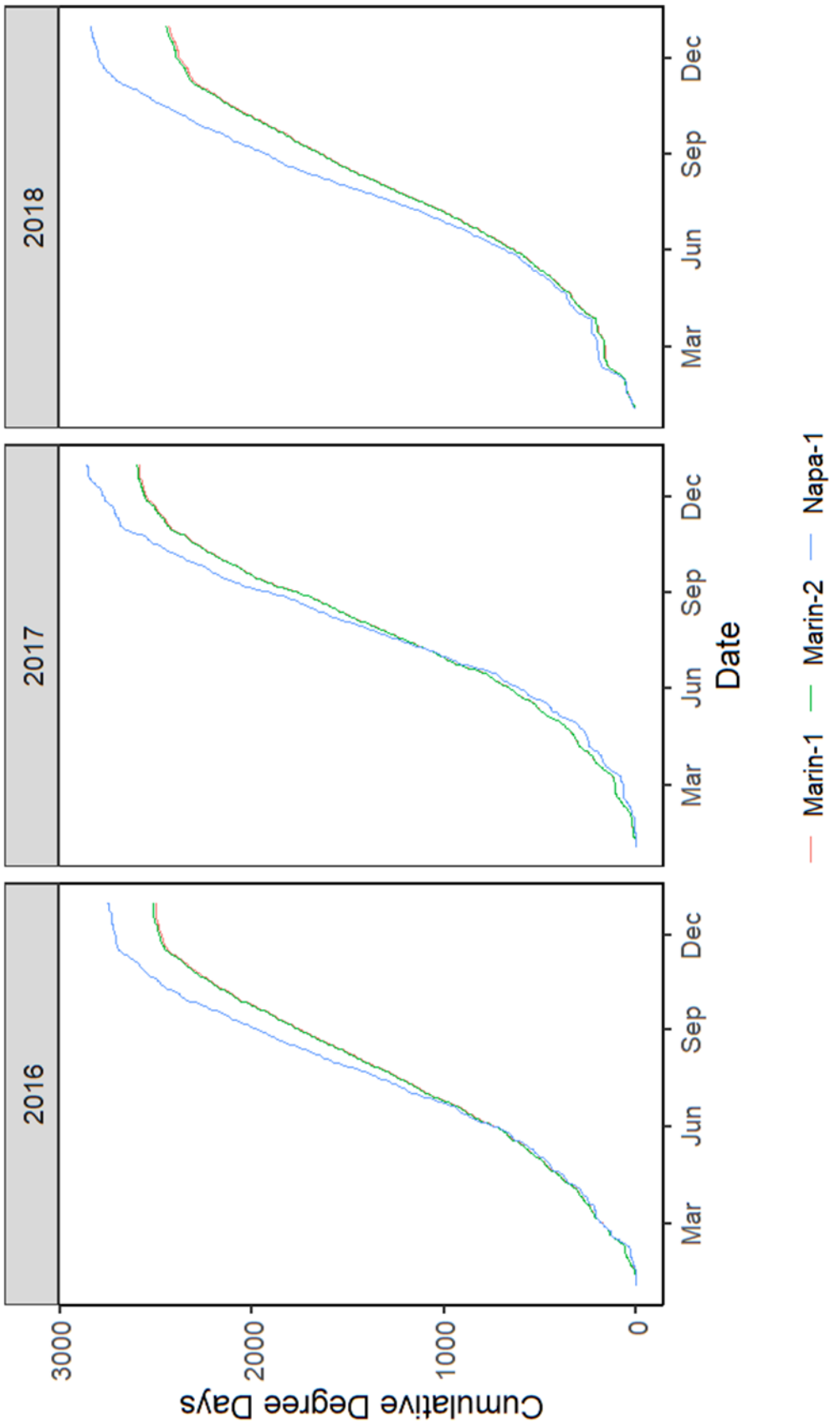

Proportion of positive test plants
0.0
0.2
0.4
0.6
0.8
1.0
2016
2017
2018
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

**Figure 2.**


Marin-1
Marin-2
Napa-1
Temperature (°C)
30
20
10
Jul16
Jan17
Jul17
Jan18
Jul18
Jan19
Date

**A.**


2016
2017
2018
Cumulative Degree Days
3000
2000
1000
0
Mar
Jun
Sep
Dec
Date
Marin-1
Marin-2
Napa-1

**B. Figure 3.**

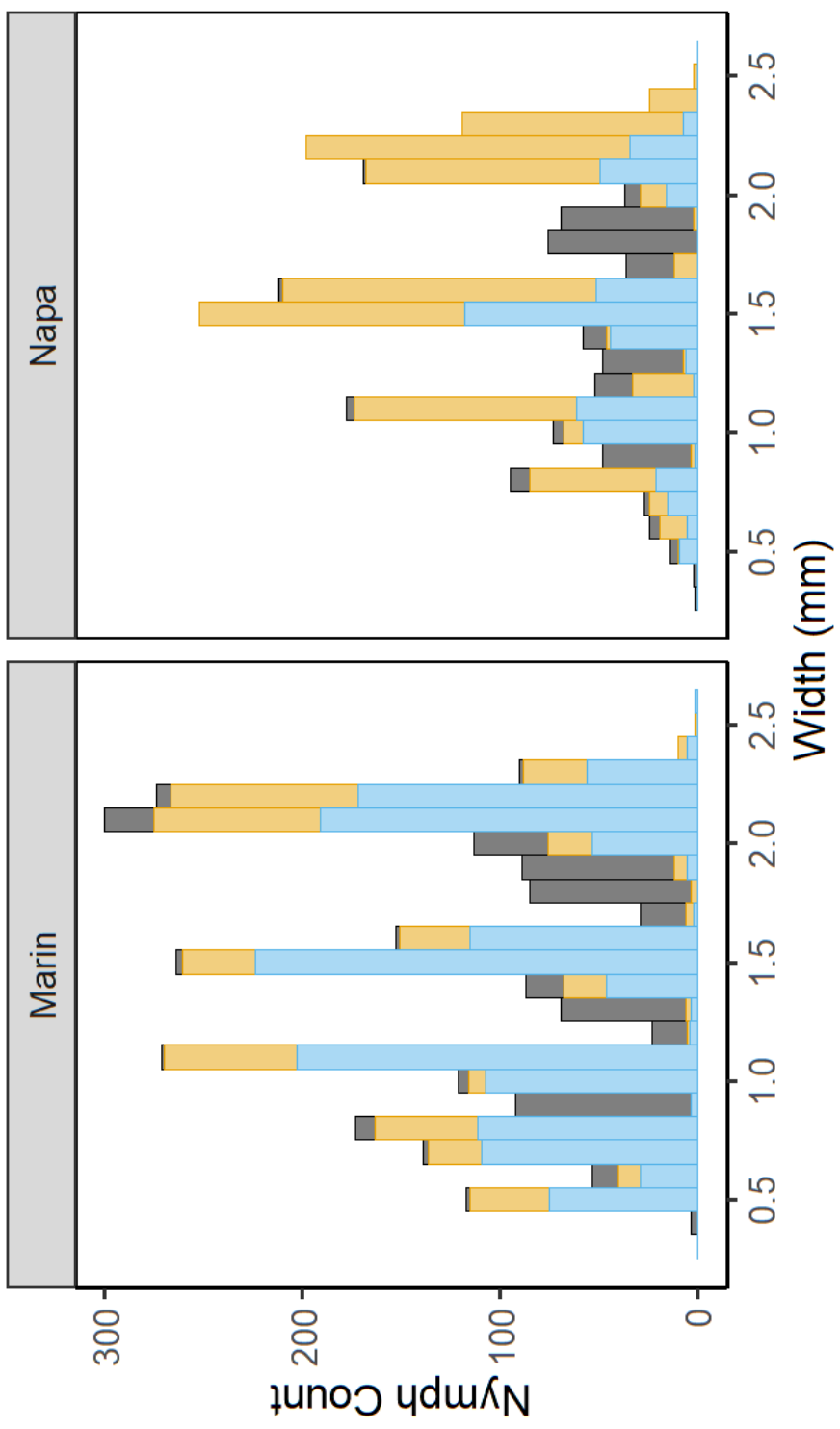
**Supplementary Table 1. Dates and associated cumulative degree days to that point (DD) of first, last, and peak occurrence of *P. spumarius* adults caught by sweep (peak dates = max no of adults sampled).**

| | | Marin-1 | | | Marin-2 | | | Napa-1 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Unit | first | peak | last | first | peak | last | first | peak | last |
| 2016 | Date | May 26 | May 26 | Oct 20 | May 2 | Oct 20 | Oct 20 | Apr 12 | May 19 | Oct 26 |
| | DD | 584.3 | 584.3 | 2142 | 521.5 | 2252.3 | 2252.3 | 369.9 | 636.4 | 2557.8 |
| 2017 | Date | May 5 | Jun 23 | Dec 2 | May 5 | May 17 | Feb 27 (2018) | May 11 | May 25 | Nov 1 |
| | DD | 464.2 | 928.8 | 2555.2 | 465.2 | 532.9 | 2767 | 441.6 | 561.3 | 2679.8 |
| 2018 | Date | May 14 | Jun 1 | Dec 13 | Apr 30 | Jun 1 | Dec 13 | May 29 | May 29 | Oct 16 |
| | DD | 509.4 | 637.7 | 2395.9 | 413.8 | 642.7 | 2410.3 | 657.6 | 657.6 | 2459.9 |

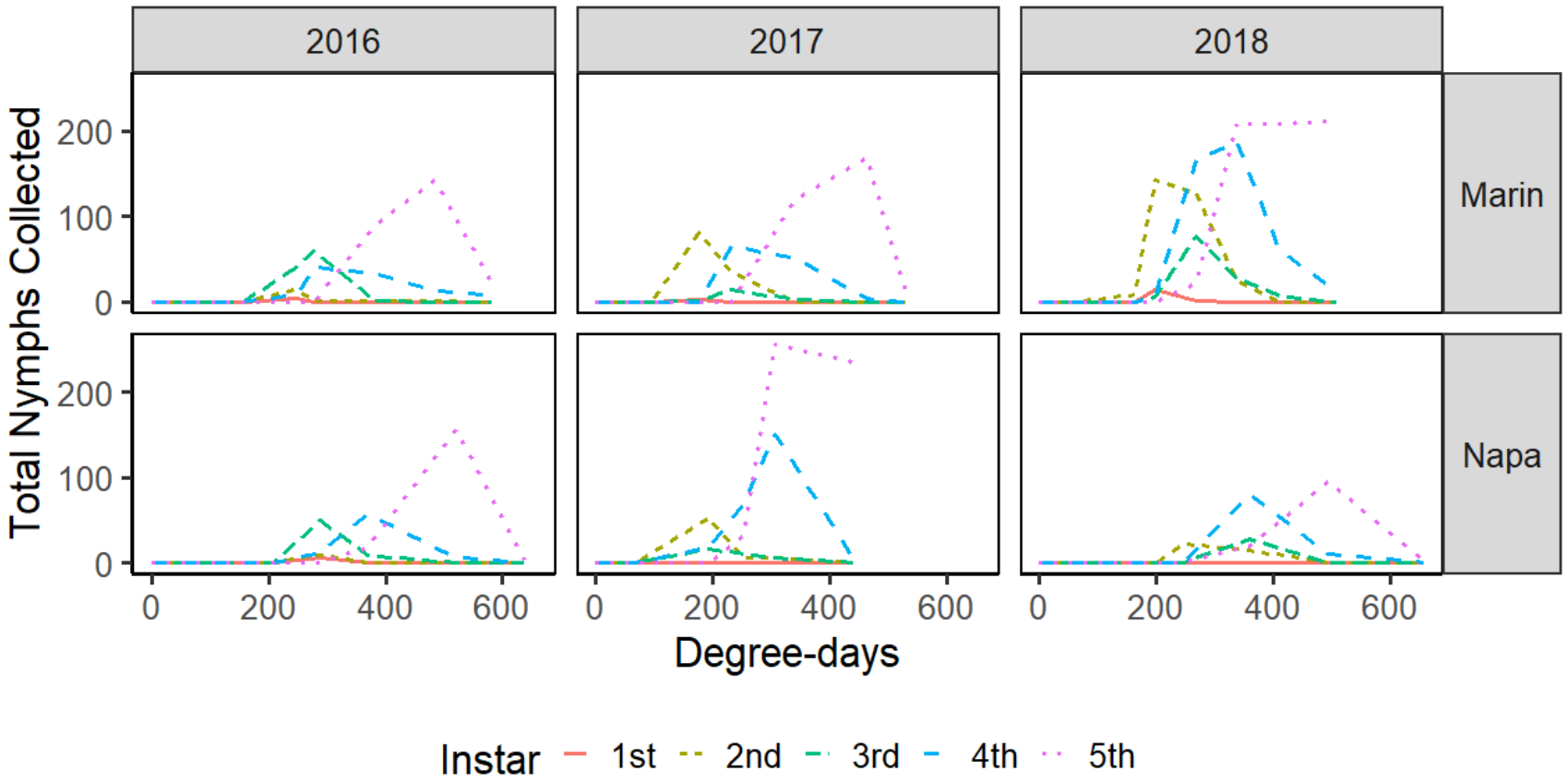
**Supplementary Table 2. Dates and associated cumulative degree days to that point (DD) of first, last, and peak occurrence of *P. spumarius* adults caught by trap (peak dates = max no of adults sampled).**

| | | Marin-1 | | | Marin-2 | | | Napa-1 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Unit | first | peak | last | first | peak | last | first | peak | last |
| 2016 | Date | Jul 21 | Jul 21 | Oct 20 | Jul 21 | Oct 20 | Dec 17 | May 19 | Aug 11 | Dec 1 |
| | DD | 1276.4 | 1276.4 | 2142 | 1284.2 | 2252.3 | 2513.2 | 636.4 | 1703.0 | 2713.8 |
| 2017 | Date | Jun 23 | Nov 11 | Dec 2 | May 5 | Nov 11 | Dec 2 | May 25 | Jul 24 | Nov 14 |
| | DD | 928.8 | 2467.4 | 2555.2 | 465.2 | 2476.1 | 2563.9 | 561.3 | 1356.6 | 2481.4 |
| 2018 | Date | Jun 21 | Aug 24 | Dec 13 | Jun 21 | Oct 22 | Dec 13 | May 29 | Aug 30 | Dec 3 |
| | DD | 826.5 | 1571.5 | 2395.9 | 832.5 | 2169.2 | 2410.3 | 657.6 | 1915.1 | 2800.8 |

