SIGNIFICANCE OF RIPARIAN PLANTS AS RESERVOIRS OF *XYLELLA FASTIDIOSA* FOR INFECTION OF GRAPEVINES BY THE BLUE-GREEN SHARPSHOOTER

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ABSTRACT

On California's North Coast, plant species in natural habitats adjacent to vineyards, namely riparian areas, are non-crop hosts of *Xylella fastidiosa* (*Xf*). The importance of a riparian host as a pathogen reservoir is related to its ability to support pathogen populations and its attractiveness to the vector, *Graphocephala atropunctata* (blue-green sharpshooter [BGSS]). We quantified BGSSs on five species (California blackberry, California grapevine, elderberry, Himalayan blackberry, periwinkle) of naturally-established plants adjacent to vineyards. We assessed the ability of the same species to support *Xf*, using controlled inoculations of potted plants kept in screenhouses in the field. No species were characterized by both an abundance of BGSSs and a high frequency of *Xf* detection. A 71% frequency of *Xf* detection in periwinkle suggests that, regardless of having the fewest BGSS (0.4 nymphs and 0.9 adults per sample), infrequent visitations may result in a high acquisition rate. California grapevine supported eight times as many nymphs and three times as many adults as periwinkle, suggesting that frequent visitations may offset its significantly lower infection rate (19%). California blackberry, elderberry, and Himalayan blackberry are likely less important pathogen reservoir because *Xf* was infrequently detected in their tissues and they hosted few BGSSs.

INTRODUCTION

In the north-coastal grape-growing region of California, *Xylella fastidiosa (Xf),* the bacterium that causes Pierce's disease (PD) (Freitag 1951), is spread to grapevines by a native vector, *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae) (blue-green sharpshooter [BGSS]; Hewitt et al. 1949; Purcell 1975). Purcell (1974, 1975) demonstrated a direct relationship between incidence of PD and proximity to riparian vegetation bordering vineyards. The distribution of diseased grapevines is associated with a high concentration of BGSS in spring in vinerows adjacent to riparian vegetation, which serves as feeding and reproductive habitat for the BGSS (Hewitt et al. 1949; Purcell 1975). Not only do some riparian plants provide habitat for BGSSs, but they also host *Xf* (Wells et al. 1987).

The spread of *Xf* from riparian hosts to grapevines is, in part, a function of the proportion of BGSSs that acquire the pathogen when feeding on infected riparian hosts. Acquisition of *Xf* is directly related to the concentration of the pathogen within the host. The minimum threshold for acquisition is 10^4 CFU of *Xf* per gram of plant tissue, increases above which result in proportionally higher transmission rates (Hill and Purcell 1997). Baumgartner and Warren (2005) found that *Rubus discolor* Weihe & Nees (Himalayan blackberry), *Vinca major* L. (periwinkle), and *Vitis californica* Benth. (California grapevine) supported populations $\geq 10^4$ CFU/g tissue, whereas *R. ursinus* Cham. & Schldl. (California blackberry) and *Sambucus mexicana* C. Presl (elderberry) did not. California grapevine, Himalayan blackberry, and periwinkle may be more important as pathogen reservoirs not only due to the high pathogen populations they support during part of the year (Baumgartner and Warren 2005), but also because they are systemic hosts of *Xf* (Purcell and Saunders 1999).

The importance of a riparian host as a pathogen reservoir is determined by the pathogen populations it supports and by the frequency of visitation by the vector. A common riparian host of *Xf* that is fed upon frequently by the BGSS likely will contribute more to the spread of PD because there will be more opportunities for acquisition of the pathogen from infected tissue. In this regard, it is noteworthy that some of the same riparian hosts that were previously recognized in field surveys as feeding hosts of the BGSS (Purcell 1976; Raju et al. 1983), namely California grapevine, Himalayan blackberry, and periwinkle, have since been identified as hosts in which *Xf* reaches high populations (Baumgartner and Warren 2005; Purcell and Saunders 1999).

OBJECTIVES

The goal of our research was to identify riparian hosts of greatest importance in the transmission of *Xf* to grapevines in the north-coastal grape-growing region of California. Our first objective was to determine if the BGSS is more abundant on some riparian hosts than others. We measured abundance of adults and nymphs in riparian areas adjacent to vineyards on five feeding and reproductive hosts: California blackberry, California grapevine, elderberry, Himalayan blackberry, and periwinkle. All five hosts are potentially important in the spread of PD because they are also systemic hosts of *Xf* (Purcell and Saunders 1999). Our second objective was to examine a possible relationship between the ability of riparian hosts to support both the BGSS and *Xf*. To address this second objective, we inoculated plants of the same riparian host species with *Xf*, transferred them to the field after confirming infection, and tested them afterwards for the presence of the pathogen. This approach was preferable to testing for *Xf* in the same naturally-established plants that we examined for BGSSs because (i) our

inoculation technique ensured that all plants were challenged by the pathogen; (ii) by sampling tissues distal to the inoculation site, *Xf*-positive identifications were known to represent systemic infections; and (iii) plants were inoculated once and, therefore, the presence of *Xf* was known to result from a single infection. In other words, our approach did not rely on natural infection by the BGSS, which likely reflects not only the hosts' abilities to maintain *Xf* infections, but also BGSS feeding behavior.

