SIGNIFICANCE OF RIPARIAN PLANTS IN THE EPIDEMIOLOGY OF PIERCE’S DISEASE

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ABSTRACT
We examined the relationship between the occurrence of Pierce’s disease (PD) in Napa Valley vineyards and both adjacent and distant vegetation types. Because the vector, Graphocephala atropunctata (blue-green sharpshooter, BGSS), is mobile and has a broad host range, disease risk is influenced by vector migration among vegetation types. Therefore, certain combinations of vegetation types surrounding vineyards are more likely to be associated with PD. To test this hypothesis, we surveyed for PD in a total of 41 vineyards located adjacent to either riparian woodland (vector habitat), urban land (vector habitat), other vineyards (vector habitat), or oak woodland (habitat status unknown). The proportions of the four vegetation types distant from the sites (within 0.5, 1, 1.5, and 2 km) were quantified with a geographical information system. Vineyards were surveyed for PD in 11/05. Pathogen presence was confirmed by ELISA. Multiple binary logistic regression showed that both adjacent and distant vegetation type significantly predicted PD presence. Vineyards were more likely to have PD if they were adjacent to riparian woodland and surrounded by more vineyards or urban land. These results suggest that vineyards and urban lands may be important in PD epidemiology. Given that uninfected vineyards adjacent to riparian woodland were also surrounded by large amounts of riparian and upland woodland, it is also possible that riparian woodland in more forested landscapes hosts lower vector densities or a lower proportion of infective vectors. Alternatively, more expansive woodland may be associated with lower PD risk because it decreases the spread of infective BGSSs.

INTRODUCTION
Riparian areas contribute to Pierce’s disease (PD) in North Coast vineyards, as evidenced by a correlation between disease incidence and proximity of vines to riparian woodland (Purcell 1974). Purcell (1975) concluded that the bluegreen sharpshooter (BGSS) acquires Xylella fastidiosa (Xf) mainly from riparian hosts in spring, as the pathogen is not detectable in vines early in the growing season (Hopkins 1981). Our findings of few infected riparian hosts in spring suggest that feeding on such hosts is unlikely to result in Xf acquisition by the BGSS at this time (Baumgartner and Warren 2005). Therefore, either BGSSs acquire Xf from riparian hosts in spring or autumn, when Xf populations are sufficient, or they acquire Xf from other hosts.

The generalist feeding habit of the BGSS (Hewitt et al. 1949) makes it difficult to predict which hosts are important inoculum sources (competent reservoirs). Xf has a broad host range that includes all winegrape varieties and some riparian plants (Hewitt et al. 1949, Severin 1949, Freitag 1951), but its limited persistence and low titers in most species means that not all hosts are competent reservoirs (Purcell and Saunders 1999, Baumgartner and Warren 2005). Xf hosts have been identified mainly from greenhouse studies (e.g. Hill and Purcell 1995). In the field, such hosts are situated within plant communities (vegetation types), where their relative abundance can vary. In addition, the Xf-conducive environment in a greenhouse likely over estimates the host range. Therefore, field-based investigations are needed to identify vegetation types that contribute most to the spread of PD.

Our aim was to determine the relationship between PD and the spatial arrangement of vineyards among other vegetation types (landscape structure). Landscape structure is 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), and the invasive forest pathogen, Phytophthora ramorum (Meentemeyer et al. 2004). Our approach was to randomly select 41 Napa Valley vineyards adjacent to riparian woodland (vector habitat), urban land (vector habitat), other vineyards (vector habitat), or oak woodlands (habitat status unknown). The proportions of the four vegetation types distant from the sites (within 0.5, 1, 1.5, and 2 km) were quantified with a geographical information system (GIS; ArcGIS v9.1, ESRI, Inc., Redlands, CA). Each site (standardized to a 500-vine block) was sampled for PD in October 2005. Xf presence was confirmed by Dr. Barry Hill, using ELISA (Hill and Purcell 1995). Multiple binary logistic regression was used to identify combinations of adjacent and distant
vegetation types that were significantly correlated with PD. We also examined the relationship between winter temperatures, adjacent and distant vegetation types, and PD.

OBJECTIVES
1. Determine if vegetation types distant from vineyards are correlated with PD occurrence in North Coast vineyards.
2. Identify combinations of adjacent and distant vegetation types that are correlated with PD occurrence in North Coast vineyards.
3. Examine the relationship between winter temperatures, adjacent and distant vegetation types, and PD.

RESULTS
Multiple binary logistic regression showed that both adjacent and distant vegetation type significantly predicted PD presence. PD was significantly more likely to be present in vineyards adjacent to riparian woodland. Conversely, PD was less likely to be present in vineyards surrounded by a higher proportion of riparian woodland. This pattern was significant at the buffer radius of 1.0 km, based on both the likelihood ratio and Wald significance tests, and it was significant at the radius of 1.5 km, based on the likelihood ratio test (Table 1). A similar, but nonsignificant pattern existed for the 0.5 km (Figure 1) and 2.0 km radii. Similarly, we found that PD was significantly likely to be absent from vineyards surrounded by a higher proportion of upland woodland at all spatial scales, based on the likelihood ratio test.