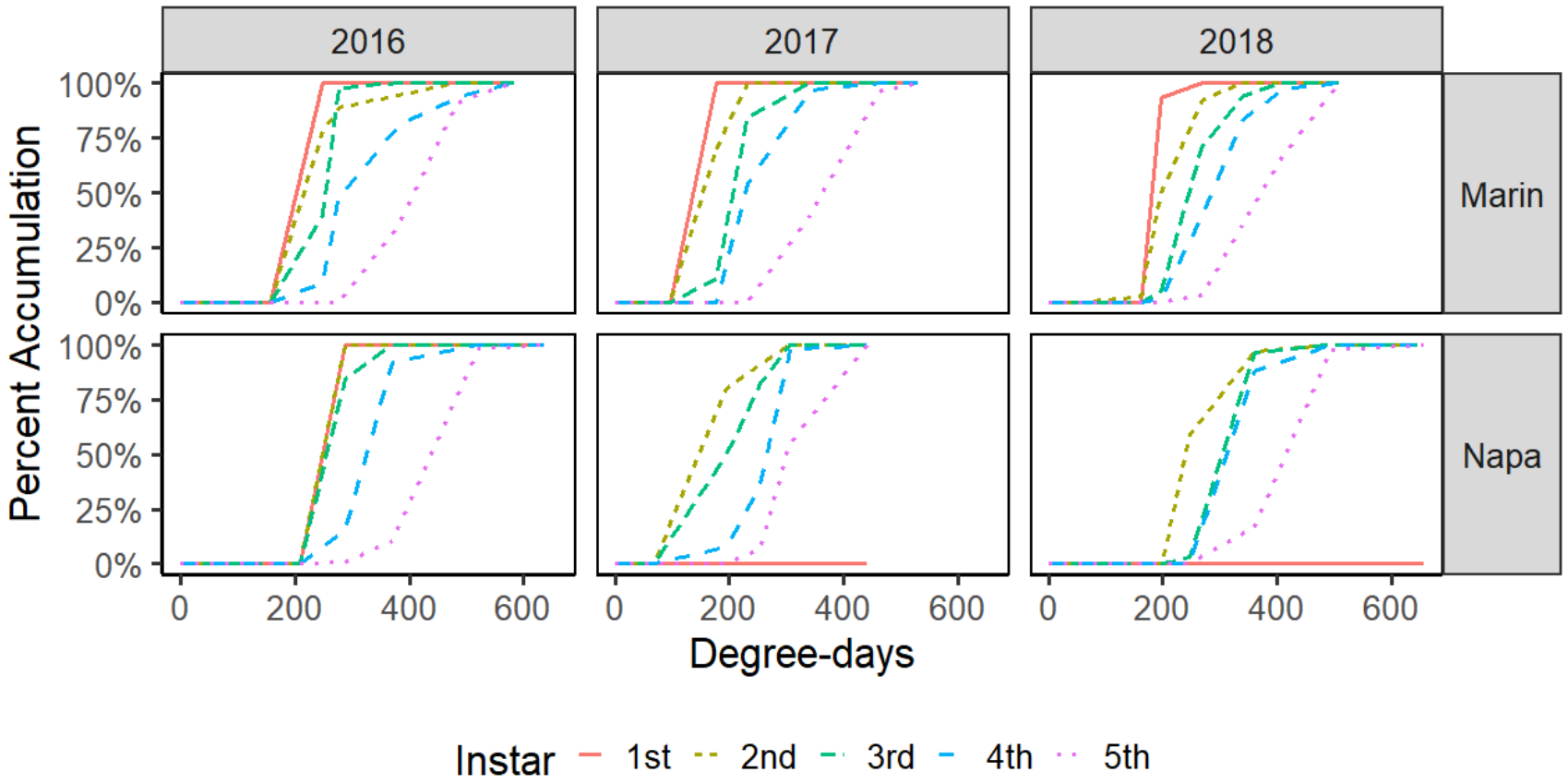
**Supplementary Figure 1: A) Histogram of *P. spumarius* nymphs binned by head width, B) observed total abundance of *P. spumarius* instars over the year, and C) cumulative abundance of nymphal instars of *P. spumarius* in relation to the accumulated degree-days over the year in Marin and Napa Vineyards for 2016, 2017, and 2018.**


Year
2016
2017
2018
Marin
Napa
Nymph Count
0
100
200
300
0.5
1.0
1.5
2.0
2.5
Width (mm)

**A.**


2016
2017
2018
Marin
Napa
Total Nymphs Collected
0
100
200
0
200
400
600
Degree-days
Instar
1st
2nd
3rd
4th
5th

B.


2016
2017
2018
Marin
Napa
Percent Accumulation
100%
75%
50%
25%
0%
0
200
400
600
Degree-days
Instar
1st
2nd
3rd
4th
5th

C.

Chapter 3: Assessment of nymphal ecology and adult *Xylella fastidiosa* transmission biology of *Aphrophora* nr. *permutata* (Hemiptera: Aphrophoridae) in California vineyards

**Abstract**

Although *Aphrophora* nr. *permutata* (Hemiptera: Aphrophoridae) is a reported vector of the plant pathogen *Xylella fastidiosa*, its ecology and role in Pierce's disease of grapevines dynamics in coastal California vineyards are poorly understood. From 2016-2020, we surveyed the abundance of *A.* nr. *permutata* nymphs among potential host plants along the vineyard floor, the field edges and adjacent vegetation in vineyards in Napa and Sonoma county. In 2019 and 2020, vineyards adjacent to woodland habitat hosted larger *A.* nr. *permutata* populations than those next to riparian habitat, while in 2017 and 2018, the nymphal populations were similar among riparian and woodland sites. Among sampled 2020 plant taxa, *A.* nr. *permutata* nymph abundance was positively associated with total area occupied by *Helminthotheca echoides*, *Vicia sativa*, and *Daucus carota* and negatively associated with total area occupied by *Taraxacum officinale*. In 2018 and 2019, we also tracked early-season occurrence and development of *A.* nr. *permutata* nymphs among potential host plants. Analyses showed a significant effect of site, year, and plant taxa on first detection of nymphs and a significant effect of site and year on the estimated development time between 1$^{st}$ and 5$^{th}$ instars. In 2019, we conducted experiments of transmission of *X. fastidiosa* from grapevine to grapevine with individual and groups of five *A.* nr. *permutata* adults. In these studies, 5% (3 of 60) individual *A.* nr. *permutata* and 7.7% (1 of 13) of groups successfully transmitted *X. fastidiosa*. This study provides preliminary evidence of potential host plant associations with *A. nr. permutata* abundance and phenology that should be explored further with field and greenhouse-based approaches.

## Introduction

Insect herbivores can be generally classified by their host range, spanning from specialists that feed on a single host (monophagy) to insects associated with a multitude of reported hosts (polyphagy). Although polyphagous insects can have tens to hundreds of hosts, these insects often associate more closely with a subset of "primary hosts" (Rafter and Walter 2020). The association between insect vectors of plant pathogens and their primary hosts can have consequences for disease spread (Purcell 1975, Purcell 1976, Walls et al. 2019). Understanding the effects of host-plant associations on pest insect abundance, development, and population dynamics is central to develop and inform potential management strategies.

The western pine spittlebug, *Aphrophora permutata* (Uhler) (Hemiptera: Aphrophoridae), is a vector of the plant pathogenic bacterium *Xylella fastidiosa* (Wells) (Xanthomonadales: Xanthomondaceae) (Severin 1950). Vectors of *X. fastidiosa* consist of xylem sap-feeding insects such as spittlebugs (Hemiptera: Cercopoidea) and sharpshooter leafhoppers (Hemiptera: Cicadellidae, subfamily Cicadellinae) (Redak et al. 2004). *Xylella fastidiosa* is a xylem-limited bacterium responsible for numerous plant diseases such as olive quick decline syndrome (OQDS), citrus variegated chlorosis (CVC), and Pierce's disease (PD) of grapevine (Sicard et al. 2018). Pierce's disease of grapevine has threatened California viticulture for over a century (Pierce 1892). California spends approximately $104 million each year on costs associated with the management of PD and its insect vectors (Tumber et al. 2014). In the California North Coast, PD outbreaks have been closely associated with the ecology of the blue green sharpshooter, *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae) (Severin 1949, Purcell 1975). Patterns of PD associated with *G. atropunctata* demonstrate a strong "edge effect" with infected vines concentrated close to riparian habitats where adults overwinter (Purcell 1974, Purcell 1975). Outbreaks of PD in Southern California over the last 25-30 years were driven by the introduction and establishment of the glassy winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) (Sorensen and Gill 1996, Blua et al. 1999). Spittlebug vectors such as *A. permutata* and *Philaenus spumarius* (Linnaeus) (Hemiptera: Aphrophoridae) were not considered important in California PD outbreaks as their occurrence in vineyards was considered rare (Delong and Severin 1950). Since 2015, PD outbreaks in the California North Coast have included cases characterized by an increased incidence of diseased vines far from the riparian border or at non-riparian sites (Almeida et al. 2019, Beal et al. 2021). These patterns have led to speculation that *X. fastidiosa* vectors other than *G. atropunctata*, such as spittlebugs, may play a role in California PD outbreaks.

Although past studies have described nymphal and reproductive hosts of *A. permutata* (Delong and Severin 1950) and its developmental biology on Monterey pine, *Pinus radiata* (D. Don) (Pinales: Pinaceae), in California (Kelson 1964), the ecology of this species in vineyards and its contribution to PD epidemics is poorly understood. Observations of *A. permutata* on vineyard groundcover far from conifers (> 5 meters) or at vineyard sites lacking conifers, a primary reproductive host (Kelson 1964, *personal communication with Dr. Vinton Thompson*), led to speculation that the *Aphrophora* spittlebugs observed might be a different species. Assessment of collected specimens along with historical *A. permutata* specimens from two prior studies conducted in California (Delong and Severin 1950, Kelson 1964) by Thompson led to the conclusion that the specimens were morphologically similar, although molecular confirmation is in progress. For clarity, we refer to the study species as *Aphrophora* nr. *permutata*.

To understand the role of *A.* nr. *permutata* in the epidemiology of PD, we characterized the ecology of this species in vineyard systems. We hypothesized that site characteristics such as surrounding habitat or the community composition of potential host plants would influence the occurrence and abundance of *A.* nr. *permutata* nymphs. We also predicted that site-to-site differences in host plant composition would affect the development of *A.* nr. *permutata* nymphs. Understanding the potential role of *A.* nr. *permutata* in the occurrence of PD epidemics also required a reassessment of the capacity of adults to transmit the pathogen from grapevine-to-grapevine. We initiated our assessment of *A.* nr. *permutata* ecology and transmission biology through a series of field and greenhouse studies.

**Materials and Methods**

**Nymphal abundance**

In 2017, we monitored *A.* nr. *permutata* nymph abundance in late April or early May at 32 vineyard sites (Table S1, Table S2) in Napa and Sonoma counties that were part of a broader project focused on PD epidemiology in coastal Northern California that was initiated in 2016 (Almeida et al. 2019). At each of the vineyards, surveys of the surrounding plant community were used to characterize the predominant habitat adjacent to the vineyard as either riparian, woodland or chaparral, or other (i.e. grassland, open space, or other vineyards; MP Daugherty, unpublished data).

The timing of nymphal surveys was selected to primarily coincide with the presence of 4th and 5th instars of *A*. nr. *permutata*, ensuring that all eggs had hatched and thatno nymphs had undergone their final molt to the adult stage. We surveyed spittlemasses, the foamy excrement that surrounds the nymphal stages of spittlebug species (Weaver and King 1954), as they could be assessed and identified through visual observations. Each site survey was comprised of up to 11 transects: six row middles (resident vegetation growing between vine rows), all four vineyard edges, and an adjacent patch of surrounding vegetation outside of the vineyard. The adjacent patch of outside vegetation could not be sampled at 9 sites due to adverse conditions such as steep slopes or poison oak (*Toxicodendron diversilobum* (Torr. & A. Gray) (Sapindales: Anacardiaceae), a common plant at these sites that produces an oil, urushiol, which can cause severe skin irritation). The six row middles were selected to be representative of the entire vineyard. Two-minute counts of *A*. nr. *permutata* spittlemasses amongst all herbaceous plant species were conducted for each transect, for a total of 20-22 min per vineyard (depending on whether the adjacent vegetation was sampled), and a total of 342 transects among all sites. We analyzed the number of nymphal spittlemasses per transect using a generalized linear mixed-effects model (GLMM), with fixed effects of site type (riparian, woodland/chaparral, or grassland/other) and location (vineyard row, vineyard edge, adjacent habitat), a random effect of transect identity nested within site to account for autocorrelation stemming from repeated measurements at each site, and a zero-inflated, negative binomial error term to account appropriately for dispersion in the data (lme4, Bates et al. 2015). All analyses were conducted in the R programming language, ver. 4.02 (R Core Team 2020.

In 2018, we conducted a survey of 31 of the same 32 vineyard sites in mid-April (Table S2). These surveys focused on spittlemass abundance along the vineyard edge and the surrounding vegetation. Row middles were not sampled given the low numbers observed in 2017. Depending on vegetation abundance, we conducted two to four transects (each covering

125 $m^2$). Fifty plants per transect, belonging to known host species of *A. permutata* (Delong and Severin 1950), were selected haphazardly and inspected for *A.* nr. *permutata* spittlemasses. At sites in which *A.* nr. *permutata* co-habitated with *P. spumarius*, we verified the spittlebug species inhabiting each spittlemass. Only spittlemasses that contained *A.* nr. *permutata* nymphs were counted. Due to time constraints, we counted spittlemasses with *A.* nr. *permutata* rather than the number of nymphs, although spittlemasses often contained 1-3 nymphs (occasionally up to 10 nymphs). Across 75 total transects among 31 sites, we analyzed the number of spittlemasses per transect using a Poisson GLMM with a fixed effect of site type and a random effect of transect id nested within site.

In 2019, we employed a similar sampling approach as in 2018 except individual nymphs were counted on each plant and only two transects were evaluated for each site. The 2019 transects were conducted at the same sixteen Sonoma sites, and at nine of the sixteen Napa sites at which *A.* nr. *permutata* was present in 2018 (Table S2). Across the 48 total transects among 24 sites, we analyzed the number of plants of all species with spittlemasses present per transect using a zero-inflated negative binomial GLMM, with a fixed effect of site type, number of unique plant taxa (w/ or w/o spittlemasses) as a covariate, and a random effect of transect nested within site. To understand the potential for host plant preference to contribute to patterns of *A.* nr. *permutata* abundance, we next analyzed the frequency of occurrence of spittlemasses on inspected plants for the 20 most common plant taxa with a binomial GLMM with a fixed effect of plant taxa and a random effect of transect within site.

Based on the apparent *A.* nr. *permutate*-host plant associations observed in 2018 and 2019, we adapted a survey methodology in 2020 that accounted for the ground area covered by individual plant species. At four Napa sites and four Sonoma sites, we identified four sampling locations (plots) among the row middles and four among the vineyard edges (Table S1, Table S2). The management status of each plot (mowed or not mowed) was recorded and, then a 0.5 m x 0.5 m quadrat was haphazardly placed at three locations within the plot. For edge plots, the distance between quadrats ranged from 10-30 m while, up to 100 m separated quadrats within rows. Within each quadrat, we estimated the area covered by each plant species (or bare soil) using a ruler, and recorded the total number of *A.* nr. *permutata* nymphs. Surveys were conducted twice for each site, in March and in April, to account for potential phenological shifts in hosts. Across 372 total quadrats among 124 plots over two months, we analyzed the number of nymphs per quadrat with a zero-inflated negative binomial GLMM with month, site type, location (vineyard row, edge) and management status (mowed or not) as fixed effects, and total number of plant taxa and total plant cover as covariates. Model simplification via likelihood ratio tests was used to determine the minimum adequate model for capturing effects on nymphal counts (Crawley 2009). To explore qualitatively the effect of plant community composition on nymph abundance at the plot scale, we used the 69 plots where *A.* nr. *permutata* nymphs were present in at least one quadrat to construct a non-metric multi-dimensional scaling (NMDS) plot (R package "vegan"; Oksanen et al. 2018). In the NMDS plot, we considered the effects of cover from the 20 most common plant taxa and bare soil on nymphal abundance, with counts per plot binned into one of four abundance categories (1-9, 10-19, 20-29, 30+). Next, we used a multiple GLMM, with a random effect of plot nested within site and Poisson error, to analyze the effects of relative cover of key taxa on nymphal abundance. The initial model included relative cover estimates, as covariates, for the 9 plant species the NMDS revealed to be most strongly

related to nymphal abundance and the amount of bare soil: curly dock (*Rumex crispus*), dandelion (*Taraxacum officinale*), geranium (*Geranium* spp.), buckhorn plantain (*Plantago lanceolata*), miner's lettuce (*Claytonia perfoliata*), grass (*Poa* spp.), vetch (*Vicia sativa*), bristly oxtongue (*Helminthotheca echoides*), and wild carrot (*Daucus carota*). Again, model simplification via likelihood ratio tests was used to determine the minimum adequate model for effects of cover on nymphal abundance.