RESULTS

Abundance of nymphs varied significantly among species ($P<0.0001$). Nymphs were significantly more abundant on California grapevine which had a mean of 3.1 nymphs per sample, compared to all other hosts, but especially compared to periwinkle and elderberry, which had means of 0.4 nymphs per sample and 0.7 nymphs per sample, respectively (Figure 1). Abundance of adults was not significantly different among species ($P=0.0676$). California grapevine, the species with the most nymphs, also had the most adults, 2.4 per sample (Figure 1). In contrast, periwinkle, the species with the fewest nymphs, also had the fewest adults, 0.9 per sample (Figure 1).

Frequency of detection of *Xf* varied significantly among species ($P<0.0001$). Periwinkle had the highest frequency of detection with 70.8% of all tested plants, averaged across three sampling periods, found to be *Xf*-positive (Figure 2). Frequency of detection of *Xf* did not vary significantly between the two detection methods, colony counts in culture and real-time PCR ($P=0.09$). Results from both detection methods showed the same relative differences among species; the interaction of species x detection method was not significant ($P=0.3582$). For example, periwinkle had the highest percentage of plants that were found to be *Xf*-positive by culture (113 out of 160 total samples tested, summed across sampling periods) and by real-time PCR (140 out of 160 total samples tested, summed across sampling periods). In contrast, none of the 202 culture attempts from elderberry samples yielded *Xf* colonies, and real-time PCR analyses of the same tissues resulted in only six *Xf*-positive samples.

Despite the lack of statistical significance for differences in abundance of adults among riparian hosts from ANOVA ($P=0.07$), there was a significant positive correlation between abundance of adults and nymphs ($r=0.96$, $P=0.01$). Samples with many nymphs also had many adults (Figure 3). There were no correlations between detection frequency of *Xf* and abundance of adults ($r=-0.44$, $P=0.45$) or nymphs ($r=-0.34$, $P=0.58$).
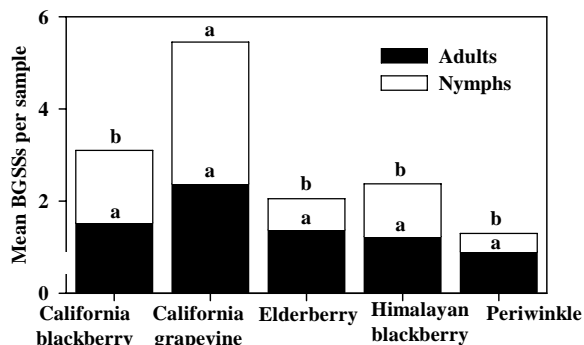

Figure 1. Abundance of BGSSs on naturally-established riparian hosts adjacent to vineyards in northern California. A sample consisted of 25 sweeps per plant; *n*=13 to 95 samples per species per year. Each column is the sum of the mean number of adults and nymphs per sample per species, averaged over years. Columns within each life stage with different letters are significantly different at $P \leq 0.05$ (Tukey's test).
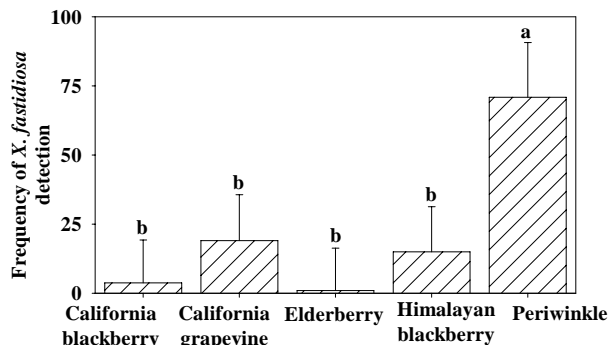

Figure 2. Frequency of detection of *Xf* from riparian hosts. Plants were inoculated in the greenhouse. Infected plants were placed in the field and subsequently tested at 3, 11, & 13 mos., by culture and real-time PCR; *n*=45-76 plants per species per sampling period. Each column is the mean percentage of plants that were *Xf*-positive, averaged over sampling periods and detection methods. Columns with different letters are significantly different at $P \leq 0.05$ (Tukey's test).

