Project title: The spatial distribution of PD across the North Coast: Patterns, causes, and implications for management.

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Abstract: *Xylella fastidiosa* can infect all winegrape varieties and some riparian plants, but its limited persistence and low titers in many species means that a small subset of all hosts are likely important inoculum sources. We examined the relationship between the abundance of riparian host species in riparian areas and the incidence of Pierce's disease in Napa Valley vineyards. Our preliminary finding of only one species, *Vinca major* (periwinkle), being positively correlated with a high incidence of Pierce's disease suggests that eradication of all twelve species recommended for removal, according to the guidelines of riparian vegetation management, may be unnecessary.

Introduction: In California's North-coastal grape-growing region, the Pierce's disease pathogen, *Xylella fastidiosa*, is vectored by a native insect, *Graphocephala atropunctata* (blue-green sharpshooter; BGSS; Purcell 1975). There are no effective controls for Pierce's disease on the North Coast. Uptake of a soilapplied insecticide, imidacloprid, is effective against the invasive vector *Homalodisca coagulata* (glassywinged sharpshooter; GWSS) in the San Joaquin Valley and southern California (Castle et al. 2005), but is known to be poor in North Coast soils (Weber et al. 2005). The egg parasitoids released in southern California to control GWSS do not attack the BGSS (Boyd and Hoddle 2006). Furthermore, no commercially-viable, resistant winegrapes yet exist.

Riparian areas contribute to Pierce's disease in North Coast vineyards as evidenced by a correlation between disease incidence and proximity of vines to riparian areas (Purcell 1974). Eradication of riparian hosts, a technique known as riparian vegetation management (Anonymous 2000), offers the promise of decreasing the pathogen reservoir and vector densities outside of vineyards. However, there are some gaps in the knowledge that make it difficult to predict the efficacy of this approach for control of Pierce's disease.

X. fastidiosa hosts have been identified primarily from greenhouse studies (Hill and Purcell 1995). Greenhouse studies are important for evaluating a pathogen's host range. However, the moderate greenhouse climate and the high pathogen concentrations used for inoculations likely over-estimate a pathogen's host range in the field. The generalist feeding habit of the BGSS (Hewitt et al. 1949) further complicates our ability to predict which hosts identified from greenhouse studies are significant inoculum sources (i.e., competent reservoirs). In the field, such hosts are situated within plant communities, where their relative abundance and, thus, importance in the spread of a pathogen, can vary. Therefore, field-based investigations are needed to identify vegetation types that contribute most to the spread of Pierce's disease. X. fastidiosa populations within infected hosts affect the probability of a vector first acquiring the pathogen while feeding on such hosts, then transmitting the pathogen to other hosts (Hill and Purcell 1997). While X. fastidiosa populations tend to be higher in some hosts (e.g., Vitis vinifera cv. Chardonnay) than in others (e.g., Artemisia douglasiana; mugwort), they are known to fluctuate according to temperature (Feil and Purcell 2001) and plant hormones (Hopkins 1985). In order for a host to serve as a significant inoculum source, vector feeding must be synchronous with X. fastidiosa populations at or above the acquisition threshold ($\geq 10^4$ colony forming units (CFU) per g petiole tissue; Hill and Purcell 1997). Such fluctuations make it difficult to determine that all hosts identified in the greenhouse are likely to host high pathogen populations during high rates of feeding activity. Given that X. fastidiosa is not detectable in vines early in the growing season (Hopkins 1981), it is possible that overwintering BGSSs acquire the pathogen from feeding on infected riparian hosts in spring (Purcell 1975). In spring, we found that X. fastidiosa was not detectable in two riparian hosts [California grapevine (Vitis californica), blue elderberry (Sambucus

mexicana)] at two North Coast locations where infected plants of both species were placed the previous summer (Baumgartner and Warren 2005). X. fastidiosa was detected in spring in three riparian hosts [California blackberry (Rubus ursinus), Himalayan blackberry (Rubus discolor), periwinkle (Vinca major)], but no plants of any of these species supported X. fastidiosa populations $\geq 10^4$ CFU/g petiole. Our findings suggest that seasonal fluctuations in X. fastidiosa populations limit the ability of species identified as hosts in the greenhouse to serve as hosts in the field.