**Nymphal development**

In 2018, four Napa and three Sonoma sites were selected for a separate survey of patterns of *A.* nr. *permutata* activity and development over the season. In 2019, an additional Napa site and two additional Sonoma sites were assessed. The site descriptions are presented in the supplementary materials (Table S1, Table S2). In mid-February of 2018, at each site, we began marking groundcover plants and weeds that contained first instar nymphs. All such plants were identified to species and inspected every other week until the end of May, when all nymphs developed into adults or otherwise disappeared. For each site, we assessed at least 40 plants (or 100 nymphs when possible), recording the number of nymphs per plant and the corresponding stage of each nymph. In 2019, the same approach was used to monitor 30 plants per site, beginning in early January.

Across all sites and surveys, a total of 558 observations of *A.* nr. *permutata* nymphs were made. We subjected this dataset to two related analyses. Because the plant taxa present at each site differed among sites and years, it was not possible to analyze some variables or interactions. To assess how early in the year *A.* nr. *permutata* nymphs became active in vineyards, we used a generalized linear model (GLM) on the week number of the first recorded observation of a nymphal spittlebug on a plant, with year, vineyard site, and plant taxa included as fixed effects, and Poisson error (Crawley 2009). Although a total of 28 host plant taxa were represented among sites and years, we limited our analysis to plants that were observed at least five times at one or more sites. This reduced our dataset to 16 plant species and 540 total observations. Next, to assess variation in development time, we used a similar Poisson GLM on the number of weeks between the first recording of a 1$^{st}$ instar and the last recording of a 5$^{th}$ instar on the same plant, with fixed effects of year, site, and plant taxa. As multiple insects were sometimes present on the same plant, this number is an approximation of the duration of nymphal development. Because 1$^{st}$ or 5$^{th}$ instars were occasionally absent on flagged plants, this reduced the total number of observations to 210 nymphs over eight sites on eight plant taxa. To explain potential differences among sites, we collected additional biological and environmental data, including temperature and relative humidity recorded using HOBO data loggers, nymph head capsule size, and measurements of vine growth stage (Appendix 1).

***Xylella fastidiosa* transmission**

To confirm the competence of *A.* nr. *permutata* as a *X. fastidiosa* vector, in 2019, we conducted grapevine-to-grapevine transmission experiments using individuals and groups of insects. Over 500 5$^{th}$ instar nymphs of *A.* nr. *permutata* were collected in Sonoma County in April and brought to greenhouses at UC Berkeley to be raised to adulthood. Nymphs were caged in BugDorms (61 cm$^3$; Bioquip Products) on a mixture of *X. fastidiosa*-free *H. echoides*, *V. sativa*, and Cabernet Sauvignon seedlings (*Vitis vinifera* (Linnaeus) (Vitales: Vitaceae)). Plants were replaced weekly to prevent the possible transmission and re-acquisition of *X. fastidiosa*, as

the nymphs were of unknown infection status. This method was repeated until all nymphs had eclosed to adulthood. Given that *X. fastidiosa* is lost after each nymphal molt (Purcell and Finlay 1979), this ensured a greenhouse population of *X. fastidiosa*-free *A.* nr. *permutata*. In late May, we caged 170 adults, in groups, for a four-day acquisition access period (AAP) on one of seven *V. vinifera* cv. Cabernet Sauvignon source plants that had been needle inoculated one month prior with the Napa-1 strain of *X. fastidiosa*. After the four-day AAP, the 125 surviving adults were caged either individually or in groups of five spittlebugs on a single, two-month-old uninfected cutting of *V. vinifera* cv. Cabernet Sauvignon for a four-day inoculation access period (IAP). For the IAP, we caged 60 individual spittlebugs and 13 groups of five spittlebugs (65 spittlebugs total). The test plants were kept for two months in a greenhouse at which point their infection status for *X. fastidosa* was determined using the live cell culturing method (Hill and Purcell 1995). We analyzed transmission frequency with a binomial GLMM with a fixed effect of group size (1 or 5) and a random effect of source plant ID to control for variability in acquisition rates due to differences in source plant infection levels or condition. We then used a binomial test to evaluate the hypothesis that the number of infected test plants was greater than zero.

**Results**

**Nymphal abundance**

Among all transects in 2017, the number of spittlemasses observed ranged from 0 to 17 per transect, with an overall mean of 0.94 spittlemasses per transect. There were significant effects of sampling location ($\chi^2$=38.257, df=2, P<0.0001) and site type ($\chi^2$=7.882, df=2, P=0.0194) on *A.* nr. *permutata* spittlemass abundance. Spittlemass number was nearly twice as high in the vegetation adjacent to vineyards than in vineyard edges, which, in turn, was more than 8-fold higher than spittlemass abundance in the row middles (Fig. 1A). Spittlemasses were approximately 20% more abundant at sites adjacent to riparian vegetation compared to woodland or chaparral habitat; both of which had more than 10 times as many spittlemasses than at sites surrounded by grassland or other vineyards (Table 1).

Analysis of 2018 spittlemasses showed a significant effect of site type ($\chi^2$=11.678, df=2, P=0.0029). Spittlemasses were similarly abundant at riparian and woodland/chaparral sites, which was more than 9 times greater than at other sites (Table 1). Spittlemasses were observed on at least 28 plant taxa, collectively, among all sites and transects (Fig. S1). *R. crispus* and shortpod mustard (*Hirschfeldia incana*) were the most prevalent host plants (occupying approximately 25% of transits and 35% of sites), followed by *H. echoides*, catsear (*Hypochaeris radicata*), poison hemlock (*Conium maculatum*), *Sonchus* spp., and *V. sativa* (Fig. S1).

In 2019, 0 to 66% of plants per transect had at least one *A.* nr. *permutata* nymph present, with an overall mean of 13.75%. The number of unique plant taxa ranged from 1 to 13 per transect. Site type again had a significant effect on the number of plants occupied by *A.* nr. *permutata* nymphs ($\chi^2$=8.594, df=2, P=0.0136), but the effect of total plant taxa richness was not significant ($\chi^2$=0.1217, df= 1, P=0.7272). Woodland or chapparal sites had the highest frequency of spittlemass-occupied plants, and riparian sites an intermediate frequency (Table 1). Among the 20 most common plant taxa, there were significant differences in the proportion with *A.* nr. *permutata* spittlemasses present ($\chi^2$=81.48, df=19, P<0.0001; Fig. 2). California poppy (*Eschscholzia californica*), *Trifolium* spp., *Geranium* spp., and *Sonchus* spp. had the highest

proportion occupied (>25%), some of the most abundant plants [*R. crispus*, Italian thistle (*Carduus pycnocephalus*), *C. maculatum*] were occupied less frequently, and spittlemasses were never observed on two moderately common taxa, cheeseweed (*Malva parviflora*) and *T. officinale* (Fig. 2).

In 2020 surveys, the number of *A.* nr. *permutata* nymphs ranged from 0 to 23 per quadrat, with a mean of 1.67. The final model for effects on nymph counts per quadrat included only significant effects of sampling location ($\chi^2$=16.359, df=1, P<0.0001) and site type ($\chi^2$=5.247, df=1, P=0.022); all other variables were eliminated during the model simplification process. At woodland/chapparal sites, there were ~4-fold more nymphs than at riparian sites (Table 1), and vineyard edges hosted ~4 times more nymphs compared to vineyard rows (Fig. 1B). Among the survey plots where *A.* nr. *permutata* nymphs were present in at least one quadrat, the highest nymphal counts occurred in plots dominated by taxa such as *D. carota*, *H. echoides*, *Trifolium* spp., *V. sativa*, soap plant (*Chlorogalum pomeridianum*), and *Sonchus* spp. (Fig. 3). The lowest number of nymphs were in plots dominated by pot marigold (*Calendula officinalis*), *E. californica*, *T. officinale*, *H. radicata*, *R. crispus*, and bare soil (Fig. 3). After model simplification, the final model for effects of cover included significant effects of only four taxa (Table 2). *H. echoides, V. sativa*, and *D. carota* were positively associated with *A.* nr. *permutata* nymph abundance while *T. officinale* was negatively associated with nymph abundance.

**Nymphal development**

Based on the 540 observations of *A.* nr. *permutata* nymphs made over the two years of development surveys, there was a significant effect of year ($\chi^2$=183.28, df=1, P<0.0001), site ($\chi^2$=32.638, df=8, P<0.0001), and plant taxa ($\chi^2$=32.351, df=15, P=0.0058) on the first detection of nymphs. Nymphs were found approximately four weeks earlier in 2019 than in 2018 (Fig. 4A), and the first detection of nymphs differed by up to seven weeks among sites (mid-February vs. early-April; Fig. 4B). Nymphs were found earliest on *D. carota*, *C. pomeridianum*, *P. lanceolata*, and horseweed (*Erigeron canadensis*; Fig. 4C), and latest on purple stem mustard (*Chorispora tenella*), filaree (*Erodium* spp.), California burclover (*Medicago polymorpha*), and *R. crispus* (Fig. 4C).

The estimated time between the first detection of 1$^{st}$ instars and that of 5$^{th}$ instars on the same plant ranged from 2 to 14 weeks, with a mean of 9.82 weeks. Development time differed significantly between years ($\chi^2$=25.747, df=1, P<0.0001) and sites ($\chi^2$=91.161, df=7, P<0.0001), but not plant taxa ($\chi^2$=7.857, df=7, P=0.3454). Development time was approximately two to three weeks shorter in 2018 than in 2019, with a difference of greater than four weeks among sites (Fig. 5). Development time ranged from less than eight weeks on chicory (*Cichorium intybus*) to ca. twelve weeks on *C. maculatum* and *H. incana*, but the site-specific nature of some plant taxa make it difficult to separate the plant effects from the site effects.

Some of the sites with more rapid *A.* nr. *permutata* development were moderately warmer during spring months (April and May) than those sites exhibiting relatively slower development (Fig. S2A), and were slightly less humid in April and May than sites with slower development rates, though one of sites with immediate development had the most consistent low humidity in the spring (Fig. S2B). At sites with more rapid *A.* nr. *permutata* development, adults emerged

when the vines were at early stages of leaf development (4-5 mature leaves) but at sites with slower development, adults emerged during the flowering period. (Table S4)

### *Xylella fastidiosa* transmission

*Xylella fastidiosa* detection in test plants revealed that 3/60 (0.05) plants exposed to individual insects harboered culturable *X. fastidiosa* while the pathogen was detected in 1/13 (0.077) test plants exposed to groups of insects. The effect of group size was not significant ($\chi^2$ = 0.0189, df = 1, $P$ = 0.8907; Fig. 6), but the overall number of infected test plants was significantly greater than zero (5.5% infected, 95% CI = 1.9% to 1, P<0.0001).

## Discussion

Our study re-assessed *A.* nr. *permutata* biology, ecology, and *X. fastidiosa* vector competence in California North Coast vineyards, expanding upon research conducted over 70 years ago (Delong and Severin 1950, Severin 1950). Our observations of a single *A.* nr. *permutata* nymphal generation from February to May each year align with previous assessments of the developmental period of this insect (Kelson 1964). Although Delong and Severin reported two generations of *A. permutata* each year in California, this second generation was later determined to be a separate species, *Aphrophora canadensis* (Walley) (Hemiptera: Aphrophoridae) (Delong and Severin 1950, Kelson 1964). Building off these initial studies of *A. permutata* biology and apparent plant hosts, we aimed to understand how site characteristics, host plant community composition, and host plant identity might explain *A.* nr. *permutata* nymph abundance, occurrence, and development.

Across the four-year study, key trends emerged. The high prevalence of *A.* nr. *permutata* spittlemasses along the edge of vineyards and in adjacent habitat suggest that those habitats and the plants within them are more supportive of this insect than the plants found within the vineyard. The observations of greater nymphal abundance along the edge of vineyards and surrounding vegetation draw parallels with the blue green sharpshooter, *G. atropunctata*, the major vector of *X. fastidiosa* in the California North Coast that overwinters and develops on a wide range of hosts found in riparian habitats bordering vineyards (Purcell 1975, Purcell 1976).

Observations of greater *A.* nr. *permutata* abundance in riparian and woodland sites suggest that the host community as whole or specific hosts associated with those site types are particularly supportive of *A.* nr. *permutata* nymphs. Year-to-year variation in site characteristics that influence the local microclimate or host composition might explain why woodland sites hosted larger numbers of *A.* nr. *permutata* than riparian sites in 2019 and 2020. Given the non-significant effect of plant taxa on *A*. nr. *permutata* numbers in 2019 and 2020, it is more likely that the prevalence or quality of certain plant species rather than overall plant richness explains the relatively high abundance of *A.* nr. *permutata* at particular sites. Some of the host plants strongly associated with *A.* nr. *permutata* abundance, such as *H. echoides*, *D. carota*, *Sonchus* spp., and *Trifolium* spp., belong to genera that are also strongly favored by a related spittlebug *Aphrophora alni* (Linnaeus) (Hemiptera: Aphrophoridae) (Bodino et al. 2020). Although *V. sativa* plant cover was also strongly associated with *A.* nr. *permutata* abundance, this plant species may be more of an indicator of site soil organic matter or nutrient content, given its common role as a cover crop species in California vineyards (Ingels et al. 1998).

Various characteristics of the sites, years, and host plants surveyed may in part explain the observed differences in *A.* nr. *permutata* nymph development time. Our exploratory characterization of climatic factors at the development survey sites suggested that sites supporting shorter development times had higher temperatures and lower humidity in the Spring. As high humidity conditions are often considered conducive to spittlebug egg and nymphal survival (Weaver and King 1954), the shortened development time at sites with low humidity may represent an attempt by *A.* nr. *permutata* to escape adverse conditions by accelerating development into the more mobile, winged adult stage. In the 1950s, *A. permutata* was reported at sites along the foggy coastal areas as well as drier interior valleys of California, suggesting the potential for this species to occupy habitats of variable temperature and humidity (Delong and Severin 1950). While it is unknown how climatic shifts over the past 70 years may have impacted the distribution of *A. permutata*, *P. spumarius* has reportedly undergone a northward shift and / or decline within California (Karban and Strauss 2004, Karban and Huntzinger 2018).

Differences in development time on various host plants may be due to their differences in nutritional quality. Spittlebug species are often associated with nitrogen fixing or actinorhizal host plants (Thompson 1994, Thompson 1999) and other spittlebugs like *P. spumarius* preferentially feed on plant components having elevated amino acid concentrations in the xylem sap (Horsfield 1977). The host plants associated with shorter *A.* nr. *permutata* development times may have higher amino acid concentrations or other key nutrients conducive for nymphal development. The abundance and survival of other spittlebug species such as the meadow spittlebug, *P. spumarius*, is impacted by the nutritional status of their hosts (Wood et al. 2020). Alternatively, host defensive structures such as trichomes may affect host selection and feeding behavior by *A.* nr. *permutata*, as it does in associations between *P. spumarius* and its host plants (Hoffman and McEvoy 1986).

