Vector Transmission of *Xylella fastidiosa*: Applying Fundamental Knowledge to Generate Disease Management Strategies

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ABSTRACT *Xylella fastidiosa* is a xylem-limited bacterium transmitted to plants by xylem sap-feeding insects. This pathogen has a wide host range, causing disease in crops such as grape, citrus, almond, and coffee; ornamental plants; and trees. Sharpshooter leafhoppers are the major vectors of *X. fastidiosa* to crops of economic importance. Transmission characteristics include the lack of a latent period, no transstadial or transovarial transmission, persistence in adults, and multiplication in the foregut of vectors. Various factors influence vector transmission of *X. fastidiosa*, including the distribution and density of bacterial populations in host plants, insect host range and plant preference, season of inoculation, and climatic conditions. The ecology of vectors can affect epidemics, as demonstrated by the large increase in Pierce's disease of grapevine incidence in California after the introduction of *Homalodisca coagulata* (Say). Disease control strategies should incorporate basic knowledge of transmission biology, vector ecology, and other interactions involved in *X. fastidiosa* diseases. We discuss basic aspects of *X. fastidiosa* transmission by vectors, the ecology of insects in relation to transmission and disease spread, and how basic research can be applied to the development of management strategies for a *X. fastidiosa* disease.

KEY WORDS Hemiptera, Cicadellidae, bacteria, plant disease, biofilm

INSECTS THAT INGEST PRIMARILY xylem sap belong to four families within the Hemiptera: Cicadellidae (subfamily Cicadellinae, sharpshooter leafhoppers), Cercopidae (spittlebugs), Machaerotidae (tube-building spittlebugs), and Cicadidae (cicadas). In general, these insects cause little damage to plants. Some cicadas can damage foliage or debilitate plants by sucking significant amounts of xylem sap when large broods emerge, whereas spittlebugs can affect pasture grasses or sugarcane. Sharpshooters rarely damage plants directly (Andersen et al. 2003) but are economically important as vectors of the plant pathogenic bacterium *Xylella fastidiosa.* Grape, almond, citrus, coffee, peach, and many forest tree species are among a large list of economically important plants in which *X. fastidiosa* induces disease (Purcell 1997). Many other agronomic, ornamental, and wild plants are also hosts of the pathogen, most without expressing symptoms (Hopkins and Purcell 2002).

Worldwide, research involving *X. fastidiosa* and its vectors has increased dramatically in recent years (Fig. 1). Epidemics of citrus variegated chlorosis (CVC) in Brazil and new outbreaks of Pierce's disease (PD) of grapevines in California driven by *Homalodisca coagulata* (Say) (Blua et al. 1999, Hopkins and Purcell 2002), and the availability of genome sequences of four strains of *X. fastidiosa* (Simpson et al. 2000; Bhattacharyya et al. 2002a,b; Van Sluys et al. 2003), are among the major reasons for such emerging interest. This recent research has rapidly advanced knowledge of *X. fastidiosa* biology and epidemiology. Recent reviews evaluate various aspects of *X. fastidiosa* diseases, including genomics (Van Sluys et al. 2002), vector ecology and disease epidemiology (Redak et al. 2004), and case studies (Purcell and Feil 2001, Hopkins and Purcell 2002). We focus this review on *X. fastidiosa* transmission by vectors, the ecology of the interactions, and how this knowledge can be incorporated into disease management strategies.

***X. fastidiosa* Transmission Characteristics.** Initial studies on PD in vineyards suggested that the disease's etiological agent was an insect-borne virus (Hewitt et al. 1946). After Newton Pierce's observations in the late 1800s and the devastation caused by the disease to southern California vineyards (Pierce 1892), interest in PD diminished until an outbreak of the disease in the Central Valley of California during the 1930s and early 1940s (Hewitt et al. 1949). Researchers during the 1940s identified sharpshooter leafhoppers and spittlebugs as vectors (Houston et al. 1947; Severin 1949, 1950).

