BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCE'S DISEASE: SIGNIFICANCE OF RIPARIAN PLANTS IN THE EPIDEMIOLOGY OF PIERCE'S DISEASE

Project Leader:
Kendra Baumgartner
USDA-ARS
Davis, CA 95616

Cooperators:
Alexander H. Purcell
Division of Insect Biology
University of California
Berkeley, CA 94720

M. Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

Reporting Period: The results reported here are from work conducted from April 1, 2002 to March 31, 2003.

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. Our first objective is to examine the epidemiological role of seasonal *Xylella fastidiosa* (*Xf*) concentration fluctuations in riparian hosts in the field, where plants are subject to seasonal temperature changes. 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. After confirming infection with PCR, we transferred infected plants to two sites in the North Coast (Napa County and Mendocino County). Populations of *Xf* reached detectable levels in all five riparian hosts located at our Napa County site in October 2003. Every replicate plant of periwinkle and California grapevine showed typical leaf scorch symptoms of PD, which is not surprising given the high concentrations of *Xf* detected among them. The fact that none of the Himalayan blackberry showed symptoms, despite high *Xf* concentrations, suggests that Himalayan blackberry is more tolerant of *Xf* infection. Assuming our results reflect that of naturally-established riparian hosts in the field, *Xf* concentrations in California grapevine, Himalayan blackberry, and periwinkle are still sufficient for acquisition by BGSS in autumn. Dilution plating results have not yet been obtained from our Mendocino County site and real-time PCR reactions have not been completed for plants from either site. We will continue to estimate *Xf* concentrations in plants at both sites using dilution plating and real-time PCR on a seasonal basis. *Xf* concentration estimates from each plant species at each site will be averaged among replicate plants. The effects of plant species, season, and location on mean *Xf* concentration will be determined using an analysis of variance. Results obtained using the two quantitation techniques will be analyzed separately.

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 that occupy the banks of rivers and streams experience the heaviest losses, but rows have fewer diseased vines with increasing distance away from riparian plants. Riparian habitat adjacent to vineyards contains plant species that serve as 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 many riparian plant species provide habitat for BGSS, but some also serve as reservoir hosts of the PD strain of *Xylella fastidiosa* (*Xf*) (Freitag 1951). A variety of common riparian plants, including native and non-native trees, shrubs, and herbaceous annuals, 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, *Rubus discolor* (Himalayan blackberry) and *Vitis vinifera* (grapevine), supported systemic *Xf* populations. These results imply that some riparian hosts are likely more important than others as reservoirs for the spread of *Xf* to grapevines.

Interactions among BGSS, *Xf*, and their host plants are likely to vary from season to season. Aside from the obvious effects of season on BGSS breeding, 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 *Vitis labrusca* (Hopkins and Thompson 1984) and they are lower in *V. vinifera* grown at cooler temperatures (Feil and Purcell 2001). The efficiency of *Xf* acquisition and transmission by BGSS is influenced by the concentration of *Xf* in the plant host; the higher the concentration of *Xf*, the higher the probability of BGSS acquiring *Xf* while feeding (Hill & Purcell, 1997). Therefore, we might expect that in

riparian hosts, 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 affect the probability of a BGSS acquiring *Xf* while feeding on an infected plant. 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 by BGSS 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. By measuring seasonal concentrations of *Xf* in riparian plants, we will determine if and when concentrations are high enough for acquisition by BGSS. We will focus on five systemic hosts of *Xf*: *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 for these five riparian hosts.

We will test the hypothesis that *Xf* concentrations are the same among five riparian hosts at two sites in the North Coast (Napa Co. & Mendocino Co.). In October 2002, we propagated California grape, California blackberry, Himalayan blackberry, blue elderberry, and periwinkle (100 plants/species) in our greenhouse at UC Davis. In February 2003, we mechanically inoculated all plants with the STL strain of *Xf* (a strain isolated from PD-symptomatic vines near Yountville, CA) in the greenhouse, where there are more uniform environmental conditions for infection development than in the field. In June 2003, we used PCR to confirm infection using PCR primers of Minsavage et al (1994) and Pooler et al (1995). In July 2003, we transferred infected plants from the greenhouse to two sites in the North Coast (50 replicate potted plants x five plant species = 250 plants/site). Plants are in 3-gallon pots and are surrounded by a fine-mesh screen enclosure. On a seasonal basis (except during the dormant season), *Xf* concentrations are estimated from petioles located distal to the stem inoculation site using dilution plating and real-time PCR. *Xf* concentration estimates from each plant species at each site are averaged among replicate plants. The effects of plant species, season, and location on mean *Xf* concentration is determined using an analysis of variance. Results obtained using the two quantitation techniques will be analyzed separately.

RESULTS

We inoculated 100 plants per riparian host in the greenhouse. Using PCR, we confirmed the following numbers of infected plants/species: Himalayan blackberry, 40; California blackberry, 12; blue elderberry, 78; periwinkle, 68; California grapevine, 37. Among infected plants, half were transferred to our Napa County site and the remaining half were transferred to our Mendocino County site.

To date, we collected our first set of petioles for *Xf* quantitation from plants at both sites. Table 1 contains culture attempt results from the Napa County site. (Plates are still incubating from Mendocino County site and real-time PCR reactions from both sites have not yet been completed). Results discussed will focus on autumn *Xf* concentrations in riparian hosts located at our Napa County site as obtained from dilution plating.