The transmission efficiency of *X. fastidiosa A.* nr. *permutata* from grapevine to grapevine was much lower than that previously reported for *A. permutata* (48-50% for individuals, 52% for groups of 3-5 insects) (Severin 1950). As the insects used in Severin's trials were collected as adults (Severin 1950), it is possible that they were naturally infected with *X. fastidiosa* prior to use, given that *X. fastidiosa*, once acquired, is persistent in the foreguts of adult vectors (Purcell and Finlay 1979). Given the impact of AAP and IAP duration on *X. fastidiosa* transmission efficiency (Purcell and Finlay 1979, Almeida and Purcell 2003, Cornara et al. 2016), differences in the duration of these periods between the two experiments (one to eight day AAP and one to six day IAP in the earlier study vs four day AAP and IAP in 2019) may partially explain these conflicting results. The poor survivorship of adult *A.* nr. *permutata* on grapevine (Severin 1950) or differences in the *X. fastidiosa* populations of source plants (Hill and Purcell 1997) might also explain the variable transmission results. During our transmission experiments ~26% (45/170) adults died during the initial AAP when caged solely on grapevine, highlighting this species' poor survival on grapevine and the challenges this poses for assessing transmission. If *A.* nr. *permutata* is a separate species or complex of species distinct from those used in the studies, species-level variations in transmission biology and behavior (Daugherty and Almeida 2009) might account for the differences in observed transmission rates.

In our experiments, the difference in transmission success between individuals and groups of *A.* nr. *permutata* was nonsignificant. Although one might expect that increased vector

number would lead to significantly more transmission events, meta-analyses of *X. fastidiosa* transmission studies with sharpshooter vectors noted that the effect of vector number on transmission varied by species (Daugherty and Almeida 2009). For some *X. fastidiosa* vectors, the length of the acquisition and inoculation access period had a greater impact on transmission than vector number and this could be the case for *A.* nr. *permutata* as well.

The low *X. fastidiosa* transmission efficiency by *A.* nr. *permutata* obtained in these experiments is sufficiently high to be of epidemiological relevance for PD in the California North Coast. The major sharpshooter leafhopper vectors associated with sustaining epidemics of CVC in Brazil transmit *X. fastidiosa* to citrus at efficiencies of 1-30% per individual per day (Krugner et al. 1998, Redak et al. 2004, Lopes and Krugner 2016). At sites where *A.* nr. *permutata* is prevalent, it is possible that this vector insect may be able to acquire and transmit *X. fastidiosa* to grapevines during the summer and fall months. Historically, *X. fastidiosa* infection events initiated after May in California were likely to recover over the winter in response to cooler temperatures (Feil et al. 2003). However, recent work on the transmission dynamics of glassy winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in the southern San Joaquin Valley (Sisterson et al. 2020) and *P. spumarius* in the California North Coast (Beal et al. 2021) found that the prevalence of *X. fastidiosa* positive vectors was greatest between June and December. In both of those studies, the authors suggested that secondary (grapevine to grapevine) *X. fastidiosa* infection events by these vectors during the summer and fall months are linked to cases of chronic PD in vineyards. Given climatic trends favoring warmer, milder winters in California (Swain et al. 2016, Wang et al. 2017), it is possible that *A.* nr. *permutata* may also be able to incite cases of chronic PD in California North Coast vineyards. Additional research on seasonal patterns of *A.* nr. *permutata* activity, infectivity, and transmission is needed to address this hypothesis.

**Conclusions**

Field surveys and greenhouse studies conducted over 4 years have provided a greater understanding of *A.* nr. *permutata* biology, ecology, and vector capacity in the California North Coast. *Aphrophora* nr. *permutata* spittlemass abundance was more often associated with riparian and woodland sites than with grassland or agricultural sites. *Aphrophora* nr. *permutata* occupancy and abundance also appears to be associated with certain host plant species suggesting a potential preference for *E. californica*, *D. carota*, *Geranium* spp., *Trifolium* spp., *Sonchus* spp., and *H. echoides*. *Aphrophora* nr. *permutata* development times varied due to site, year, and occasionally host plant. Sites having higher temperatures and lower humidity were more likely to have hastened *A.* nr. *permutata* development. Similar to other spittlebug species, host plant nutritional status (Horsfield 1977, Wood and Jones 2020) may explain why *A.* nr. *permutata* development time was shorter on some host plant species than others. Although *A.* nr. *permutata X. fastidiosa* transmission efficiency to grapevine was lower than 10%, this species may still contribute to the ecology of PD at sites in which it is abundant. Should this insect present itself as a greater threat to California North Coast viticulture in the future, this study provides the initial resources to inform or guide potential management efforts.

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**Table 1.** Mean (±SE) *A.* nr. *permutata* abundance over four years of vineyard surveys among site type categories (riparian, woodland/chaparral, grassland/other). Measured as number of spittlemasses per transect (2017, 2018), number of plants with one or more spittlemass present (out of 50 inspected; 2019), and number of nymphs per quadrat (2020).

| Site type | 2017 spittlemasses[1] | 2018 spittlemasses[1] | 2019 plants[1] | 2020 nymphs[1] |
|---|---|---|---|---|
| riparian | 1.195 (0.235)a | 15.795 (3.668)a | 4.900 (0.998)a | 0.561 (0.166)a |
| forests | 1.000 (0.287)a | 18.542 (6.886)a | 12.286 (3.426)ab | 2.275 (0.291)b |
| other | 0.033 (0.033)b | 1.750 (1.486)b | 2.75 (2.75)a | --[2] |

[1]different letters denote significant differences among site types within a given year
[2]only riparian and woodland/chaparral sites were surveyed in 2020

**Table 2.** Plant taxa, effect sizes, and test statistics for the variables retained in the final model of plant cover on the abundance of spittlebug nymphs among plots.

| Taxon | Slope (± SE)[1] | Intercept (± SE)[1] | $\chi^2$ | df | P |
|---|---|---|---|---|---|
| *Helminthotheca echoides* | 0.291 (± 0.081) | 1.256 (± 0.157) | 12.819 | 1 | 0.0003 |
| *Taraxacum officinale* | -0.482 (± 0.224) | 1.256 (± 0.157) | 3.896 | 1 | 0.0484 |
| *Vicia sativa* | 0.224 (± 0.071) | 1.256 (± 0.157) | 10.208 | 1 | 0.0013 |
| *Daucus carota* | 0.414 (± 0.089) | 1.256 (± 0.157) | 21.389 | 1 | <0.0001 |

[1]Variables are from a Poisson generalized linear mixed-effects model with estimates presented on the $\log_{10}$ transformed scale.

**Figure legends**

Figure 1: Mean (±SE) number of A) spittlemasses per transect among three locations (row middles, vineyard edge, or adjacent habitat) in 2017 and B) nymphs per quadrat in two locations in 2020.

Figure 2: Mean (±SE) frequency of spittlemass presence on individual plants of the 20 most common taxa in 2019 surveys. Plant taxa are ordered according to the overall frequency of those plant taxa among all transects (denoted by the numbers above the bars). Plant taxa: cd (*Rumex crispus*), it (*Carduus pycnocephalus*), ph (*Conium maculatum*), bo (*Helminthotheca echoides*), st (*Sonchus* spp.), pl (*Plantago major*), fi (*Erodium* spp.), bc (*Medicago polymorpha*), cp (*Eschscholzia californica*), ch (*Cichorium intybus*), sm (*Hirschfeldia incana*), pr (*Lactuca serriola*), ml (*Malva parviflora*), dn (*Taraxacum officinale*), sp (*Chlorogalum pomeridianum*), gn (*Geranium* spp.), cl (*Trifolium* spp.), wr (*Raphanus raphanistrum*), ce (*Hypochaeris radicata*), fn (*Foeniculum vulgare*).

Figure 3: Non-metric multidimensional scaling plot depicting associations between plant community composition and *A*. nr. *permutata* abundance. Points represents all plots among the sites, with the size of the point reflecting the relative abundance of nymphs, binned into one of four categories. Letter codes denote the 20 most common plant taxa (further defined in Table S3), with points located closer to a given code reflecting that plot being dominated by that taxa. Surveyed groundcover: bs (bare soil), bc (*Medicago polymorpha*), bo (*Helminthotheca echoides*), bp (*Plantago lanceolata*), ca (*Calendula officinalis*), cd (*Rumex crispus*), ce (*Hypochaeris radicata*), cl (*Trifolium* spp.), cp (*Eschscholzia californica*), dn (*Taraxacum officinale*), fi (*Erodium* spp.), gn (*Geranium* spp.), gr *(Poa* spp.), it (*Carduus pycnocephalus*), mn (*Claytonia perfoliata*), pr (*Lactuca serriola*), sp (*Chlorogalum pomeridianum*), st (*Sonchus* spp.), ve (*Vicia sativa*), wc (*Daucus carota*), wr (*Raphanus raphanistrum*).

Figure 4: Mean (±SE) timing of first detection of *A.* nr. *permutata* nymphs A) between years, B) among vineyard sites, and C) among plant taxa. Plant taxa: bc (*Medicago polymorpha*), bo (*Helminthotheca echoides*), bp (*Plantago lanceolata*), cd (*Rumex crispus*), ch (*Cichorium intybus*), cp (*Eschscholzia californica*), fi (*Erodium* spp.), hw (*Erigeron canadensis*), it (*Carduus pycnocephalus*), me (*Wyethia mollis*), ph (*Conium maculatum*), pm (*Chorispora tenella*), sm (*Hirschfeldia inca*), sp (*Chlorogalum pomeridianum*), st (*Sonchus* spp.), wc (*Daucus carota*).

Figure 5: Mean (±SE) number of weeks from first detection of 1$^{st}$ instar nymph through last appearance of 5$^{th}$ instars A) between years, B) among vineyard sites.

Figure 6: Transmission efficiency (±SE) of *X. fastidiosa* among individuals or groups of five *A.* nr. *permutata* adults.

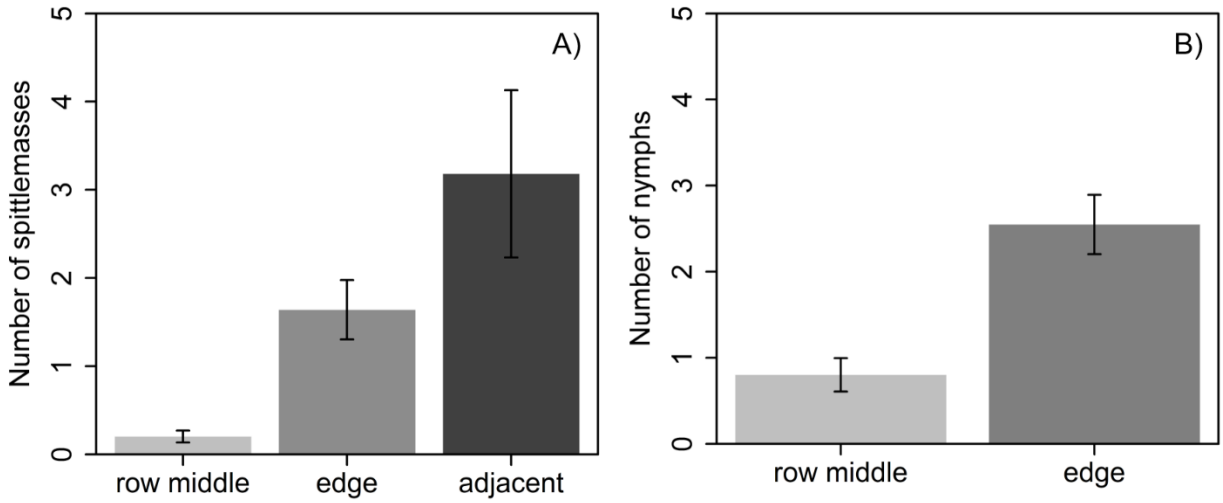

A)
Number of spittlemasses
0
1
2
3
4
5
row middle
edge
adjacent
B)
Number of nymphs
0
1
2
3
4
5
row middle
edge

**Figure 1**.

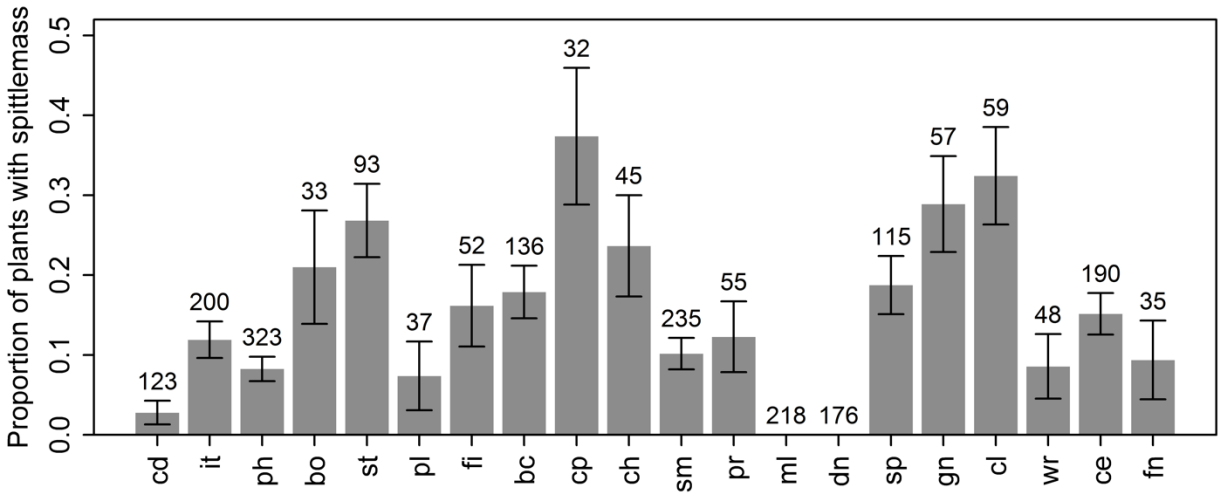

Proportion of plants with spittlemass
0.0
0.1
0.2
0.3
0.4
0.5
123
200
323
33
93
37
52
136
32
45
235
55
218
176
115
57
59
48
190
35
cd
it
ph
bo
st
pl
fi
bc
cp
ch
sm
pr
ml
dn
sp
gn
cl
wr
ce
fn