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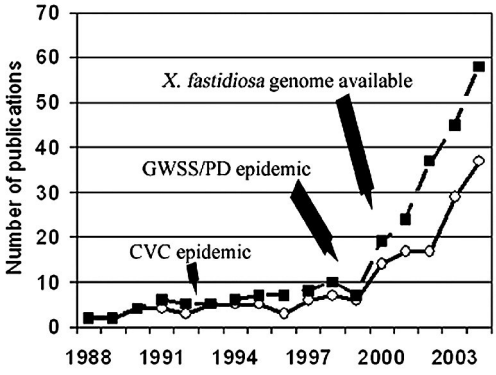

Fig. 1. Number of publications retrieved after a '*Xylella*' term was used as a keyword to search the ISI Web of Knowledge (Web of Science) database in December 2004. Solid line (empty circles) represents number of hits with 'Title' only option selected; dashed line (solid squares) are results for general 'Topic' search.

The identification of a group of vectors considered xylem feeders suggested that the etiological agent was limited to xylem, as was later demonstrated by experimentally blocking vector transmission to xylem tissues with a metal foil barrier (Houston et al. 1947). Davis et al. (1978) identified *X. fastidiosa* as the etiological agent of PD. Vector ability seems to require only that the insect is a xylem sap-feeding specialist. Although phloem and mesophyll feeders have been tested for *X. fastidiosa* transmission to plants (Purcell 1980), including leafhopper species known to penetrate and ingest from xylem, e.g., *Euscelidius variegatus* (Kirschbaum) (Naito 1977), none of these insect groups transmitted the pathogen. In contrast, most Cicadellinae species tested in sufficient numbers were proven to be vectors. Observations that *X. fastidiosa* has little, if any, species specificity within xylem-feeding specialists (Purcell 1989) suggest that distinctive probing behaviors or foregut morphological characteristics shared by xylem feeders enable bacterial transmission.

X. fastidiosa is unique in its transmission characteristics among vector-borne plant pathogens in that it does not require a latent period yet is persistently transmitted. Severin (1949) showed that adult sharpshooters remained infective for long periods. However, the lack of transstadial transmission or a latent period (Purcell and Finlay 1979) strongly suggested that the pathogen is inoculated into plants from the foregut of vectors, the cuticle of which is shed at each molt. Although the bacterium multiplies in the foregut (Purcell et al. 1979, Hill and Purcell 1995), which explains the persistence of transmission in adults, very few live bacterial cells in the vector's foregut are required for transmission (Hill and Purcell 1995), an observation that agrees with the lack of a latent period. Freitag (1951) demonstrated the lack of *X. fastidiosa* transovarial transmission.

The length of both acquisition and inoculation access periods affects transmission efficiency, or vector competency (Purcell and Finlay 1979). Longer access periods result in higher transmission efficiency. *X. fastidiosa* is irregularly distributed in infected tissues (Hopkins 1989), thus longer plant access time may increase chances of the vector probing infected vessels. Because *X. fastidiosa* must attach to the foregut of vectors in a fast moving environment with fluid speed of 5–10 cm/s (Purcell et al. 1979), longer access periods also can increase the number of cells ingested by the insect, and, proportionally, the opportunities for attachment to occur. By contrast, two other factors may be associated with the increase in inoculation efficiencies over time. First, attached cells in the foregut would have more opportunities to detach from the cuticle and be inoculated into a susceptible vessel with longer access times relative to shorter ones. Second, it may be that cells are frequently inoculated into plants, but most die before proliferating and generating disease. The number of cells inoculated and the probability of establishment would increase with access time. Both factors could operate independently. The basis of the transmission mechanism may have some similarities with that of semipersistently transmitted (foregut-borne) viruses (Nault 1997).

Vector Foregut and Its Relationship to Transmission. Sharpshooters have typical hemipteran mouthparts adapted for feeding on liquids. The maxillary stylets interlock to form parallel food and salivary canals. The mandibular stylets surround and support the maxillary stylets and cut through plant tissues during probing. The mouthpart morphology of *H. coagulata* has been described in detail previously (Leopold et al. 2003). Sap enters the stylets and is transferred to the precibarium, a narrow tube connecting the food canal to the cibarium (pumping chamber). The precibarial valve, located about midway in the precibarium, opens to admit ingested sap and closes to prevent backflow while food is pumped to the gut. Backus and McLean (1982, 1983) described the morphology of the precibarial valve and a likely ingestion mechanism. The precibarium canal continues after the valve and ends in the cibarium. The cibarium connects the precibarium to the esophagus or true mouth. From the esophagus, food is pushed through the cardiac/esophageal valve into the midgut.

