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

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ABSTRACT

The goal of this research is to evaluate the significance of riparian hosts in the epidemiology of Pierce's disease (PD) in the North Coast grape-growing region of California. The first objective is to examine the epidemiological role of seasonal changes in *Xylella fastidiosa* (*Xf*) concentrations in riparian hosts. Among systemic riparian hosts, differences in seasonal *Xf* concentrations and *Graphocephala atropunctata* (blue-green sharpshooter, BGSS) feeding preference affect their importance as *Xf* reservoirs. Temperature affects *Xf* concentrations in plant hosts and, in turn, *Xf* concentrations affect the probability of a BGSS acquiring *Xf* while feeding on an infected plant. We focused on *Xf* concentrations in five systemic hosts: *Rubus discolor* (Himalayan blackberry), *R. ursinus* (California blackberry), *Sambucus mexicana* (blue elderberry), *Vinca major* (periwinkle), and *Vitis californica* (California grapevine). We needle inoculated potted plants of California grape, California blackberry, Himalayan blackberry, blue elderberry, and periwinkle in the greenhouse and transferred all infected plants to two sites in the North Coast (Napa County, Mendocino County). *Xf* was not detected in the majority of plants after several months in the field (from July to Oct. 2003), except for periwinkle which maintained a high number of infected plants through all seasons. *Xf* concentrations were highest in periwinkle in all seasons and at both sites, and were sufficient for BGSS acquisition year-round. California grape and Himalayan blackberry supported *Xf* concentrations that were sufficient for BGSS acquisition in autumn and summer, but not in spring. These results suggest that BGSS likely acquires *Xf* from riparian hosts in autumn, instead of spring.

INTRODUCTION

Past research (Purcell 1976, 1981) demonstrated the direct relationship between incidence of Pierce's Disease (PD) in *Vitis vinifera* and proximity to riparian plants bordering vineyards in the North Coastal grape-growing region of California. Vineyard rows closest to riparian plants (plant species that occupy the banks of rivers and streams) experience the heaviest losses, but there are fewer diseased vines farther away from riparian plants. Riparian habitat adjacent to vineyards contains plants that are feeding and breeding hosts for *Graphocephala atropunctata* (blue-green sharpshooter, BGSS), the most efficient vector of PD in the Napa Valley (Hewitt et al 1949, Purcell 1975). Not only do riparian plants provide habitat for BGSS, but some are also reservoir hosts of the PD strain of *Xylella fastidiosa* (*Xf*) (Freitag 1951). A variety of common riparian plants are capable of maintaining *Xf* infections without expressing disease symptoms. Purcell and Saunders (1999) found that *Xf* populations are, generally, lower in riparian hosts than in grapevines. The ability of *Xf* to multiply and spread within a plant host varies from species to species. After screening several breeding hosts of BGSS for systemic movement of *Xf*, Hill and Purcell (1995) found that only two tested, *Rubus discolor* (Himalayan blackberry) and *V. vinifera*, supported systemic infections. These results imply that some riparian hosts are more important than others as *Xf* reservoirs.

Interactions among BGSS, *Xf*, and their host plants are likely to vary with season. Seasonal changes in BGSS flight activity have been documented (Feil et al 2000). Seasonally variable levels of plant hormones (Hopkins 1985) and changes in temperature (Feil and Purcell 2001) can have major effects on *Xf* concentrations in host plants. *Xf* concentrations change on a seasonal basis in *V. labrusca* (Hopkins and Thompson 1984), and they are lower in *V. vinifera* grown at cooler temperatures (Feil and Purcell 2001). Transmission by BGSS is influenced by *Xf* concentrations in the plant host; the higher the concentration, the higher the probability of BGSS acquiring *Xf* (Hill & Purcell, 1997). Therefor, we might expect that seasonal fluctuations of *Xf* concentrations may influence the spread of PD to grapevines by affecting the proportion of BGSS that acquire *Xf* when feeding on riparian hosts.

OBJECTIVES

The goal of this research is to evaluate the significance of riparian hosts in the epidemiology of PD in the North Coast. Among systemic riparian hosts, differences in seasonal *Xf* concentrations and vector feeding preference affect their importance as *Xf* reservoirs. Temperature affects *Xf* concentrations in plant hosts; *Xf* concentrations, in turn, affect the probability of *Xf* acquisition by BGSS. Probability of *Xf* acquisition is also influenced by how attractive a host is to BGSS; a systemic riparian host that is fed upon more frequently will likely serve as a more significant source of *Xf*. The first objective is to examine the epidemiological role of seasonal *Xf* concentration fluctuations in riparian hosts in the field, where plants are subject to seasonal temperature changes. We focused on *Xf* in five systemic hosts: *Rubus discolor* (Himalayan blackberry), *Rubus ursinus* (California blackberry), *Sambucus mexicana* (blue elderberry), *Vinca major* (periwinkle), and *Vitis californica* (California grapevine). Future research will focus on BGSS feeding preference.