**Figure 2**.

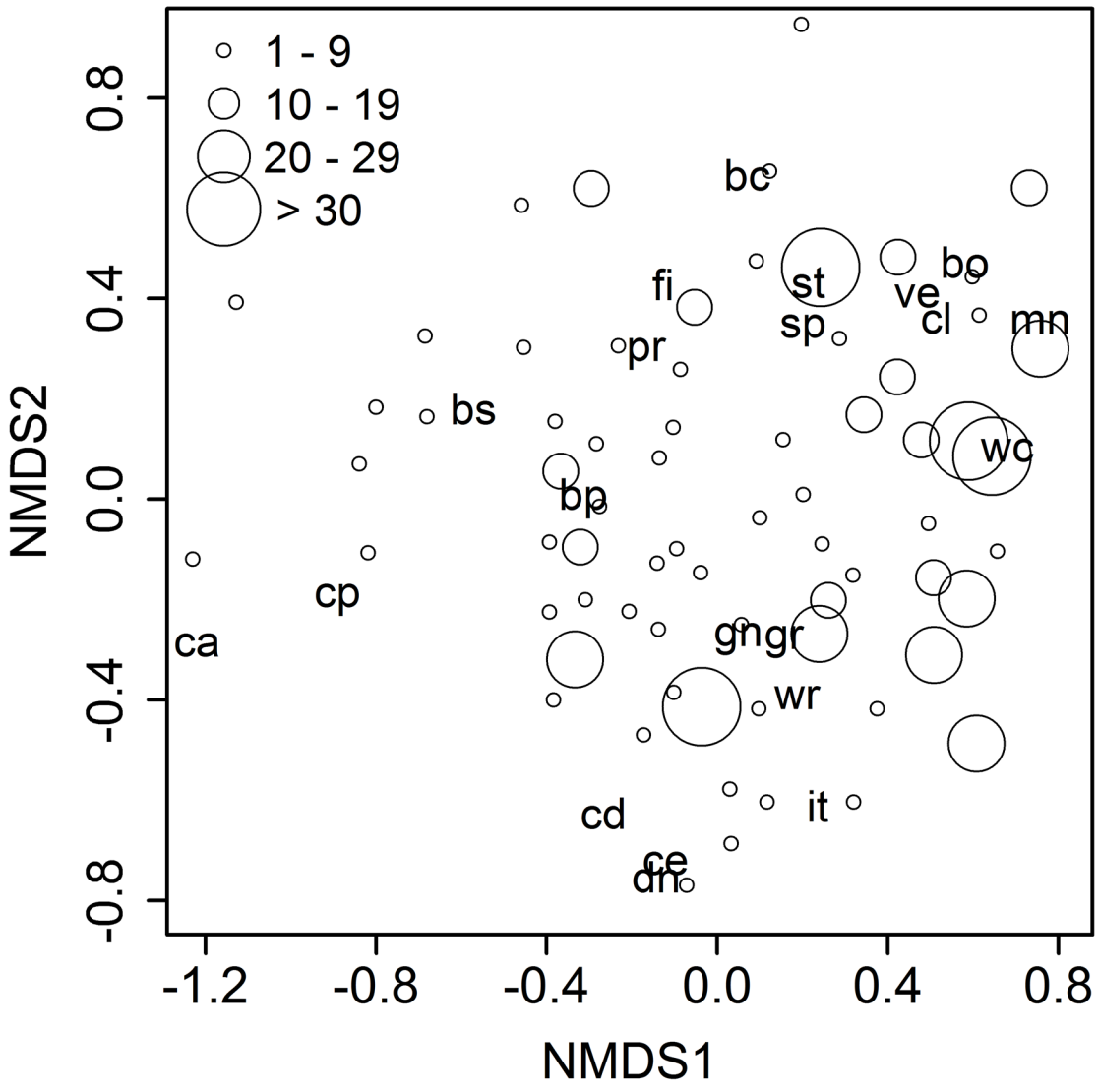

1 - 9
10 - 19
20 - 29
> 30
bc
fi
st
ve
bo
cl
mn
sp
pr
bs
wc
bp
cp
ca
gn
gr
wr
cd
it
ce
dn
NMDS1
NMDS2
-1.2
-0.8
-0.4
0.0
0.4
0.8

**Figure 3**.

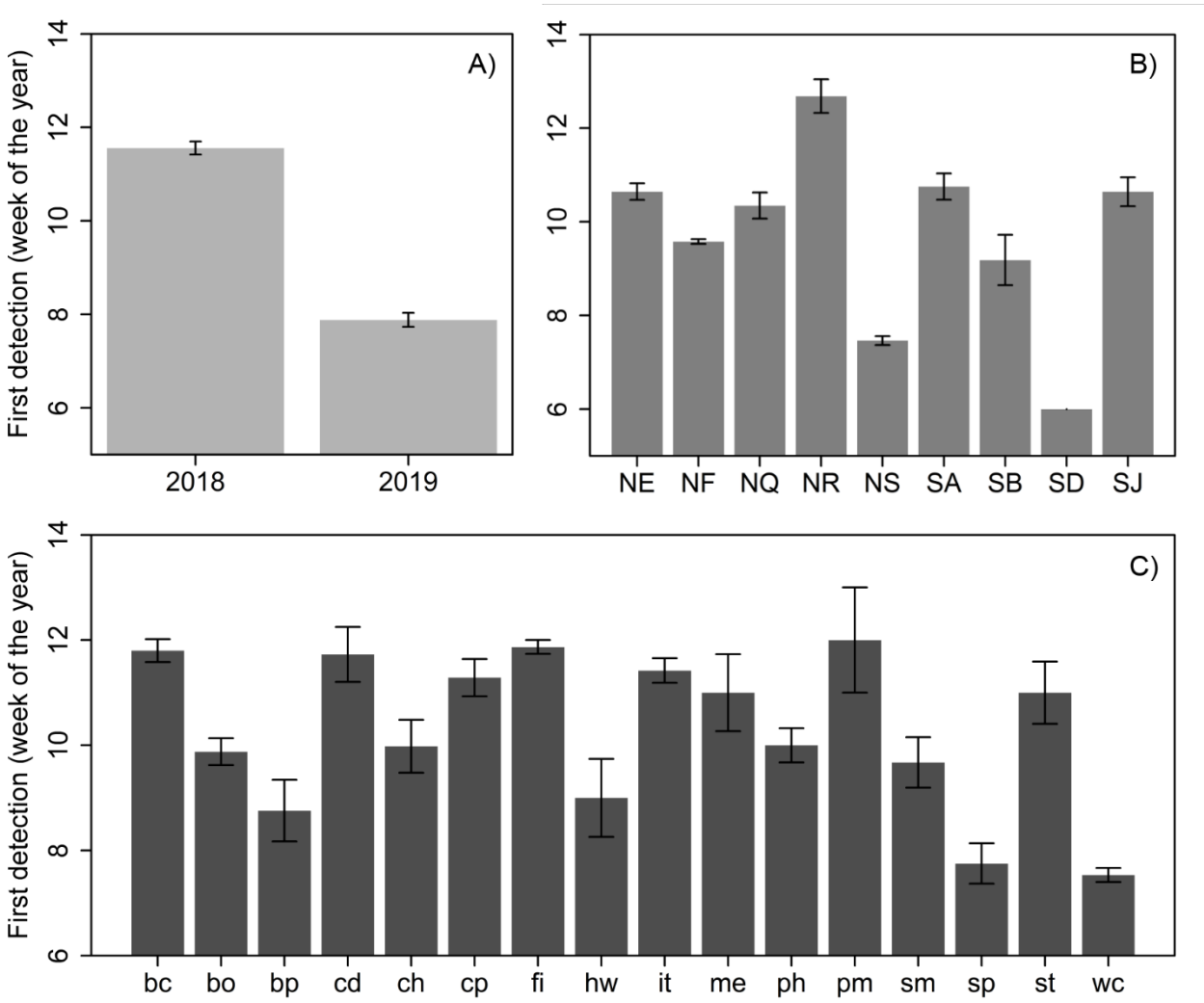

A)
B)
C)
First detection (week of the year)
6
8
10
12
14
2018
2019
NE
NF
NQ
NR
NS
SA
SB
SD
SJ
bc
bo
bp
cd
ch
cp
fi
hw
it
me
ph
pm
sm
sp
st
wc

**Figure 4**.

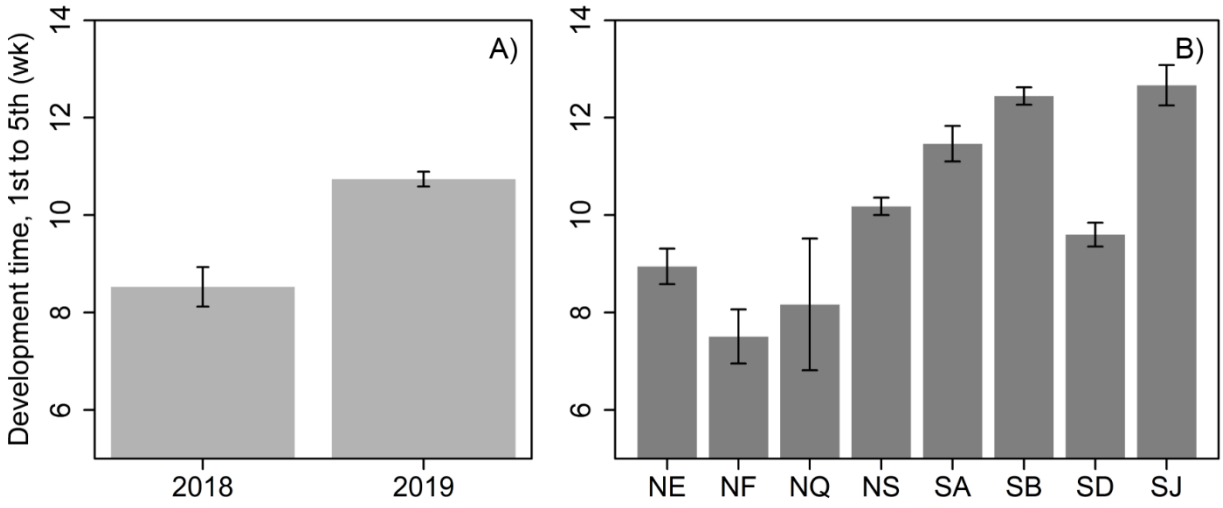

Development time, 1st to 5th (wk)
6
8
10
12
14
A)
2018
2019
B)
NE
NF
NQ
NS
SA
SB
SD
SJ

**Figure 5**.

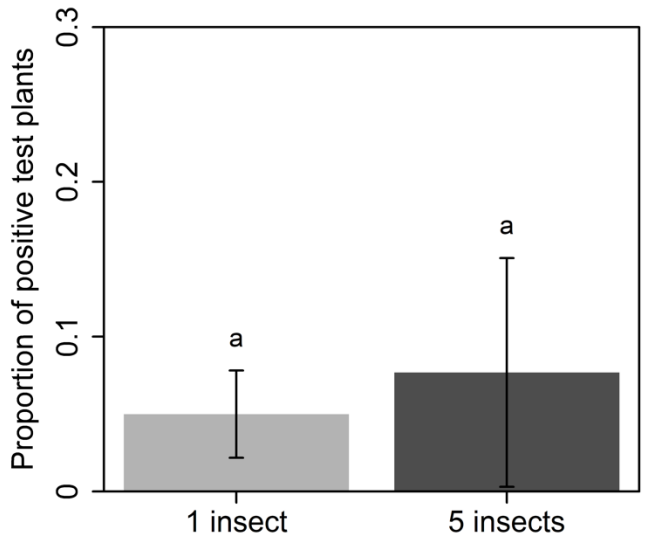

Proportion of positive test plants
0
0.1
0.2
0.3
a
a
1 insect
5 insects

**Figure 6.**

**Appendix 1: Supplementary Materials and Figures-**

In 2019, at seven nymphal development sites (four in Napa: NE, NF, NQ, NS. three in Sonoma: SA, SB, SJ) HOBO U10 temp/RH data loggers (Onset Computer Corporation, Bourne, MA) were set up at each site to collect temperature and humidity data from February to June (Fig. S2). The data loggers were placed inside of a solar shield (Onset Computer Corporation, Bourne, MA) mounted onto a metal vineyard stake.

In 2019, we collected 12-20 *A.* nr. *permutata* nymphs from non-flagged plants at each nymphal development site. We identified the plants from which each nymph was collected to the species level. The sampling interval was usually every two weeks although rain and other impediments occasionally lead to sampling intervals between seven and 21 days. Nymphs were collected and placed in 95% ethanol. For each site and sampling date, all nymphs collected during that period were stored in a single vial. Measurements of nymphal head capsule occurred at the Napa and Sonoma Cooperative extension offices using a reticular lens (Klarman Rulings #207 (25 mm with 10 mm ruler of 100 divisions)) mounted on a dissecting microscope (Nikon SMZ 745). Head capsule width was measured from the outer edge of the right eye to the outer edge of the left eye. Micrometer units were recorded and converted to millimeter to determine the ranges for each corresponding instar stage. Nymphs were binned by head width to the nearest 0.1 mm and the distribution of nymphal head widths among the surveyed populations was visualized by site in a histogram (Fig. S3).

At the nymphal development sites, we recorded the vine growth stage on the week of first observed adult emergence in each county. In 2018, measurements were recorded on April 19$^{th}$ and 20$^{th}$ in Sonoma and April 27$^{th}$ in Napa. Ten healthy vines were selected at random to cover the edge closest to the source of *A.* nr. *permutata*. Stunted vines were avoided (due to the assumption that vectors avoid PD vines). For each vine, two shoots were selected as follows: a) cane trained vines: shoot at 3$^{rd}$ cane node and terminal shoot; b) cordon trained vines: a top or bottom shoot of the 3$^{rd}$ spur and terminal spur on one side of cordon. When cordon pruned, both positions were chosen from same cordon; when cane pruned, both positions were chosen from same cane. When cordon pruned, the selection of the position on the spur (1 or 2) was done at random. Each shoot was evaluated with the modified E-L scale (Pearce and Coombe 2004). The median of 10 data points per shoot position in each study site was calculated (Table S4). In 2019, we followed this same methodology at Napa on April 26$^{th}$ while at Sonoma, we did not sample until May 17$^{th}$, when a majority of the *A.* nr. *permutata* population had emerged as adults.

**Table S1:** Site information of vineyards surveyed from 2017-2020.

| Site | County | Cultivar (*Vitis vinifera*) | Rootstock | Planting Date | Acres |
|---|---|---|---|---|---|
| NA | Napa | Cabernet Sauvignon | 039-16 | 2014 | 9.07 |
| NB | Napa | Chardonnay | 101-14 | 2010 | 5 |
| NC | Napa | Merlot | 101-14 | 1997 | 3.03 |
| ND | Napa | Cabernet Franc | 3309 & other | 1999 | 6.37 |
| NE | Napa | Cabernet Sauvignon | 3309, St George | 2004, 2005 | 7.44 |
| NF | Napa | Cabernet Sauvignon | 110R | 2000 | 1.92 |
| NG | Napa | Petit Verdot | 110R | 2004 | 0.89 |
| NH | Napa | Cabernet Sauvignon | 101-14 | 2004 | 2.84 |
| NI | Napa | Cabernet Franc | 039-16 | 2002 | 2.5 |
| NJ | Napa | Cabernet Sauvignon | 101-14 | 2004 | 4.88 |
| NK | Napa | Chardonnay | 101-14 | 2000 | 4.6 |
| NL | Napa | Malbec | 420A | 2004 | 1.69 |
| NM | Napa | Cabernet Sauvignon | 3309, 1103P, 110R | 1991/1993 | 4.42 |
| NN | Napa | Chardonnay | 1103P | 2008 | 2.6 |
| NO | Napa | Cabernet Sauvignon | 101-14 | 2013 | 5 |
| NP | Napa | Cabernet Sauvignon | Riparia Gloire, 420A, 101-14, 3309C | 2000, 1994, 1993, 1990 | 7.23 |
| NQ | Napa | Cabernet Sauvignon | | | 1.77 |
| NR | Napa | Cabernet Sauvignon | 101-14 | 2007 | 9.23 |
| NS | Napa | Cabernet Sauvignon | 110R | 2002 | 2.26 |
| NT | Napa | Merlot, Petit Verdot, Malbec | 3309 | 2004, 1998, 1997, 1996, 2018 | 1.46 |
| NU | Napa | Merlot | 3309, 420A | | 2.86 |
| NV | Napa | Petit Verdot, Malbec, Merlot | | | |
| SA | Sonoma | Chardonnay | 5C | 1998 | 5.11 |
| SB | Sonoma | Chardonnay | 039-16 | 2011 | 5.45 |
| SC | Sonoma | Zinfandel | 110-R | 2001 | 1.1 |
| SD | Sonoma | Zinfandel | 039-16, 110-R | 1995 | 4.27 |
| SE | Sonoma | Chardonnay | 1103P | 2001 | 3.78 |
| SF | Sonoma | Gruner Veltliner | 101-14 | 2011 | 4.93 |
| SG | Sonoma | Merlot, Cabernet Sauvignon | 039-16 | 1993, 2000 | 5.8 |
| SH | Sonoma | Pinot Noir | 101-14 | 2007 | 3.97 |
| SI | Sonoma | Merlot, Cabernet Sauvignon | 5C | 1996 | 3.79 |
| SJ | Sonoma | Chardonnay | 5C | 2001 | 7.5 |
| SK | Sonoma | Malbec | 039-16 | 2008 | 4.25 |
| SL | Sonoma | Chardonnay | 3309C | 2008 | 2.25 |

| Site | County | Cultivar (*Vitis vinifera*) | Rootstock | Planting Date | Acres |
|---|---|---|---|---|---|
| SM | Sonoma | Chardonnay | AXR, 5C | 1986, 1992, 1993, 1994 | 5 |
| SN | Sonoma | Chardonnay | 101-14 | 2003 | 4.16 |
| SO | Sonoma | Chardonnay | 101-14 | 2005, 2003 | 4.08 |
| SP | Sonoma | Chardonnay | S04 | 2007 | 2.75 |