Populations of *Xf* reached detectable levels in all five riparian hosts located at our Napa County site in October 2003 (Table 1). Every replicate plant of periwinkle and California grapevine showed typical leaf scorch symptoms of PD, which is not surprising given the high concentrations of *Xf* detected among them. The fact that none of the Himalayan blackberry showed symptoms, despite high *Xf* concentrations, suggests that Himalayan blackberry is more tolerant of *Xf* infection. *Xf* concentrations of at least 10^4-10^5 CFU/g of plant tissue are required for acquisition by BGSS (Hill and Purcell 1997). Assuming our results reflect that of naturally-established riparian hosts in the field, estimated *Xf* concentrations in California grapevine, Himalayan blackberry, and periwinkle are sufficient for acquisition by BGSS in early autumn. 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 grapevine, 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. Late season infections of grapevines are unlikely to result in chronic disease and infected canes are pruned out during the dormant season (Purcell 1981). However, young adult BGSSs that acquire *Xf* in mid summer to early autumn and survive the winter are still capable of transmitting *Xf* the following spring after budbreak.

Table 1. Culture of *Xylella fastidiosa* from riparian hosts in the field following mechanical inoculation.

Species	Number Infected	Number Contaminated	Mean CFU/g [a]	Range CFU/g	Incubation(days) [b]
Himalayan blackberry	9	0	6×10^5	6.7×10^1 to 2.9×10^6	210
California blackberry	3	0	2×10^2	2×10^2	210
Blue elderberry	1	0	2×10^2	2×10^2	210
Periwinkle	30	0	1.3×10^7	6.6×10^3 to 8.0×10^7	210
California grapevine	5	0	6.6×10^6	6.4×10^3 to 9.7×10^6	210

[a] Colony forming units per gram of petiole tissue.
[b] Number of days between inoculation in spring 2003 and culture attempt in autumn 2003.

CONCLUSIONS

Riparian Revegetation Management is a method of PD control that focuses on removal of host plants of BGSS and *Xf* (host plants other than grapevines), followed by revegetation with native, non-host plants. This method of PD control has been shown to reduce local populations of BGSS (unpublished research, 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 interested in pursuing this method of PD control 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 of PD control has some positive aspects. With lower BGSS populations, fewer insecticide applications are used in the vineyard. Some of the plants targeted for removal, such as Himalayan blackberry and periwinkle, are invasive weeds. However, removal of large sections of riparian vegetation is very disruptive to wildlife, it increases the probability of streambank erosion, and some of the riparian hosts are extremely difficult to eradicate.

We only have general knowledge of the role of riparian hosts in the epidemiology of PD. Overwintering hosts of *Xf* likely 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). Based on the results of this past research, we anticipate that removal of overwintering hosts from riparian habitat adjacent to vineyards will decrease disease incidence. However, not all of these hosts are known to attain adequate *Xf* concentrations for acquisition early in the growing season.

The fewer riparian plants removed before revegetation, the less disruption to wildlife habitat. The success of revegetation management depends on a thorough understanding of how riparian hosts contribute to the spread of PD. We know which plants are *Xf* hosts. We don't know if or when these hosts attain high enough concentrations of *Xf* for acquisition (10^4 colony forming units/g of plant tissue) by BGSS in the field. Some hosts may not attain acquisition concentrations of *Xf* until late summer. Late summer transmission of *Xf* to grapevines is thought to result in local infections of short sections of canes that go dormant (and are then pruned off) before *Xf* has a chance to establish a systemic infection. Systemic infection leads to permanent disease, while local infections, if pruned out, do not. If the results of this research reveal that only a few of the riparian hosts recommended for removal serve as major sources of *Xf* in spring, grape-growers can concentrate on removing fewer riparian plants, thereby reducing the total amount of riparian habitat disruption.

REFERENCES

Feil, H., W.S. Feil, and A.H. Purcell. 2000. Effects of temperature on the flight activity of *Graphocephala atropunctata* (Hemiptera: Cicadellidae). Journal of Economic Entomology 93: 88-92.

Feil, H., and A.H. Purcell. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa in vitro* and in potted grapevines. Pl Dis 85: 1230-1234.

Freitag, J.H. 1951. Host range of Pierce's disease virus of grapes as determined by insect transmission. Phytopathology 41: 920-934.

Hewitt, W.B., N.W. Frazier, and J.H. Freitag. 1949. Pierce's disease investigations. Hilgardia 19: 207-264.

Hill, B.L., and A.H. Purcell. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 85: 1368-1372.

Hill, B.L., and A.H. Purcell. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology 87: 1197-1201.

Hopkins, D.L. 1985. Effects of plant growth regulators on development of Pierce's disease symptoms in grapevine. Pl Dis 69: 944-946.

Hopkins, D.L., and C.M. Thompson. 1984. Seasonal concentration of the Pierce's disease bacterium in 'Carlos' and 'Welder' muscadine grapes compared with 'Schuyler' bunch grape. HortScience 19: 419-420.

Minsavage, G.V., C.M. Thompson, D.L. Hopkins, R.M.V.B.C. Leite, and R.E. Stall. 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84: 456-461.

Pooler, M., J.S. Hartung, and R. Fenton. 1995. Genetic relationships among strains of *Xylella fastidiosa* from RAPD-PCR data. Curr Microbiol 31: 134-137.
Purcell, A.H. 1975. Role of the blue-green sharpshooter, *Hordnia circellata*, in the epidemiology of Pierce's disease of grapevines. Environ Entomol 4: 745-752.
Purcell, A.H. 1976. Seasonal changes in host plant preference of the blue-green sharpshooter *Hordnia circellata* (Homoptera: Cicadellidea). Pan-Pacific Entomology 52: 33-37.
Purcell, A.H. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. Phytopathology 71: 429-435.
Purcell, A.H., and S.R. Saunders. 1999. Fate of Pierce's Disease strains in common riparian plants in California. Pl Dis 83: 825-830.

FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service.