Sap ingestion is regulated by the precibarial and cardiac (esophageal) valves and the cibarium dilator musculature, which originates at the insect clypeus and inserts along a medial line distal to the elastic cibarial diaphragm (Purcell et al. 1979). Contraction of the dilator muscle raises the diaphragm to create negative pressure in the cibarium chamber, sucking in fluid from the food source (e.g., xylem vessel) via the stylets to the cibarium. Negative pressure collapses shut the cardiac valve, serving as a one-way check valve that opens with positive pressure and closes with negative pressure from the cibarium. Relaxation of the dilator muscle collapses the top of the cibarium chamber, pushing fluid into two directions, along a groove on the floor of the cibarium, toward the esophagus and cardiac valve and the precibarial valve. Positive pressure opens the cardiac valve to admit fluid

into the midgut. Small amounts of fluid present distally to the precibarial valve and in the food canal could be pushed back into plants during the closure of the precibarial valve.

Studies documenting the relationship between *X. fastidiosa* distribution in the foregut of vectors and its transmission to plants have only been conducted a few times (e.g., Purcell et al. 1979). Newman et al. (2004) showed a correlation between bacterial presence in the precibarium of vectors and its transmission to plants. Likewise, Almeida and Purcell (unpublished data) found a strong correlation between transmission to plants and bacterial presence in the precibarium. Furthermore, *X. fastidiosa* also was frequently observed in the precibarium by using scanning (Brlansky et al. 1983) and confocal microscopy with a strain expressing a green-fluorescent protein (Newman et al. 2003).

Based on microscopy studies, *X. fastidiosa* forms a biofilm on the foregut of vectors, where cells are polarly attached to the cuticle in a monolayer (Purcell et al. 1979, Brlansky et al. 1983, Newman et al. 2004). Polar attachment may increase the amount of nutrients cycling through the biofilm. Interestingly, the bacterial growth seems to be most dense where fluid velocities along the cuticle are likely most rapid, i.e., in the narrowest portions of the foregut, such as the precibarium, or a groove in the floor of the cibarium chamber leading from the precibarium to the esophagus, or the irregular grooves in the cibarial chamber internal to the insertions of the cibarial dilator muscle. Although biofilm formation may be a characteristic of *X. fastidiosa* colonization of vectors, it is not required for successful transmission because inoculation can occur before there is sufficient time for a biofilm to form, as evidenced by the lack of latent period. Details on detachment of cells from the foregut for inoculation of healthy plants have not been addressed; although it can be hypothesized that leafhopper behavior may induce or facilitate such detachment.

Requirements for a *X. fastidiosa* Transmission Hypothesis. Although vector transmission of *X. fastidiosa* would seem to be a relatively simple matter of bacterial attachment and detachment from the foregut, numerous observations and experimental findings show that transmission is a complex process with many requirements. Transmission also can be analyzed based on results of biological experiments conducted mostly under laboratory conditions, for which a mechanistic hypothesis must accommodate existing data. Thus, any hypothesis must consider: 1) lack of latent period, 2) inoculum persistence over time, 3) transmission to plants with xylem sap under negative tension or under positive root pressure (dormant grapevines), 4) lack of transmission after molting, 5) spatial distribution of *X. fastidiosa* in the foregut of vectors, and 6) the failure of sap-sucking insects that are not xylem specialists (but none the less probe xylem) to transmit. Successful transmission to plants with positive root pressure suggests that vector probing behavior is related to inoculation (Almeida et al. 2005). Thus, a specific probing behavior may dislodge cells attached to the precibarium and inoculate them into a host plant. The use of electronic means to elucidate sharpshooter probing behaviors will provide useful information on the mechanisms of pathogen acquisition and inoculation (Almeida and Backus 2004, Backus et al. 2004).

Bacterial Colonization of Host Plant and Its Relationship to Transmission. *X. fastidiosa* transmission is the result of three events: acquisition from a source plant, attachment and retention to vector's foregut, and detachment and inoculation into a new host. A successful transmission event also includes development of an infection (i.e., multiple cycles of bacterial multiplication) after inoculation. Although factors affecting acquisition are relatively well understood, primarily the effect of different source plants and bacterial populations within them on acquisition efficiency, little is known about inoculation and persistence in plants.