The opposite pattern existed for vineyards and urban land. Vineyards were significantly more likely to have PD if they were adjacent to riparian woodland and if more vineyards were present within 0.5, 1.0, 1.5, or 2.0 km (Table 1; Figure 2). Similarly, vineyards surrounded by more urban land were significantly more likely to have PD at all spatial scales.

There were correlations between some vegetation types at some spatial scales (data not shown). Proportions of upland woodland and riparian woodland were positively correlated ($P<$0.05) at 1.0, 1.5, and 2.0 km. Proportions of urban land and vineyard were also positively ($P<$0.05) correlated at 1.0, 1.5, and 2.0 km. All other pairs of independent variables (riparian woodland vs. vineyard, upland woodland vs. vineyard, upland woodland vs. urban, riparian woodland vs. urban) were negatively correlated ($P<$0.05) at all four spatial scales.

PD was more likely to be present in vineyards with colder minimum January temperatures. Multiple binary logistic regression with minimum January temperature and type of adjacent vegetation as independent variables showed that the overall model was significant ($X^2=14$, $P=0.001$; Nagelkerke $R^2=0.43$). Both minimum January temperature (Wald=6.9, $P=0.008$) and type of adjacent vegetation (Wald=4.6, $P=0.03$) contributed significantly to the model. Minimum January temperature was positively correlated with the proportion of total woodland within 0.5, 1.0, 1.5, and 2.0 km of the vineyards ($P<$0.05).

CONCLUSIONS
Our results show a clear pattern - vineyards are more likely to have PD if they are adjacent to riparian woodland and surrounded by more vineyards or urban land. Several mechanisms, not mutually exclusive, may result in this pattern. For example, because all winegrapes and certain ornamental plants can host both the vector and the pathogen, vineyards and urban lands may be important in PD epidemiology. Northern California vineyards and gardens, which are irrigated in the summer, may provide relatively attractive forage for the BGSS. In addition, reports of pathogen titers show that grapevines are among the highest (Hill and Purcell 1995). Therefore, the focal host species in this disease system, the grapevine, may itself be a competent reservoir. However, given that the proportions of vineyards and urban lands were significantly positively correlated, and that both were negatively correlated with the proportion of woodland, it is not possible to determine if spatial patterns of PD result from the presence of vineyards and urban lands and/or the absence of woodland.

Although PD was more common in vineyards adjacent to riparian woodland, the concomitant absence of PD from the majority of such vineyards (63%) suggests that either not all riparian woodland contributes to PD risk or that factors other than proximity to riparian woodland are more important. Given that uninfected vineyards adjacent to riparian woodland were also surrounded by large amounts of riparian and upland woodland, it is possible that riparian woodland in more forested landscapes hosts lower vector densities. Similar results were found in Lyme disease studies, where both vector density and the proportion of infective vectors are higher in smaller forest patches (Allan et al. 2003). Riparian woodland is thought to be preferred BGSS habitat because of the succulent vegetation. In vineyards situated in more forested landscapes, BGSS densities may be controlled by natural enemies. Insect predators are often more vulnerable to loss of natural habitat than are their prey (Hunter 2002). This differential effect of habitat fragmentation on natural enemies and prey would be expected to result in high BGSS densities in riparian woodland adjacent to vineyard only when natural habitat has been lost in the surrounding landscape.

Smaller patches of riparian woodland may be associated with higher PD risk because they may host a higher density of competent reservoirs, compared to larger woodland patches. More expansive woodlands are typically characterized by higher plant diversity, possibly resulting in lower densities of competent reservoirs via a dilution effect (Schmidt and Ostfeld 2001). PD is characterized by several features required for such a relationship to exist between disease risk and host diversity.
Temperature is a potentially confounding variable in our study. Grapevines are more likely to recover from infection by *Xf* in regions with colder winters (Feil et al. 2003). Thus, if colder vineyards were more likely to be situated in landscapes dominated by woodland, winter temperature might have explained the relationship between woodland and PD. We found the opposite pattern; colder vineyards were more likely to be infected with PD and were more likely to be in landscapes with low amounts of woodland. Since our results are limited to correlative relationships among these factors (not causal relationships), we can only conclude that the relationship between PD and winter temperature may be more complex than previously thought. It is possible that factors other than winter temperatures, that are also correlated with the amount of nearby woodland, are responsible for increased risk of PD.

From an epidemiological perspective, our findings suggest that both local and remote factors have interactive effects on the risk of PD. The occurrence of PD is not correlated with the presence of an adjacent riparian woodland unless the surrounding landscape is also dominated by vineyards or urban lands. This complicates management decisions, which are targeted at the vineyard scale. If vegetation types and their proportions within 2 km of a vineyard have significant effects on PD, then control practices focused on the vineyard and its immediately surrounding area have to counteract these distant factors.