**Table S2:** Replicates conducted for each site by survey year and project.

| Site | 2017 Survey[1] | 2018 Survey[2] | 2019 Survey[2] | 2020 Survey[3] | 2018 Development[4] | 2019 Development[4] |
|---|---|---|---|---|---|---|
| NA | 11 | 2 | 2 | | | |
| NB | 11 | 3 | 2 | | | |
| NC | 11 | 2 | 2 | | | |
| ND | 10 | 2 | | | | |
| NE | 11 | 3 | 2 | | 105 | 30 |
| NF | 11 | 2 | 2 | | 37 | 30 |
| NG | 10 | 2 | | | | |
| NH | 10 | 2 | | | | |
| NI | 11 | 2 | 2 | | | |
| NJ | 11 | 3 | 2 | | | |
| NK | 10 | 2 | | | | |
| NL | 11 | 2 | 2 | | | |
| NM | 10 | 2 | 2 | 24 | | |
| NN | 10 | 2 | | | | |
| NO | 11 | 3 | | | | |
| NP | 11 | 3 | | | | |
| NQ | | | | | 37 | 30 |
| NR | | | | | 19 | |
| NS | | | | 24 | | 30 |
| NT | | | | 24 | | |
| NU | | | | | | |
| NV | | | | 24 | | |
| SA | 11 | 2 | 2 | 24 | 40 | 30 |
| SB | 11 | 2 | 2 | | 40 | 30 |
| SC | 10 | 4 | 2 | | | |
| SD | 11 | 4 | 2 | 24 | | 30 |
| SE | 10 | | | | | |
| SF | 11 | 3 | 2 | 24 | | 30 |
| SG | 10 | 2 | 2 | | | |
| SH | 11 | 4 | 2 | | | |
| SI | 10 | 2 | 2 | | | |
| SJ | 11 | 2 | 2 | 24 | 40 | 30 |
| SK | 11 | 2 | 2 | | | |
| SL | 11 | 2 | 2 | | | |
| SM | 11 | 2 | 2 | | | |
| SN | 11 | 3 | 2 | | | |
| SO | 11 | 2 | 2 | | | |
| SP | 11 | 2 | 2 | | | |

[1] Number of 2-minute surveys per site
[2] Number of transects per site
[3] Number of quadrats per site
[4] Number of plants flagged and followed per site

**Table S3:** List of common plant taxa identified during the 2016-2020 surveys.

| Common name | Scientific name | Study code |
|---|---|---|
| California burclover | *Medicago polymorpha* (Linnaeus) (Fabales: Fabaceae) | bc |
| bristly oxtongue | *Helminthotheca echoides* (Linnaeus) (Asterales: Asteraceae) | bo |
| buckhorn plantain | *Plantago lanceolata* (Linnaeus) (Lamiales: Plantaginaceae) | bp |
| rough seed buttercup | *Ranunculus muricatus* (Linnaeus) (Ranunculales: Ranunculaceae) | bu |
| pot marigold | *Calendula officinalis* (Linnaeus) (Asterales: Asteraceae) | ca |
| curly dock | *Rumex crispus* (Linnaeus) (Caryophyllales: Polygonaceae) | cd |
| Catsear | *Hypochaeris radicata* (Linneaus) (Asterales: Asteraceae) | ce |
| Chicory | *Cichorium intybus* (Linnaeus) (Asterales: Asteraceae) | ch |
| clover | *Trifolium* spp. | cl |
| California poppy | *Eschscholzia californica* (Cham.) (Ranunculales: Papaveraceae) | cp |
| dandelion | *Taraxacum officinale* (Linnaeus) (Asterales: Asteraceae) | dn |
| Filaree | *Erodium* spp. | fi |
| Fennel | *Foeniculum vulgare* (Mill.) (Apiales: Apiaceae) | fn |
| Geranium | *Geranium* spp. | gn |
| Grass | *Poa* spp. | gr |
| horseweed | *Erigeron canadensis* (Linnaeus) (Asterales: Asteraceae) | hw |
| Italian thistle | *Carduus pycnocephalus* (Linnaeus) (Asterales: Asteraceae) | it |
| mules ear | *Wyethia glabra* (A.Gray) (Asterales: Asteraceae) | me |
| | | |

| Common name | Scientific name | Study code |
|---|---|---|
| Marigold | *Calendula arvensis* (Linnaeus) (Asterales: Asteraceae) | mg |
| little mallow (cheeseweed) | *Malva parviflora* (Linnaeus) (Malvales: Malvaceae) | ml |
| miner's lettuce | *Claytonia perfoliata* (Donn) (Caryophyllales: Montiaceae) | mn |
| Blessed milk thistle | *Silybum marianum* (Linnaeus) (Asterales: Asteraceae) | mt |
| Mugwort | *Artemisia douglasiana* (Besser) (Asterales: Asteraceae) | mw |
| poison hemlock | *Conium maculatum* (Linnaeus) (Apiales: Apiaceae) | ph |
| broadleaf plantain | *Plantago major* (Linnaeus) (Lamiales: Plantaginaceae) | pl |
| purple stem mustard | *Chorispora tenella* (Pall.) (Brassicales: Brassicaceae) | pm |
| perennial pepperweed | *Lepidium latifolium* (Linnaeus) (Brassicales: Brassicaceae) | pp |
| prickly lettuce | *Lactuca serriola* (Linnaeus) (Asterales: Asteraceae) | pr |
| pacific sanicle | *Sanicula crassicaulis* (Poepp.) (Apiales: Apiaceae) | ps |
| shortpod mustard | *Hirschfeldia incana* (Linnaeus) (Brassicales: Brassicaceae) | sm |
| soap plant | *Chlorogalum pomeridianum* (DC.) (Asparagales: Asparagaceae) | sp |
| sowthistle | *Sonchus* spp. | st |
| Vetch | *Vicia sativa* (Linnaeus) (Fabales: Fabaceae) | ve |
| wild carrot | *Daucus carota* (Linnaeus) (Apiales; Apiaceae) | wc |
| wild radish | *Raphanus raphanistrum* (Linnaeus) (Brassicales: Brassicaeae) | wr |
| willow weed | *Persicaria lapathifolia* (Linnaeus) (Caryophyllales: Polygonaceae) | ww |

**Table S4:** Median E-L Growth Stage at time of adult emergence in 2018 and 2019.

| Site | Training | 2018 | | 2019 | |
|---|---|---|---|---|---|
| | | 3rd[1] | Terminal[1] | 3rd[1] | Terminal[1] |
| SA[2] | Cordon- Spur Pruned | 7 leaves separated | 7 leaves separated | 17-20 leaves separated, Flowering, 50% caps off | 17-20 leaves separated, Flowering, 50% caps off |
| SB[2] | Cordon- Spur Pruned | 7 leaves separated | 7 leaves separated | 16 leaves separated, Beginning of Flowering, 30% caps off | 16 leaves separated, Beginning of Flowering |
| SD[2] | Cordon- Spur Pruned | | | 12 leaves separated, Well-developed inflorescence, single flowers separated | 12 leaves separated, Well-developed inflorescence, single flowers separated |
| SF[2] | Cane Pruned | | | 10 leaves separated | 12 leaves separated, Well-developed inflorescence, single flowers separated |
| SJ[2] | Cane Pruned | 6 leaves separated | 10 leaves separated | 16-17 leaves separated, 30-50% caps off | 17-20 leaves separated, Flowering, 50% caps off |
| NE | Cordon- Spur Pruned | 7 leaves separated | 7 leaves separated | 4-5 leaves separated, inflorescence clear[3] | 4-5 leaves separated, inflorescence clear[3] |
| NF | Cane Pruned | 9 leaves separated, shoot elongating rapidly, single flowers in compact groups | 9 leaves separated, shoot elongating rapidly, single flowers in compact groups | | |

| Site | Training | 2018 | | 2019 | |
|---|---|---|---|---|---|
| | | **3rd[1]** | **Terminal[1]** | **3rd[1]** | **Terminal[1]** |
| NQ | Cane Pruned | 7-8 leaves separated, shoot elongating rapidly, single flowers in compact groups | 7-8 leaves separated, shoot elongating rapidly, single flowers in compact groups | 5 leaves separated, inflorescence clear | 5 leaves separated, inflorescence clear |
| NR | | 8 leaves separated, shoot elongating rapidly, single flowers in compact groups | 8 leaves separated, shoot elongating rapidly, single flowers in compact groups | | |
| NS | Cordon- Spur pruned | | | 6-7 leaves separated | 6-7 leaves separated |

[1] For cane trained vines: shoot at 3rd cane node and terminal shoot; For cordon trained vines: a top or bottom shoot of the 3rd spur and terminal spur on one side of cordon

[2] For Sonoma sites in 2019, E-L measurements were made on May 17th, when a majority of *A.* nr. *permutata* had already emerged as adults.

[3] For NE in 2019, E-L measurements were made on April 16th, before adults emerged around April 26th.

**Figure S1.** Relative frequency of the top 20 occurring plant species on which spittlemasses were noted in the 2018 surveys sorted by frequency among transects (A.) and frequency among sites (B.). Letter codes denote plant taxa (further defined in Table S3). Surveyed Taxa: cd (*Rumex crispus*), sm (*Hirchfeldia incana*), bo (*Helminthotheca echoides*), ce (*Hypochaeris radicata*), ph (*Conium maculatum*), st (*Sonchus* spp.), ve (*Vicia sativa*), bc (*Medicago polymporpha*), fi (*Erodium* spp.), bp (*Plantago lanceolata*), ch (*Cichorium intybus*), pl (*Plantago major*), dn (*Taraxacum officinale*), gn (*Geranium* spp.), mg (*Calendula arvensis*), cp (*Eschscholzia californica*), hw (*Erigeron canadensis*), ml (*Malva parviflora*), mt (*Silybum marianum*), pr (*Lactuca serriola*), ps (*Sanicula crassicaulis*), sp (*Chlorgalum pomeridianum*), bu (*Ranunculus muricatus*), fn (*Foeniculum vulgare*), me (*Wyethia mollis*), wc (*Daucus carota*), wr (*Raphanus raphanistrum*), ww (*Persicaria lapathifolia*).

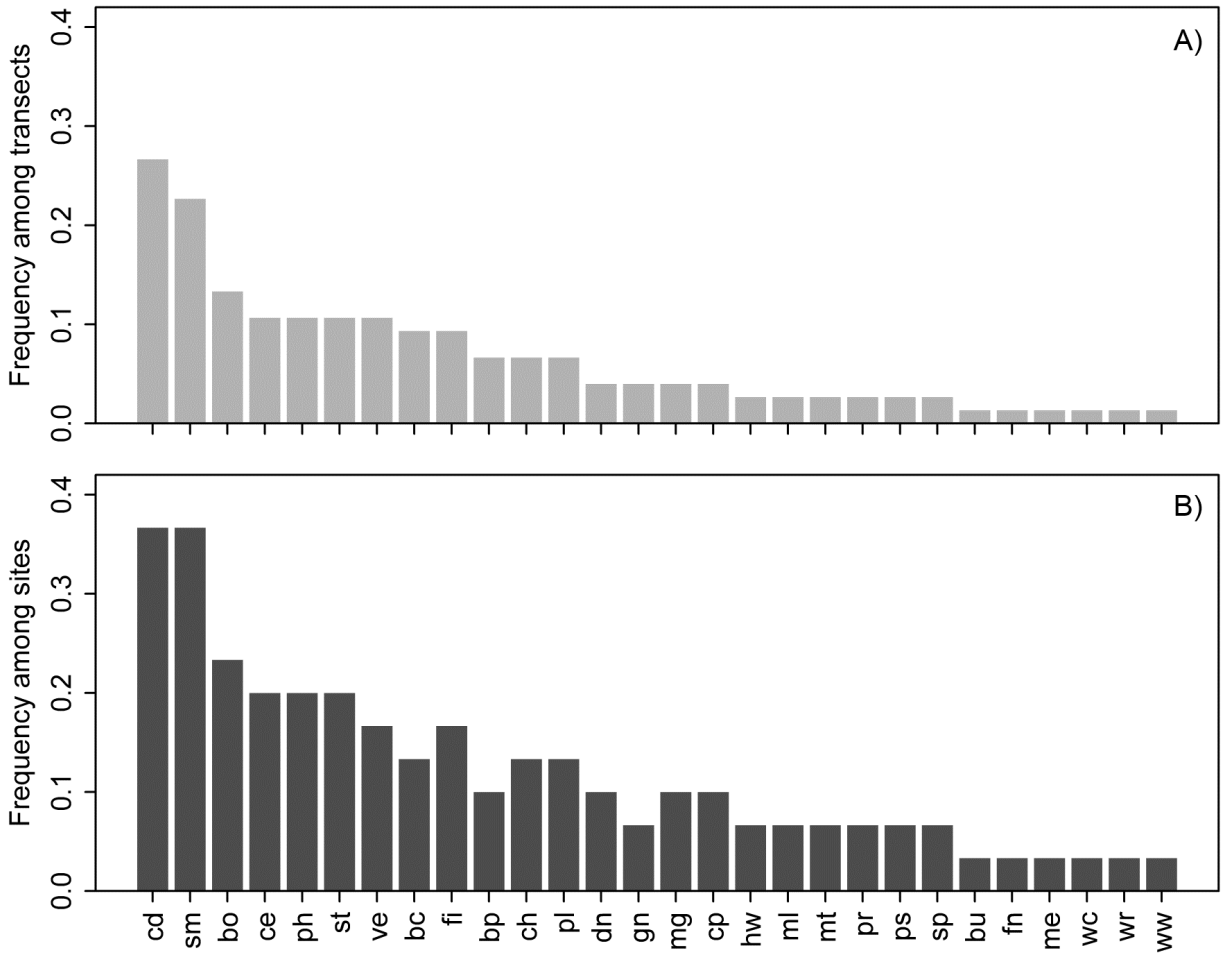


**Figure S2.** Mean temperature (A) and Mean relative humidity over the late-winter and spring months of 2019 at 7 of the development survey sites.