Bacterial populations within host plants affect acquisition efficiency from grape (Hill and Purcell 1997). In general, higher numbers of live bacteria found in plants (using culturing methods for quantification) have been associated with higher acquisition efficiency. The minimum acquisition threshold in grape was 10^4 colony forming units (CFU)/g of plant tissue, and acquisition efficiency increased with increasing bacterial numbers (Hill and Purcell 1997). Transmission efficiency for CVC vectors is low, usually <10% transmission efficiency even with long acquisition and inoculation periods (reviewed in Redak et al. 2004). Estimated *X. fastidiosa* live populations in symptomatic citrus leaves were $\approx 10^6$ CFU/g, 100- to 1000-fold lower than in grapes (Almeida et al. 2001, Oliveira et al. 2002). Bacterial populations within almonds are also lower ($\approx 10^7$ CFU/g) (Almeida and Purcell 2003a). Correspondingly, transmission of *X. fastidiosa* from almond to almond by *H. coagulata* was less efficient (≈5% per insect per day) than from grape to grape (≈20% per insect per day) (Almeida and Purcell 2003b, c). Nonpersistent *X. fastidiosa* hosts, in which *X. fastidiosa* eventually die out (Purcell and Saunders 1999), could function as significant reservoirs of bacterial inoculum only if they harbor large numbers of vectors. However, they are usually not considered of major epidemiological importance.

***X. fastidiosa*–Vector Molecular Interactions.** The recent surge in molecular research on *X. fastidiosa* has provided new tools and approaches to study bacterial transmission. The development of techniques to genetically transform *X. fastidiosa* is arguably one of the most important achievements in recent years (Monteiro et al. 2001, Neto et al. 2002, Guilhabert and Kirkpatrick 2003). Site-specific mutants have enabled studies on bacterial attachment to various surfaces, biofilm formation, and regulation of several pathways associated with pathogenesis in plants (Souza et al. 2003). Use of reporter genes, such as a strain of *X. fastidiosa* expressing green fluorescent protein, has facilitated microscope observations and real-time analysis of bacterial biology (Newman et al. 2003). Linking these studies to transmission experiments

undoubtedly will provide important insight into *X. fastidiosa*'s mechanisms of attachment, persistence, and detachment on the vector's cuticle. *X. fastidiosa* mutants have been created to disable targeted genes eliminated by different transformation methods; however, most mutants have not been tested for insect transmissibility. Although it is possible to mechanically inoculate mutants into plants to test for pathogenicity, adding a vector component to such studies is of interest. After disabling the *rpf*F gene of *X. fastidiosa,* which is necessary for the bacterium to produce a cell-to-cell signal, Newman et al. (2004) found that the *rpf*F mutant caused more severe disease when mechanically inoculated into grapevines but was greatly impaired for vector transmission, probably because of its inability to attach and colonize the vector's foregut. The availability of microarrays for gene expression studies (Souza et al. 2003) will be useful to identify genes associated with *X. fastidiosa*'s colonization of insect vectors. Because of limitations in the number of cells that are present in an insect foregut, obtaining enough bacterial nucleic acids directly from insect heads for such tests may be challenging. The use of flow cell and other artificial systems with surfaces that mimic sharpshooter's cuticle may be a good alternative for these studies. The comparison of various *X. fastidiosa* genomes and the use of nontransmissible isolates also should permit researchers to identify genes required for successful vector transmission, as has already been done for plant pathogenicity (Koide et al. 2004).

Vector Ecology as a Basis for Spread of *X. fastidiosa* Diseases. The development of *X. fastidiosa* diseases is determined by multiple interactions among several elements that constitute the pathosystems, including host plants, vectors, environment, and the pathogen. The interactions of vectors with the other elements are essential for survival and spread of the bacterium among host plants. In both natural and agricultural systems, *X. fastidiosa* spread depends on suitable xylem-feeding vectors. Because of the low vector specificity and high diversity of Cicadellinae species in tropical and temperate regions of the Americas, some crops affected by *X. fastidiosa* (e.g., grapevine, almond, and citrus) are visited by numerous hopper species that can act as vectors. Up to 27, 22, and 11 species of sharpshooters and spittlebugs have been reported as vectors associated with PD, alfalfa dwarf disease, and CVC, respectively (Redak et al. 2004). Given the broad group specificity for vector transmission (Purcell 1989), these numbers probably depend more on how many species have been tested than on how many species are capable of transmitting *X. fastidiosa.*