Figure 3. Correlation of abundance of adult BGSSs with mean abundance of nymph BGSSs (r=0.96, P=0.0093) on naturally-established riparian hosts adjacent to vineyards in northern California. A sample consisted of 25 sweeps per plant; n=13 to 95 samples per species per year. Each symbol represents abundance of BGSSs per sample per species per year.
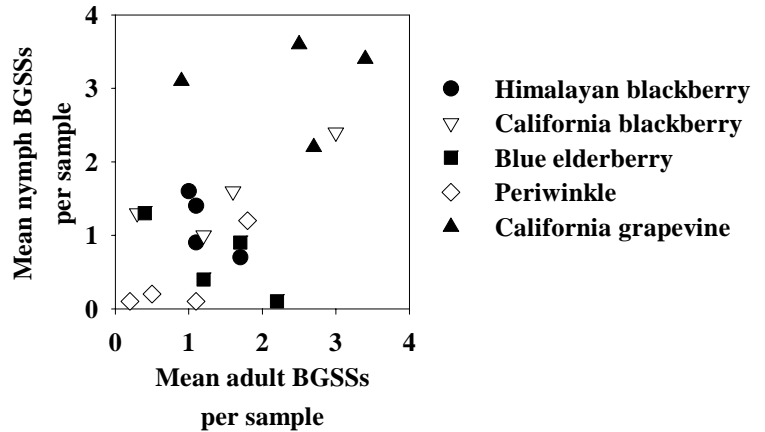
CONCLUSIONS

We measured abundance of the BGSS on five species (California blackberry, California grapevine, elderberry, Himalayan blackberry, and periwinkle) of naturally-established plants in riparian areas adjacent to vineyards on the North Coast of California. We assessed the ability of the same species to support *Xf*, based on results from controlled inoculations of potted plants kept in screenhouses in the field. None of the species were characterized by both an abundance of BGSSs and a high frequency of *Xf* detection. California grapevine and periwinkle may be more important pathogen reservoirs than California blackberry, elderberry, and Himalayan blackberry. Despite a significantly lower frequency of *Xf* detection in California grapevine, 19%, this species supported eight times as many nymphs and three times as many adults as periwinkle, suggesting that more frequent visitations by the BGSS may result in a high probability of acquisition of *Xf* from California grapevine. While periwinkle supported the fewest BGSSs, 71% of tested plants were *Xf*-positive, suggesting that a high percentage of transmission events result in systemic infection and that infrequent visitation by the vector may, nonetheless, result in a high acquisition rate. California blackberry, elderberry, and Himalayan blackberry are likely less important pathogen reservoirs because *Xf* was infrequently detected in their tissues and BGSSs were rare on these species.

Our finding that abundance of nymphs, but not that of adults, differed significantly among the riparian hosts we examined are consistent with those of Purcell (1976) who found that nymphs utilize fewer species than do adults. We might expect that nymph BGSSs have more restricted host ranges than adults based on different feeding requirements, as has been demonstrated for *Homalodisca coagulata* (Say) (glassy-winged sharpshooter) (Brodbeck et al. 1995), an introduced vector of PD in southern California (Blua et al. 1999). The significance of nymph BGSSs in the spread of PD is not known. Although nymphs lose infectivity after molting (Purcell and Finlay 1979), this may not preclude their importance in the epidemiology of PD relative to that of adults, which are infective for life once they acquire *Xf* (Purcell and Finlay 1979). The low mobility of nymphs, due to their flightlessness and small size, likely results in more transmission of *Xf* within an infected host than between hosts. Consequently, nymphs may spread *Xf* to new tissues within an infected host faster than the pathogen can move systemically. Systemic hosts of *Xf* on which nymphs are abundant, such as California grapevine, may support infections in more tissues than hosts on which nymphs are rare and, therefore, may serve as important sources of *Xf* for acquisition by adults.

It is possible that *Xf* infection of the species we examined through controlled inoculations are different in naturally-established plants of the same species. Natural levels of infection are related to a host's ability to support *Xf* and its attractiveness to the BGSS. California grapevine, for example, may have higher levels of infection in the field than we measured in our inoculated plants, based on the high number of BGSSs we found on this species. There are few published surveys of *Xf* in naturally-established plants (Raju et al. 1983; Raju et al. 1980). In one such study of 28 native and non-native species in riparian areas in Napa County, *Xf* was detected in only four species: Himalayan blackberry, periwinkle, *Fragaria vesca* L. (wood strawberry), and *Claytonia perfoliata* Willd. (miner's lettuce) (Raju et al. 1983). Although they surveyed California grapevine and elderberry, hosts that we also examined, their study was designed with the objective of identifying reservoir hosts, as opposed to comparing natural levels of infection among species.