We tested the hypothesis that seasonal *Xf* concentrations were the same among riparian hosts at two sites in the North Coast (Napa County and Mendocino County). In July 2003, we transferred infected plants from the greenhouse to the field. Plants were in 3-gallon pots and were surrounded by a fine-mesh screen enclosure. *Xf* concentrations were estimated seasonally from petioles and stems, using dilution plating and real-time PCR. The effects of plant species, season, and location on *Xf* concentrations were determined using an analysis of variance (ANOVA). Results from the two quantitation techniques were analyzed separately. Real-time PCR results for summer 2004 are still in progress. This report focuses on culture data.

RESULTS

Xf infections were not sustained in the majority of infected plants, not just from autumn 2003 to the following summer 2004, but also in the short time between transferring infected plants to the field (July 2003) and the first culture attempt in autumn 2003 (Table 1). These results suggest that both cold winter temperatures and high summer temperatures can negatively affect *Xf*. Very few *Xf*-positive plants were detected by culture in winter 2004 and spring 2004 (Table 1). In fact, periwinkle was the only species with enough *Xf*-positive stems in winter 2004 and spring 2004 to make comparisons with summer 2004 data. Based on culture results, *Xf* concentrations were consistently high in periwinkle stems at both sites, with the highest detected in summer (Figure 1), suggesting that periwinkle is an excellent reservoir host, year-round.

Figure 1. Seasonal *X. fastidiosa* concentrations in periwinkle stems, as determined by culture. See Table 1 for sample sizes. Error bars are 95% confidence limits.

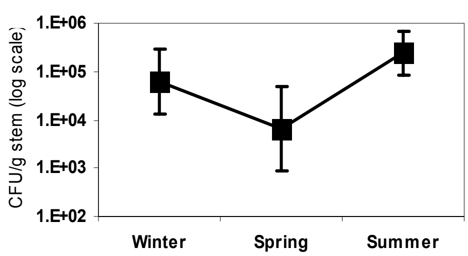
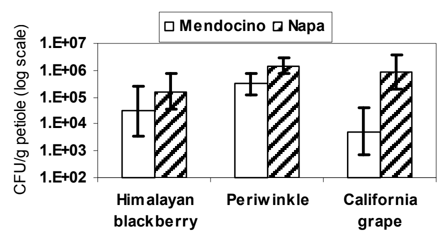


Figure 2. *Xylella fastidiosa* concentrations in petioles of riparian hosts at two North Coast sites, as determined by culture. Means are averages of autumn 2003 and summer 2004 data. See Table 1 for sample sizes. Error bars are 95% confidence limits.



In autumn 2003 and summer 2004, when there were more *Xf*-positive plants detected by culture than in winter 2004 and spring 2004, we found subtle relative differences among plant species at the two sites. For example, in Mendocino, California grape petioles had significantly lower *Xf* concentrations than periwinkle petioles, but there were no significant differences in *Xf* concentrations among periwinkle, Himalayan blackberry, or California grape in Napa (Figure 2). Assuming our results reflect that of naturally-established riparian hosts, spring *Xf* concentrations are sufficient for acquisition by BGSS in periwinkle, but not in any of the other riparian hosts tested.

Our autumn culture attempt coincided with the increased flight activity of young adult BGSS, which peaks in mid summer and remains high through early autumn (Feil et al 2000). Assuming BGSS feeds on California grape, Himalayan blackberry, and periwinkle in early autumn, *Xf* may be transmitted from infected riparian plants to adjacent vineyards before the end of the growing season. While late season infections of grapevines are unlikely to result in chronic disease before infected canes are pruned out in winter (Purcell 1981), young adult BGSSs that acquire *Xf* in autumn and survive the winter are still capable of transmitting *Xf* the following spring.

Real-time PCR results detected more *Xf*-positive plants and much higher *Xf* concentrations than culture results for all species, seasons, and tissues (*data not shown*). Real-time PCR is more sensitive than culture, so we expect that this DNA-based technique would detect *Xf*-positive plants with very low *Xf* concentrations. Detection of higher *Xf* concentrations by real-time PCR as compared to culture is likely a function of *Xf* cells being sticky; the assumption that one colony results from one cell when culturing *Xf* likely leads to underestimates of actual *Xf* concentrations.

Table 1. Number of tested plants confirmed infected with *X. fastidiosa*, as determined by culture and real-time PCR from petioles and stems for four consecutive seasons. All plants were infected upon transfer from the greenhouse to the field in July 2003.