We previously examined the relationship between Pierce's disease and the spatial arrangement of vineyards and other habitats (i.e., landscape structure). Our objective was not to disprove the association between riparian areas and Pierce's disease, but to clarify the influence of other habitats (vineyards, residential neighborhoods, wineries, etc..) known to support both the pathogen and the vector. Little work has been done on the influence of landscape structure on the spread of plant diseases (e.g., the invasive forest pathogen, Phytophthora ramorum; Meentemeyer et al. 2004). Nonetheless, landscape structure is known to be a key factor in the spread of vector-borne mammalian diseases, such as Lyme disease (Allan et al. 2003), bubonic plague (Collinge et al. 2005), and malaria (Guerra et al. 2006). Lyme disease and Pierce's disease share some similarities. Both pathosystems are characterized by a generalist pathogen, a generalist vector, and numerous alternate hosts that occupy different habitat types. Natural habitats with more diverse host populations are likely to support lower densities of alternate hosts via a dilution effect, and this is thought to contribute to lower incidence of Lyme disease in urban areas surrounded by such habitats (Schmidt and Ostfeld 2001). Based on the results of binary logistic regression analyses, we found that Pierce's disease was more likely to occur in Napa Valley vineyards situated in a landscape surrounded by more vineyard and residential development, regardless of their proximity to riparian habitat (Greenleaf et al. In review). Given that vineyards and urban areas support high densities of BGSS and X. fastidiosa hosts, specifically grapevines (Davis et al. 1978) and landscape plants (Hewitt et al. 1949; Severin 1949; Freitag 1951), it is possible that these habitats are more important in the spread of Pierce's disease than previously thought. Indeed, reports of pathogen titers in various hosts show that grapevines support among the highest (Hill and Purcell 1995).

In 2006 and 2007, we investigated the relationship between plant community composition in riparian areas and disease incidence in adjacent vineyards (Figure 1). Our study was the first to investigate which riparian plants are correlated with a high incidence of Pierce's disease in vineyards.

Objectives:

- 1) Quantify the relative abundance of plant species in riparian areas adjacent to vineyards with a high incidence of Pierce's disease and vineyards with a low to zero incidence,
- 2) Quantify the incidence of Pierce's disease in the vineyards,
- 3) Examine the correlation between the relative abundance of riparian species and Pierce's disease in adjacent vineyards.

Results: Based on real-time PCR analyses of X. fastidiosa from symptomatic vines in 50 vineyards, we found that the incidence of Pierce's disease is significantly greater in vineyards adjacent to riparian areas where periwinkle is more abundant (multiple regression model summary: $R^2 = 0.37$, p = 0.014; Figure 2). In riparian areas adjacent to vineyards with Pierce's disease, periwinkle was 21 times more abundant, on average, than in riparian areas that were adjacent to disease-free vineyards. In contrast, neither California blackberry, California grape, elderberry, Himalayan blackberry, mugwort, nor stinging nettle were associated with a high incidence of Pierce's disease in nearby vineyards.

Conclusions: In applying knowledge of the *X. fastidiosa* and BGSS host ranges to development of management strategies for control of Pierce's disease in the field, we find that there are some important gaps to fill. Over the past several years, our efforts have been aimed at testing hypotheses that fill such gaps, in order to help growers evaluate their riparian vegetation management efforts. Our preliminary results from this previous year show that periwinkle is significantly correlated with a high incidence of Pierce's disease, and that other riparian hosts known to harbor the pathogen are not. It is possible, therefore, species may be more important in the spread of Pierce's disease than previously thought.

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Figure 1. Study design for sampling the plant community composition in riparian areas and Pierce's disease in vineyards. We studied 50 randomly selected vineyards in Napa, Sonoma, and Mendocino Counties; each was adjacent to riparian vegetation. In Summer 2006, at each site we examined 500 grapevines for Pierce's disease symptoms and confirmed the presence of *X. fastidiosa* by real-time PCR among symptomatic vines. In addition, we surveyed the riparian vegetation adjacent to each vineyard. Along each of four transects per site, we measured the percent cover per riparian host within each of 20 0.5 x 0.5-m quadrats.

Coefficientsa

		Unstandardized Coefficients		Standardized Coefficients		
Model		В	Std. Error	Beta	t	Sia.
1	(Constant)	-3.575	6.384		560	.578
	VIMI	.350	.121	.415	2.895	.006
	ARDO	122	.183	091	666	.509
	RUDI	.015	.121	.016	.124	.902
	RUUR	.148	.153	.135	.968	.339
	SAME	.014	.106	.017	.134	.894
	URDI	.414	.252	.212	1.642	.108
	VICA	.131	.102	.177	1.279	.207

a. Dependent Variable: PD

Figure 2. Multiple regression analyses of the relationship between relative abundance of riparian hosts (Vinca, VIMI; mugwort, ARDO; Himalayan blackberry, RUDI; California blackberry, RUUR; blue elderberry, SAME; stinging nettle, URDI; California grape; VICA) and the incidence of Pierce's disease in adjacent vineyards. Variables were measured at a total of 50 vineyards according to the sampling scheme described in Figure 1.