Throughout the growing season, BGSSs occur in both riparian areas and vineyards (Freitag and Frazier 1954; Purcell 1975, 1976). Their whereabouts and behavior outside the growing season, when the population consists of adults (Purcell 1975; Severin 1949), are not well understood, mainly because cold temperatures limit BGSS flight activity (Feil et al. 2000) and, thus, hamper monitoring efforts. We measured BGSS abundance from spring to early summer, as this time of the year is characterized by BGSS flight activity, the presence of both adults and nymphs, and active growth of the five riparian host species we examined. Although we detected no significant differences in abundance of BGSSs on the five riparian hosts we

examined, a previous survey of 16 species in riparian areas in Napa showed that BGSSs were more common on California blackberry, California grapevine, elderberry, and Himalayan blackberry, than on periwinkle from April to July (Purcell 1976). From September to March, BGSSs were more common on periwinkle (Purcell 1976). Differences in our results may be due to differences in locations, study years, or sampling methods.

Successful long-term management of PD may require removal of certain reservoir hosts, given that insecticides do not significantly reduce the spread of the disease (Purcell 1979) and that resistant winegrape varieties are not available. Wistrom and Purcell (2005) ranked the most important reservoir hosts, in terms of vector acquisition, as those that are feeding hosts of the BGSS, are frequently infected after transmission events, are systemic hosts of *Xf*, and support high pathogen populations. Revegetation of a riparian area adjacent to a diseased vineyard offers the potential to reduce the pathogen reservoir outside the vineyard, but it may be of limited efficacy in controlling the disease when infected grapevines remain in the vineyard. Grapevines satisfy all of Wistrom and Purcell's (2005) criteria of important reservoir hosts and, thus, serve as a source of the pathogen for acquisition by BGSSs, even if riparian hosts are removed from an adjacent riparian area. Furthermore, removal of reservoir hosts may not diminish the ability of a riparian area to support BGSSs. Riparian areas are considered to be a habitat of the BGSS; they harbor many feeding and reproductive hosts (Freitag and Frazier 1954; Purcell 1975, 1976), in addition to plants that provide shelter for the overwintering adults.

REFERENCES
Baumgartner, K. and J. G. Warren. 2005. Persistence of *Xylella fastidiosa* in riparian hosts near northern California vineyards. Plant Dis. 89: 1097-1102.
Blua, M. J., P. A. Phillips and R. A. Redak. 1999. A new sharpshooter threatens both crops and ornamentals. California Agric. 53: 22-25.
Brodbeck, B. V., P. C. Andersen and R. F. Mizell. 1995. Differential utilization of nutrients during development by the xylophagous leafhopper, *Homalodisca coagulata*. Entomol. Exp. Appl. 75: 279-289.
Feil, H., W. S. Feil and A. H. Purcell. 2000. Effects of temperature on the flight activity of *Graphocephala atropunctata* (Hemiptera: Cicadellidae). J. Econ. Entomol. 93: 88-92.
Freitag, J. H. 1951. Host range of Pierce's disease virus of grapes as determined by insect transmission. Phytopathology 41: 920-934.
Freitag, J. H. and N. W. Frazier. 1954. Natural infectivity of leafhopper vectors of Pierce's disease virus of grape in California. Phytopathology 44: 7-11.
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. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology 87: 1197-1201.
Purcell, A. H. 1974. Spatial patterns of Pierce's disease in the Napa Valley. Amer. J. Enol. Vitic. 25: 162-166.
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-Pacif. Entomol. 52: 33-37.
Purcell, A. H. 1979. Control of the blue-green sharpshooter and effects on the spread of Pierce's Disease of grapevines. J. Econ. Entomol. 72: 887-892.
Purcell, A. H. and A. Finlay. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathology 69: 393-395.
Purcell, A. H. and S. R. Saunders. 1999. Fate of Pierce's Disease strains in common riparian plants in California. Plant Dis. 83: 825-830.
Raju, B. C., A. C. Goheen and N. W. Frazier. 1983. Occurrence of Pierce's disease bacteria in plants and vectors in California. Phytopathology 73: 1309-1313.
Raju, B. C., S. F. Nomé, D. M. Docampo, A. C. Goheen, G. Nyland and S. K. Lowe. 1980. Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. Amer. J. Enol. Vitic. 31: 144-148.
Severin, H. H. P. 1949. Life history of the blue-green sharpshooter, *Neokolla circellata*. Hilgardia 19: 187-189.
Wells, J. M., B. C. Raju, H. Y. Hung, W. G. Weisberg, L. Mandelco-Paul and D. J. Brenner. 1987. *Xylella fastidiosa* gen. nov, sp. nov.: gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. Int. J. Sys. Bacteriol. 37: 136-143.
Wistrom, C. and A. H. Purcell. 2005. The fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts. Plant Dis. 89: 994-999.