			# *X. fastidiosa*-infected plants			
			Culture		Real-time PCR	
Season	Location/ species	Plants tested	Petiole	Stem	Petiole	Stem
Autumn 2003	Mendocino					
(Oct. 1-22)	Himalayan blackberry	30	3	NC[y]	8	NC
	California blackberry	30	0	NC	0	NC
	Blue elderberry	30	0	NC	4	NC
	Periwinkle	30	28	NC	30	NC
	California grape	30	4	NC	13	NC
	Napa					
	Himalayan blackberry	29	9	NC	13	NC
	California blackberry	30	0	NC	4	NC
	Blue elderberry	28[w]	0	NC	0	NC
	Periwinkle	30	30	NC	30	NC
	California grape	25	5	NC	6	NC
Winter 2004	Mendocino					
(Jan. 27-Feb. 11)	Himalayan blackberry	29	0	4	1	26
	California blackberry	26	0	0	1	12
	Blue elderberry	38	0	0	0	1
	Periwinkle	30	1	6	29	30
	California grape	NC	--	--	--	--
	Napa					
	Himalayan blackberry	29	0	3	2	18
	California blackberry	34[x]	0	0	0	15
	Blue elderberry	34	0	0	0	0
	Periwinkle	30	4	19	29	30
	California grape	NC	--	--	--	--
Spring 2004	Mendocino					
(May 24-June 9)	Himalayan blackberry	30	0	1	0	2
	California blackberry	26	0	0	0	0
	Blue elderberry	37	0	0	0	0
	Periwinkle	26	1	5	14	19
	California grape	29	0	0	0	0
	Napa					
	Himalayan blackberry	29	0	6	1	7
	California blackberry	34	0	0	0	3
	Blue elderberry	34	0	0	0	0
	Periwinkle	19	1	6	8	11
	California grape	24	0	0	0	0
Summer 2004	Mendocino					
(Aug. 2-17)	Himalayan blackberry	34	2	3	IP[z]	IP
	California blackberry	29	0	2	IP	IP
	Blue elderberry	38	0	0	IP	IP
	Periwinkle	30	9	18	IP	IP
	California grape	38	2	5	IP	IP
	Napa					
	Himalayan blackberry	43	3	9	IP	IP
	California blackberry	36	0	2	IP	IP
	Blue elderberry	38	0	0	IP	IP
	Periwinkle	29	21	21	IP	IP
	California grape	40	5	8	IP	IP

[w]For real-time PCR, 24 Blue elderberry were tested.
[x]For culture, 33 California blackberry were tested.
[y]Not collected. In Autumn 2003, all plants were too young to collect stem tissue. In Winter 2004, California grape was dormant.
[z]In progress.

CONCLUSIONS

Riparian Revegetation Management is a method of PD control that focuses on removal of host plants of BGSS and *Xf*, followed by revegetation with native, non-hosts. This method has been shown to reduce local populations of BGSS (*unpublished research*, Dr. Alexander H. Purcell, Division of Insect Biology, UC Berkeley), but its impact on the riparian area as a reservoir of *Xf* has not been quantified. To obtain approval for a Lake and Streambed

Alteration Agreement (1600 permit) from the California Department of Fish and Game, grape-growers develop a management plan that includes characterizing the plant community in the riparian area, targeting individual plants for removal, and selecting replacement plant species that will provide a similar habitat for wildlife, as a source of shelter, food, and nesting sites. This method has some positive aspects: with lower BGSS populations, fewer insecticide applications are used. Some plants targeted for removal, such as Himalayan blackberry and periwinkle, are invasive weeds. However, removal of riparian vegetation is very disruptive to wildlife, it increases streambank erosion, and some riparian hosts are extremely difficult to eradicate.

Overwintering hosts of *Xf* are thought to play an important role in the epidemiology of PD in providing a source of bacteria for spring infections, especially near vineyards where infective adult BGSS do not survive the winter (Purcell and Saunders 1999). BGSS transmission of *Xf* from riparian plants to grapevines in spring is more likely than mid- or late-season infections to result in chronic disease (Purcell 1981). Given low spring *Xf* concentrations in the riparian hosts we tested, it seems likely that BGSSs acquire *Xf* in autumn instead of in spring.

We found very few *Xf*-positive blue elderberry, California blackberry, and California grape at both sites. Given that these plants were infected upon transfer to the field, it seems that hot temperatures in between transferring them to the field in July and the first sampling date in October were sufficient to prevent *Xf* infections from becoming permanent, especially since the numbers of *Xf*-positive plants among these three species stayed low throughout the rest of the sampling dates at both sites. It is possible that blue elderberry, California blackberry, and California grape do not maintain sufficient *Xf* concentrations for BGSS transmission in the field, even in autumn and summer. Given these findings, it may be more important to focus on Himalayan blackberry and periwinkle for control of PD. The fewer riparian plants removed before revegetation, the less disruption to wildlife habitat.

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