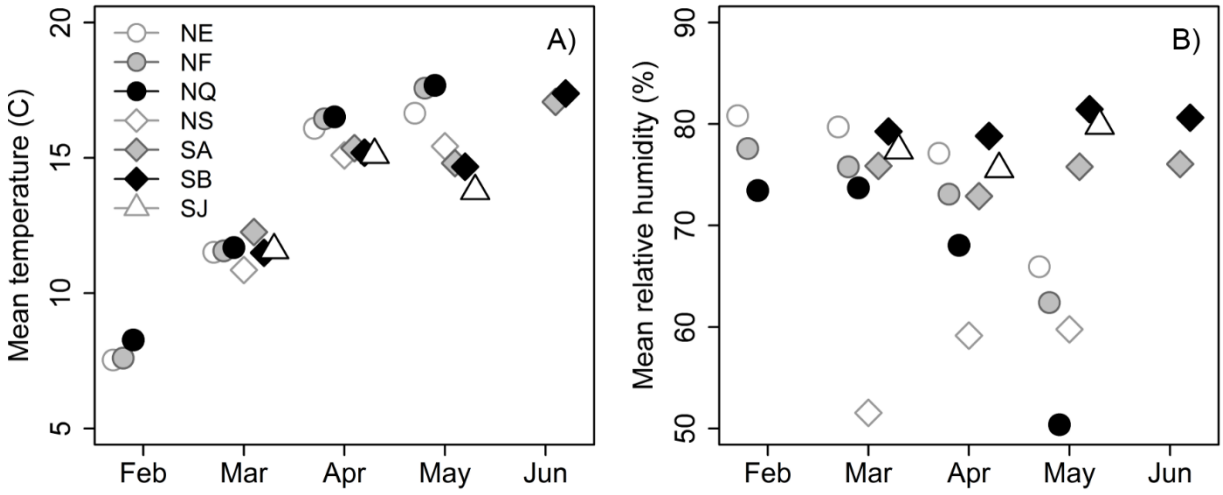
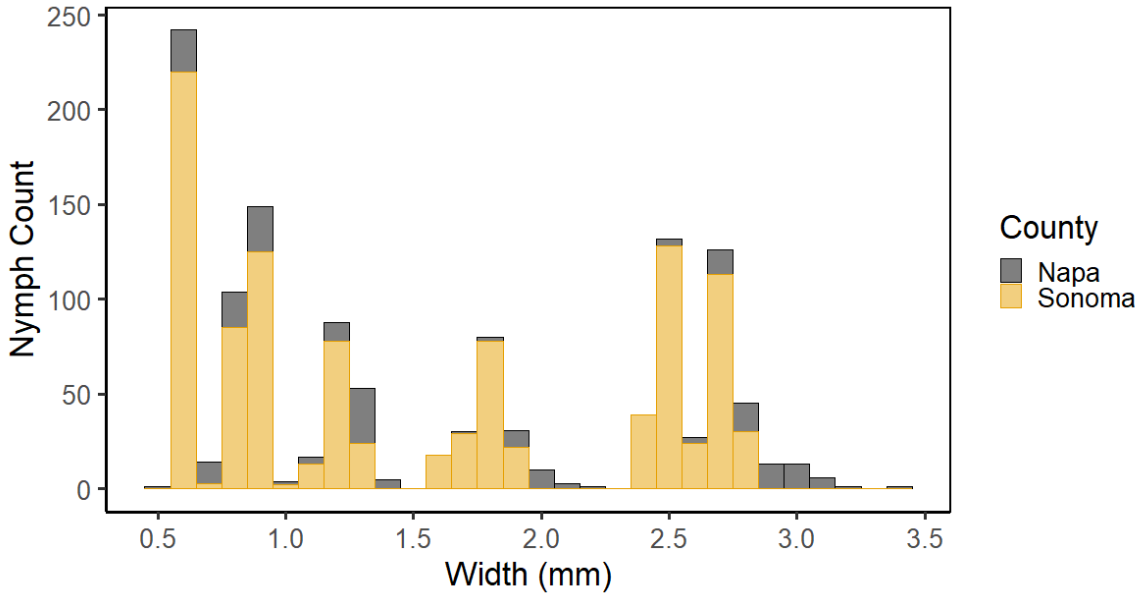



**Figure S3.** Histogram of *A.* nr. *permutata* nymphs binned by headcase width, collected from Napa(black) and Sonoma(yellow) Developmental Survey Sites in 2019.



Chapter 4: Time after acquisition differentially impacts *Xylella fastidiosa* transmission efficiency in spittlebug and sharpshooter vectors

**Abstract**

Insect vector transmission of the plant pathogen *Xylella fastidiosa* (Wells) (Xanthomonadales: Xanthomonadaceae) is influenced by numerous interactions between the insects, the bacterium, and their respective plant hosts. In the present study, we aimed to characterize the post-acquisition transmission dynamics of two vectors of *X. fastidosa*, *Philaenus spumarius* (Linnaeus) (Hemiptera: Aphrophoridae), the meadow spittlebug, and *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), the blue green sharpshooter, under greenhouse conditions. For *P. spumarius* in 2018 and *G. atropunctata* in 2019 and 2020, we measured the proportion of plants infected by individual vectors at set two-day inoculation access intervals following the acquisition access period. There was a significant effect of time after acquisition on the proportion of plants infected by *P. spumarius*. Immediately following acquisition, *P. spumarius* transmitted the pathogen to 5.8 % (8/136) of plants but by six and eight days after acquisition, spittlebugs transmitted to 7.5 % (9/120) and 11.5% (13/113) of plants respectively. In contrast, the effect of days after acquisition on *G. atropunctata* transmission was not significant. *Graphocephala atropunctata* transmission was always higher than that of *P. spumarius*, ranging from 19 % (15/79) to 35 % (28/80) in 2019 and from 30.4 % (21/69) to 55.5 % (30/54) in 2020. We discuss how aspects of vector biology such as feeding behavior and mouthpart fluid dynamics may explain the observed transmission dynamics.

## Introduction

Plant to plant transmission is a fundamental component governing the ecology and biology of pathogenic microbes. For vector-borne pathogens, the transmission efficiency of a given vector insect can have consequences for disease spread and intensity. Transmission efficiency can be influenced by a variety of factors including pathogen population density within the host (Hill and Purcell 1997), pathogen genotype (Esteves et al. 2019), temperature (Daugherty et al. 2009), length of acquisition and inoculation periods (Daugherty and Almeida 2009), and vector number (Daugherty and Almeida 2009). Such associations are dependent on the interactions between a given vector species, the pathogen, and their respective hosts (Lopes et al. 2009). In some, but not all, vector-pathogen systems, pathogen multiplication and eventual population size within a vector insect are important variables in determining transmission efficiency (Medica and Sinnis 2005, Rotenberg et al. 2009, Argüello Caro et al. 2013, Ammar et al. 2016, Han et al. 2019).

The plant pathogenic bacterium *Xylella fastidiosa* (Wells) (Xanthomonadales: Xanthomondaceae) is responsible for numerous plant diseases such as Olive Quick Decline Syndrome (OQDS), Citrus Variegated Chlorosis (CVC), and Pierce's disease (PD) of grapevine (Sicard et al. 2018). As a xylem limited pathogen, *X. fastidiosa* is transmitted by xylem sap-feeding insects such as sharpshooter leafhoppers (Hemiptera: Cicadellidae, mostly subfamily Cicadellinae) and spittlebugs (Hemiptera: Cercopoidea) (Redak et al. 2004). While bacterial populations within host plants are considered one of the most defining predictors of *X. fastidiosa* transmission efficiency (Hill and Purcell 1997), the role of bacterial population within insect mouthparts is less clear. Past transmission experiments of *X. fastidiosa* by an efficient vector, *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), did not find any relationship between vector transmission efficiency and the population of cells in the insect mouthparts. (Hill and Purcell 1995). In those experiments, some insects having large bacterial populations ($10^3$ – $10^5$ culture forming units (CFU)) did not transmit the pathogen. Conversely, 40% of transmitting insects had bacterial populations below the detection threshold of 100 CFUs. The ability of vectors of *X. fastidiosa* to transmit it without a latent period (Purcell and Finlay 1979) has also been presented as evidence that transmission efficiency is not influenced by *X. fastidiosa* population size within vector mouthparts. However, more recent transmission experiments with *G. atropunctata* (Labroussaa et al. 2017) and *Philaenus spumarius* (Linnaeus) (Hemiptera: Aphrophoridae) (Cornara et al. 2016) that used qPCR to quantify *X. fastidiosa* populations found that transmission efficiency increased with bacterial populations in the vector, although their experiments were not explicitly designed to test that association.

To explain these conflicting results, we hypothesize that population growth of *X.* fastidiosa within vectors during the period following acquisition would influence transmission efficiency, a time when bacterial populations have not yet colonized vector mouthparts. Following acquisition, *X. fastidiosa* cells attach to surfaces in the foregut of their insect vectors (Almeida and Purcell 2006), where they subsequently multiply and form a biofilm (Killiny and Almeida 2009). Such a process occurs until about four to seven days after acquisition (Hill and Purcell 1995, Almeida and Purcell 2006, Cornara et al. 2016). We predict that if there is a positive relationship between bacterial population size within the insect mouthparts and transmission efficiency, transmission efficiency would increase during the initial days post-acquisition and eventually stabilize after seven to ten days once insects are fully colonized by *X.*

*fastidiosa*. To test this hypothesis, we measured the *X. fastidiosa* transmission efficiencies of two vector species, *P. spumarius* and *G. atropunctata*, during this initial post-acquisition period in a series of greenhouse experiments.

## Materials and Methods

### Transmission Experiments

Greenhouse populations of *P. spumarius* were established from nymphs and adults collected in the California counties of Alameda and Marin from April to June 2018. Nymph and adult populations were raised separately on barley (*Hordeum vulgare* (Linnaeus) (Poales: Poaceae)), and transferred to new plants weekly. To account for possible acquisition of *X. fastidiosa* by adult spittlebugs prior to the start of the experiment, all insects were pre-screened. Thirty four groups of 25 adult spittlebugs (~850) were caged for a four-day inoculation access period (IAP) on separate pre-screened plants (*X. fastidiosa* free cuttings of *Vitis vinifera* cv. Cabernet Sauvignon (Linnaeus) (Vitales: Vitaceae)). Following the pre-screening period, the 34 groups were caged on separate *X. fastidiosa* source plants (*V. vinifera* cv. Cabernet Sauvignon infected for 12 weeks with *X. fastidiosa* strain STL) for a one-day acquisition access period (AAP). We selected a one-day AAP, to reduce variation in the initial timing of vector mouthpart colonization, as longer AAPs (2-4 days) would have allowed insects to initially acquire bacteria over several days and lead to variable amounts of growth before assessments began. Immediately following the AAP, four insects from each source plant block (136 total) were caged individually on test plants (*X. fastidiosa*-free cuttings of *V. vinifera* cv. Cabernet Sauvignon) for a two-day IAP. Cages consisted of either a cloth net or a plastic tube cage that allowed full access of the insects to the test plant. The remaining insects in each group (ca. 21) were allowed to continue to feed on *X. fastidiosa*-free *H. vulgare*. A two-day IAP was chosen as this access time was previously associated with increased vector transmission efficiency (Purcell and Finlay 1979, Cornara et al. 2016) while also enabling bacterial population growth over the post-acquisition period to be accessed (Almeida and Purcell 2006). Additional two-day IAPs were then repeated at two, four, six, and eight days after the AAP. Due to mortality encountered over the course of the experiment, the total insects tested in these four later IAPs were 136, 136, 120, and 113 respectively.

We collected *G. atropunctata* adults from wild populations in the California counties of Alameda, Napa, and Humboldt from May to July 2019 to establish greenhouse populations. The descendants of the wild caught adults were raised from egg to adult on *X. fastidiosa*-free basil plants (*Ocimum basilicum* (Linnaeus) (Lamiales: Lamiaceae)). Given that insects were transferred to new plants weekly and *X. fastidiosa* is lost at each nymphal molt (Purcell and Finlay 1979), the descendant *G. atropunctata* adults were free of *X. fastidiosa* infection. At the start of the transmission experiments, we caged five groups of 70 *G. atropunctata* adults on five separate *X. fastidiosa* source plants (*V. vinifera* cv. Cabernet Sauvignon) for a one-day AAP. After the AAP, we transferred 16 adults from each source plant (80 total) and caged them individually on a healthy *V. vinifera* cv. Cabernet Sauvignon test plant for a two-day IAP. The ~54 remaining insects from each source plant were then separately maintained on healthy *O. basilicum* plants for the duration of the experiment. Two-day IAPs were repeated after two, four, and eight days after the AAP. For these three subsequent IAP treatments, insects were sourced from each *O. basilicum* plant. Due to insect mortality encountered during the experiment, the three subsequent IAPs consisted of 80, 74, and 79 total insects respectively.

We conducted a second *G. atropunctata* transmission experiment in 2020. Greenhouse populations were descendants of *G. atropunctata* nymphs and adults collected from June to August 2020 at the same locations sampled in 2019. We caged eight groups of 40 *G. atropunctata* adults on separate *X. fastidiosa* source plants for a one-day AAP. At the conclusion of the AAP, we tranferred 7-9 individual adults from each source plant (70 total) to cages on healthy *V. vinifera* cv. Cabernet Sauvignon test plants for a two-day IAP. The remaining adults on the source plants (~71-73) were maintained on *O. basiculum* for the duration of the experiment. Two-day IAPs were then repeated at four days, eight days, and twelve days after the AAP. Due to insect mortality over the course of the experiment, the three subsequent IAPs consisted of 70, 70, and 54 total insects respectively.

**Detection of *X. fastidiosa***

Following the conclusion of each IAP, insects were collected from each test plant, the status of the insect (alive, dead, or missing) was noted, and samples were stored at -80°C. 30 healthy *V. vinifera* cv. Cabernet Sauvignon cuttings were randomly sampled from the greenhouse population to serve as negative plant controls. Uninoculated, pre-screen, and test plants were maintained in greenhouses for two months to allow for symptom development and to enable bacterial populations within the plants to reach levels detectable by culturing methods (Hill and Purcell 1995). Following established lab protocols (Hill and Purcell 1995, Hill and Purcell 1997), surface sterilized petioles were homogenized in 2 mL of succinate-citrate-phosphate (SCP) buffer) using a Polytron homogenizer (Brinkman Instruments, Inc, Westbury, NY). Two droplets (20 μL each) were then spread on PD3 media (Davis et al. 1981) and the plates were incubated at ~27°C for 5-10 days until *X. fastidiosa* colonies were observed. Six hundred forty-one total test plants were processed in 2018. The 34 pre-screen plants and 30 negative control plants were also subjected to the same culturing methods to verify that the pathogen was not present in these plants. Three hundred thirteen total test plants were processed in 2019. In 2019, we again selected 30 healthy *V. vinifera* cv. Cabernet Sauvignon plants to serve as negative plant controls. Due to plant mortality during the incubation period in 2020, 1 of 70 plants from the second IAP and 3 of 70 plants from the third IAP died before culturing could be undertaken. As we could not confirm whether those plants died due to *X. fastidiosa* infection or another cause, they were removed from our analyses. In the first IAP of 2020, 3 of 70 plants were also removed as their insects were mistakenly reported missing and had access to their test plant for an additional four days. Thus in 2020, 257 total test plants were processed. In 2020, due to limited test plant availability, we could only select 16 healthy *V. vinifera* cv. Cabernet Sauvignon to serve as negative plant controls.

**Statistical Analyses**

We analyzed the transmission efficiency data of each vector species with separate binomial generalized linear mixed-effects models (GLMM) with days after AAP as a continuous fixed effect and *X. fastidiosa* source plant as a random effect (lme4, Bates et al 2015). The binomial GLMM of *G. atropunctata* transmission efficiency included an additional fixed effect of year to account for variation between the 2019 and 2020 replicates. All analyses were conducted in the R programming language, ver. 4.02.

## Results

### *Philaenus spumarius* Transmission

In 2018, none of the pre-screened plants (0 of 34) tested positive for *X. fastidiosa*. Likewise, *X. fastidiosa* was not isolated by culture from the 30 negative control plants. For *P. spumarius*, there was a significant effect of days after AAP on the proportion of *X. fastidiosa* infected test plants ($\chi^2 = 5.270$, df = 1, $P = 0.022$). Immediately after the AAP, ~5.9% (8 of 136) of *P. spumarius* transmitted *X. fastidiosa* to test plants. Although we observed only 2.2% (3 of 136) transmission at two and four days after the AAP, transmission increased to ~7.5% (9 of 120) at six days after the AAP and 11.5% at eight days after the AAP (13 of 113) (Figure 1A).