**REFERENCES**


**FUNDING AGENCIES**

Funding for this project provided by the CDFA Pierce’s Disease and Glassy-winged Sharpshooter Board.
Table 1. Results from multiple binary logistic regression of the relationship between PD occurrence, adjacent vegetation type, and distant vegetation type (within 0.5, 1, 1.5, and 2 km of each vineyard site).

<table>
<thead>
<tr>
<th>Continuous independent variable</th>
<th>Likelihood ratio test of significance of continuous independent variable</th>
<th>Type of adjacent vegetation (riparian vs. non-riparian)</th>
<th>Overall model</th>
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<tr>
<td>Variable</td>
<td>Radius (km)</td>
<td>Nagelkerke R²</td>
<td>Parameter estimate*</td>
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<tr>
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<td>-5.6</td>
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</table>

*Negative parameter estimate, likelihood of PD decreases with increasing proportions of the vegetation type; positive parameter estimate, likelihood of PD increases with increasing proportions of the vegetation type.

Figure 1. Relationship between PD, type of adjacent vegetation, and the proportion of riparian woodland within 500 m. Closed circles, vineyards adjacent to riparian woodland; open circles, vineyards adjacent to urban land or other vineyards; y-axis, PD presence/absence. A similar pattern was found for the proportion of riparian woodlands within 1, 1.5, and 2 km (data not shown).

Figure 2. Relationship between PD, type of adjacent vegetation, and the proportion of vineyards within 500 m. Closed circles, vineyards adjacent to riparian woodland; open circles, vineyards adjacent to urban land or other vineyards; y-axis, PD presence/absence. A similar pattern was found for the proportion of vineyards within 1, 1.5, and 2 km (data not shown).
FUNCTIONAL GENOMICS OF THE GRAPE-XYLELLA INTERACTION: TOWARD THE IDENTIFICATION OF HOST RESISTANCE DETERMINANTS

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ABSTRACT
Susceptible Vitis vinifera responds to Xylella infection with a massive re-direction of gene transcription involving >800 genes with strong statistical support. This number is increased from previous estimates based on use of a more sensitive and robust statistical method known as linear models for microarray data (LIMMA). The transcriptional response to Xylella infection is characterized by increased transcripts for phenylpropanoid and flavonoid biosynthesis, ethylene production, adaptation to oxidative stress, and homologs of pathogenesis related (PR) proteins, and decreased transcripts for genes related to photosynthesis. A survey of 22 transcripts by means of in situ hybridization reveals that a majority of transcriptional activity is associated with phloem and cortical tissues, consistent with the presence of the pathogen in adjacent xylem elements. DNA sequence analysis of regions 5' to the transcription site for ~200 differentially expressed genes provides a rich source of new gene promoters and the possibility of in silico analysis of regulatory cis-elements.

In addition to highlighting potential metabolic and biochemical changes that are correlated with disease, the results suggest that susceptible genotypes respond to Xylella infection by induction of a limited, but apparently inadequate, defense response. We have also tested the hypothesis that Pierce's disease results from pathogen-induced drought stress. We compared the transcriptional and physiological response of plants treated by pathogen infection, low or moderate water deficit, or a combination of pathogen infection and water deficit. Although the transcriptional response of plants to Xylella infection was distinct from the response of healthy plants to moderate water stress, we observed synergy between water stress and disease. In particular, water stressed plants exhibit a stronger transcriptional response to the pathogen. This interaction was mirrored at the physiological level for aspects of water relations and photosynthesis, and in terms of the severity of disease symptoms and pathogen colonization, providing a molecular correlate of the classical concept of the disease triangle.

INTRODUCTION
All organisms adapt to external stressors by activating the expression of genes that confer adaptation to the particular stress. In the case of Pierce's disease, such genes are likely to include those coding for resistance or susceptibility to Xylella fastidiosa (Xf).

Genomics technology offers an opportunity to monitor gene expression changes on a massive scale (so-called "transcriptional profiling"), with the parallel analysis of thousands of host genes conducted in a single experiment. In the case of Pierce's disease of grapes, the resulting data can reveal aspects of the host response that are inaccessible by other experimental strategies. In May of 2004, the first Affymetrix gene chip was made available for public use, with ~15,700 Vitis genes represented. This gene chip has been developed based primarily on collaboration between the Cook laboratory and researchers at the University of Nevada-Reno (Goes da Silva et al., 2005). With the arrival of the Affymetrix gene chip, we are poised to make a quantum leap in the identification of host gene expression in response to Xf.

In addition to enumerating differences between susceptible and resistant genotypes of Vitis, this research is testing a long-standing but largely untested hypothesis that pathogen-induced drought stress is one of the fundamental triggers of PD symptom development. The utility of this type of data will be to inform the PD research community about the genes and corresponding protein products that are produced in susceptible, tolerant and resistant interactions. Differences in the transcriptional profiles between these situations are expected to include host resistance and susceptibility genes, and thus provide the basis for new lines of experimental inquiry focused on testing the efficacy of specific host genes for PD resistance. It should be possible, for example, to determine the extent to which resistance responses in grapes are related to well-characterized defense responses in other plant species (e.g., Maleck et al 2002; Tao et al 2003; de Torres et al 2003).