### *Graphocephala atropunctata* Transmission

The 30 negative control plants in 2019 were confirmed to free of *X. fastidiosa*. In 2020, *X. fastidiosa* was not found in any of the 16 negative controls. The effect of days after AAP ($\chi^2 = 0.140$, df = 1, $P = 0.70839$) on the proportion of plants that became infected with *X. fastidiosa* was not significant while the effect of year ($\chi^2 = 3.283$, df = 1, $P = 0.07002$) was marginally significant. In 2019, ca. 35% of *G. atropunctata* individuals (28 of 80) transmitted the pathogen immediately after the AAP, although this frequency decreased to ~27% (20 of 74) and ~19% (15 of 79) of adults successful in transmitting the pathogen by six and eight days after the AAP respectively (Figure 1B). Approximately 43% of *G. atropunctata* individuals (29 of 67) transmitted immediately after the AAP in 2020. Although 30% of *G. atropunctata* adults (21 of 69) transmitted the pathogen by four days after the AAP, transmission increased to ~ 55% of adults at eight and twelve days after the AAP (37 of 67 and 30 of 54 individuals) (Figure 1B).

## Discussion

We compared the transmission efficiency of *X. fastidiosa* by two insect vectors shortly after they acquired it from infected plants. We observed a significant effect of time after the AAP on transmission efficiency for *P. spumarius*, but not for *G. atropunctata*. We consider the biological associations between each vector species, their plant hosts and *X. fastidiosa* to explain the observed transmission patterns.

The distinct feeding behavior of vectors on their host plants may explain the differential transmission dynamics we observed. During an eight-hour electrical penetration graph (EPG) recording of *P. spumarius* conducted on *Olea europaea* (Linnaeus) (Lamiales: Oleaceae) an average of four to five total probes (Cornara et al. 2018) were noted. On *V. vinifera*, *P. spumarius* was noted to make two to three successful probes over a six-hour EPG recording (Markheiser et al. 2020). Although few in number, the duration of *P. spumarius* probing events can be quite long (eight to ten hours per twelve hour EPG recording) (Sandanayaka et al. 2017. Compared to uninfected *P. spumarius* feeding on healthy *O. europaea*, *X. fastidiosa*-infected *P. spumarius* had shorter periods of xylem ingestion and spent less time probing (Cornara et al. 2020). Given that *X. fastidiosa* infection of the insect may have decreased the frequency of *P. spumarius*' feeding events, the bacterial population inoculated during each feeding event may become a crucial determinant of inoculation success. Notably, the feeding behavior of infectious *P. spumarius'* on grapevine has not been characterized, so the behavior of infectious vectors on that host may differ. Like *P. spumarius*, *G. atropunctata* feeding behavior on host plants may influence *X. fastidiosa* transmission. *Vitis vinifera* is a suitable host that supports the development of large populations of *Graphocephala atropunctata* (Hewitt et al. 1949) and *G.*

*atropunctata* can transmit *X. fastidiosa* with high efficiencies (70-90% per insect per day) when feeding on infected plants (Hill and Purcell 1995). This insect readily feeds on grapevine, conducting 5 to 27 probes over 20 hours (Almeida and Backus 2004). In comparisons with European sharpshooters and spittlebugs that feed on the same host plant, sharpshooter species consistently conducted a greater number of probing events (personal communication with Daniele Cornara, unpublished data). The number of inoculation events rather aggregate number of pathogen cells introduced into plants is a more important measure of *G. atropunctata* inoculum supply and transmission success by *G. atropunctata* (Daugherty et al. 2009). In another sharpshooter species, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), number of probing events was a significant predictor of number of *X. fastidiosa* cells introduced into plants, while probing duration and *X. fastidiosa* population in vector mouthparts were not predictive of the number of *X. fastidiosa* cells introduced into plants (Jackson et al. 2008). As an efficient vector feeding on a preferred host, the post-acquisition transmission dynamics of *G. atropuncata* are likely due to day-to-day variation in probing events driven by insect-plant interactions.

Bacterial population dynamics within vector mouthparts might also influence transmission efficiency. Morphometric analyses of *P. spumarius*' mouthparts (Ranieri et al. 2020) suggested that it had an available surface area capable of supporting far larger populations of *X. fastidiosa* than those typically observed in spittlebugs (ca. $10^3$ CFU per individual) (Cornara et al. 2016). Low *X. fastidiosa* populations within *P. spumarius* mouthparts are likely due to factors related to the fluid dynamics and surface chemistry of this species' mouthparts (Cornara et al. 2016, Ranieri et al. 2020). The long duration of spittlebug ingestion events and the alternating contraction and rest of the cibarial pump are associated with turbulence that might hinder the attachment and multiplication of *X. fastidiosa* within spittlebug mouthparts (personal communication with Daniele Cornara, unpublished data). As the population size of *X. fastidiosa* within *P. spumarius* increases from less than $10^2$ CFUs to $10^3$ CFUs by four – seven days after acquisition (Cornara et al. 2016), differences in bacterial multiplication during this time could impact transmission. The low bacterial populations within *P. spumarius* mouthparts might affect the inoculative dose delivered in each transmission event. The presence rather than prevalence of *X. fastidiosa* in *G. atropunctata* mouthparts may be another driving force affecting transmission dynamics. The predicted effect of complete colonization of vector mouthparts on fluid dynamics was much more pronounced for *G. atropunctata* than for *P. spumarius,* increasing velocity in the precibarium from 1.47 m/s to 4.57 m/s (Ranieri et al. 2020). Even low levels of bacterial colonization (5 to 20% of maximal colonization) was predicted to increase flow rates within *G. atropunctata* mouthparts. Although primarily driven by the frequency of probing events, the high transmission rates of *G. atropunctata* might be due to its maintenance of large bacterial population sizes that are supportive of transmission throughout the post-acquisition period. *Xylella fastidiosa* populations in *G. atropunctata* multiply to reach populations of $10^4$ CFUs within four to ten days (Killiny and Almeida 2009, Labroussaa et al 2017), and some mouthparts have been found with populations of $10^4$ cells immediately after the AAP, albeit in low frequency (Hill and Purcell 1995). Typically, population sizes of $10^4$ CFUs within host plants are considered the threshold for *X. fastidiosa* acquisition and transmission (Hill and Purcell 1997). Under this scenario, transmission events during the initial post-acquisition period would be driven primarily by free *X. fastidiosa* cells acquired from the host plant, but not yet established as a biofilm in mouthparts. As *X. fastidiosa* cells colonized the

precibarium and multiplied into a mature biofilm, the large and persistent population would become the primary inoculum supply for later transmission events.

**Conclusions**

Through a series of transmission experiments, we characterized the transmission efficiency of *X. fastidiosa* of *P. spumarius* and *G. atropunctata* after their acquisition of the pathogen from infected plants. We observed a significant effect of time after the AAP on the proportion of plants infected by *P. spumarius*. However, there was little effect of time after the AAP on the proportion of plants infected by *G. atropunctata*. We hypothesize that the different feeding behaviors (Almeida and Backus 2004, Cornara et al. 2018, Cornara et al 2020) and mouthpart fluid dynamics (Ranieri et al 2020) of these two insect vectors may explain the distinct transmission patterns. The limited growth of *X. fastidiosa* in *P. spumarius* mouthparts (Cornara et al. 2016) may influence the number of cells inoculated in each feeding event. Conversely, the favorable feeding behavior of *G. atropunctata* on grapevine (Almeida and Backus 2004) supports the conclusion that a high number of inoculation events rather than bacterial population in the mouthparts is a major predictor of transmission success for that species (Daugherty et al. 2009). Overall, our results support the conclusion that the complexity of interactions among insect vectors, pathogens, their hosts and environmental factors lead to highly divergent transmission outcomes (Lopes et al. 2009).

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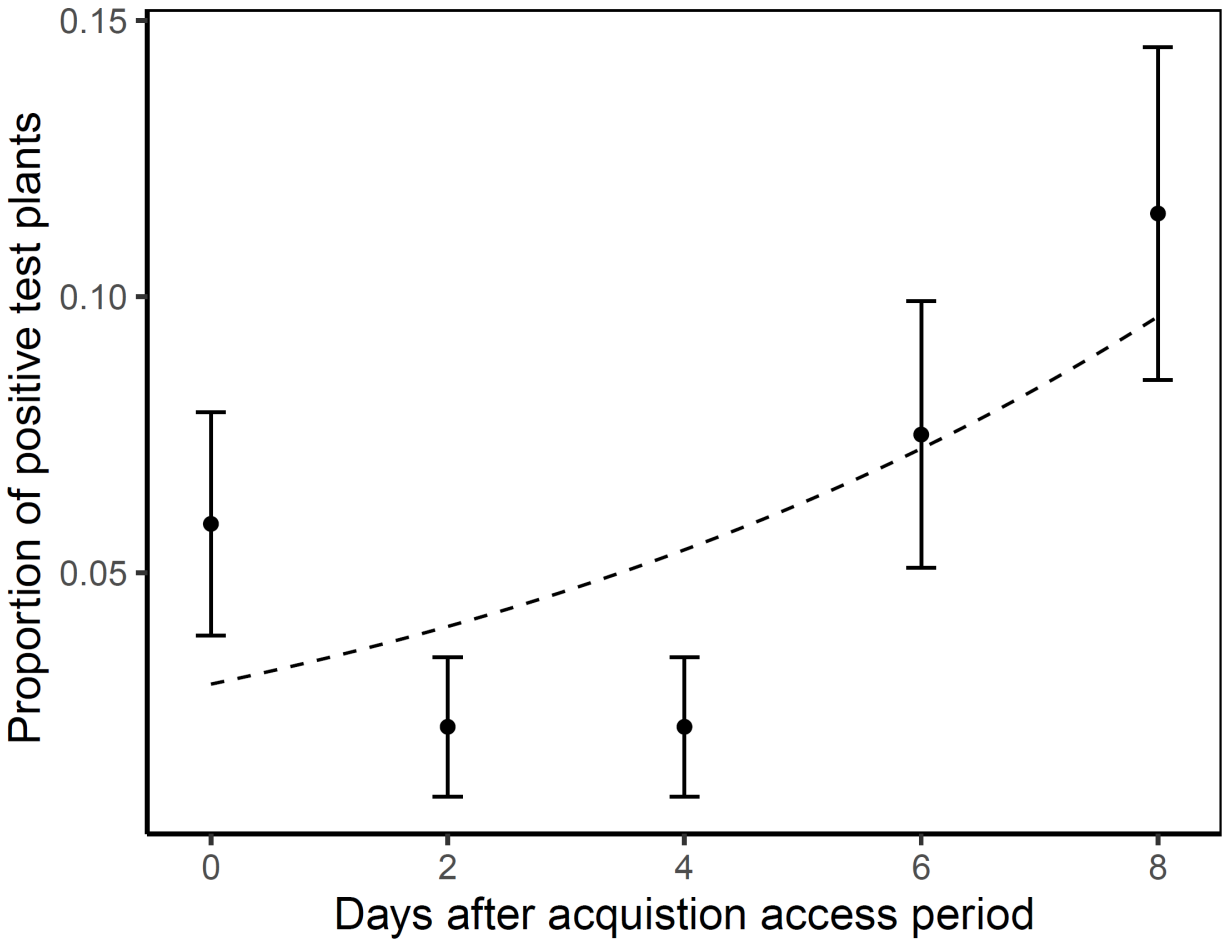
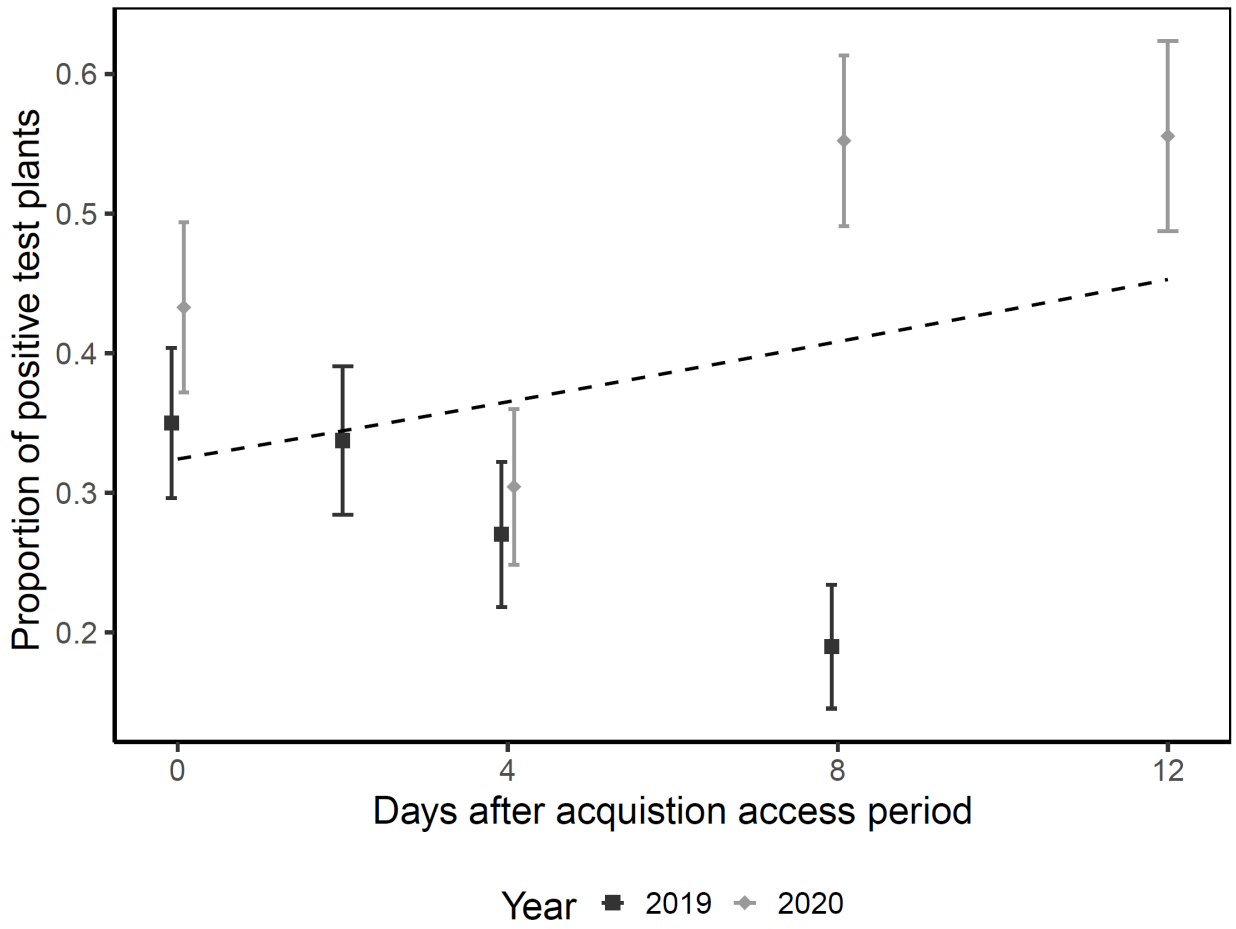
**Figure legends**

Figure 1: Transmission Efficiency reported as proportion of positive test plants infected in the days following the acquisition access period for *Philaenus spumarius* in 2018 (1A) and *Graphocephala atropunctata* (1B) in 2019 (black squares) and 2020 (gray diamonds).


0.15
0.10
0.05
Proportion of positive test plants
0
2
4
6
8
Days after acquistion access period

**A.**


Proportion of positive test plants
0.6
0.5
0.4
0.3
0.2
0
4
8
12
Days after acquistion access period
Year
2019
2020

**B. Figure 1.**