Despite the large number of potential vectors, however, only a few species are likely to play a significant role in any particular crop disease in a region. The relevance of specific sharpshooter vectors for spreading *X. fastidiosa* diseases depends not only on their competence to transmit the pathogen but also on ecological interactions with host plants and the environment. Although vector competence is determined by feeding and physiological processes that allow acquisition, retention, and inoculation of *X. fastidiosa,* as discussed in previous sections, the importance of vectors in natural spread of the diseases is influenced mainly by ecological attributes such as habitat and host selection, vector density and mobility, and spatial and temporal distribution (Purcell 1985).

Habitat and Host Selection. Habitat and host preferences limit the economic importance of many vectors of bacterial pathogens (Rosenberger 1982). Less than one-third of the 43 documented vector species of *X. fastidiosa* are associated with crop disease epidemics (Redak et al. 2004). The most important vector species for spreading *X. fastidiosa* diseases are generally those most often found on or near the affected crops. Among the vectors associated with PD in the United States, *H. coagulata* is now considered the most important because of its high mobility, extreme polyphagy, and wide distribution on various crops that are susceptible to *X. fastidiosa* (Redak et al. 2004), despite its relatively inefficient transmission of *X. fastidiosa* (Almeida and Purcell 2003c). *Graphocephala atropunctata* (Signoret) is also an important PD vector, but it is somewhat restricted to coastal areas of California, where vineyards are bordered by riparian woods, which serve as overwintering habitats for this sharpshooter (Purcell 1975). Likewise, *Draeculacephala minerva* Ball and *Xyphon fulgida* Nottingham are more closely associated with epidemics in vineyards neighboring irrigated pastures and alfalfa fields in the Central Valley of California (Hewitt et al. 1942). Of 11 sharpshooters known to transmit *X. fastidiosa* in Brazilian citrus, five [*Acrogonia citrina* Marucci & Cavichioli, *Bucephalogonia xanthophis* (Berg), *Dilobopterus costalimai* Young, *Macugonalia leucomelas* (Walker), and *Oncometopia fascialis* (Signoret)] are considered to be key vectors for CVC spread. These five species are commonly found on citrus (Paiva et al. 1996), which seems to be the major source of inoculum for CVC spread (Laranjeira et al. 1998). In contrast, *Ferrariana trivittata* (Signoret) and *Plesiommata corniculata* Young are two vector species that are very abundant on weedy grasses in citrus orchards but are not associated with CVC epidemics and are rarely found on the citrus trees (Paiva et al. 1996, Lopes 1999).

Vector preference influences the density of insects and their residence time on plants; thus, the preference of particular vector species for feeding on hosts of *X. fastidiosa* should increase the probability of infection. Vector preference expressed in field and laboratory studies, however, failed to explain differences in PD incidence among grape cultivars of differing susceptibility. Instead, the plant's rate of overwinter recovery and the speed of bacterial colonization after initial infection seemed to be more important (Purcell 1985). Vector probing behavior on plants also may be critical for acquisition and inoculation success. Several factors can influence vector preference, including plant age, nutritional status, and pathogen infection. *B. xanthophis* is the prevalent sharpshooter species in citrus nurseries and groves up to 2 yr old (Roberto et

al. 2000, Yamamoto et al. 2001), whereas other vectors associated with CVC prefer older trees (Gravena et al. 1998). The preference for young citrus plants makes this sharpshooter an important vector, because bacterial inoculation in small trees enhances the probability and speed of systemic infection in the trunk, which precludes plant recovery by pruning. In addition, its propensity to inoculate nursery trees allows long-distance spread of the pathogen through contaminated nursery stocks. Nutritional quality of hosts also impacts sharpshooter preference. Because the concentration of nutrients in plant xylem sap is very low and can vary significantly depending on many factors such as season, time of the day, fertilization, and irrigation (Andersen and Brodbeck 1991, Andersen et al. 1992, Brodbeck et al. 1993), sharpshooters are likely to select plant species that offer a nutritionally balanced or rich diet at a given time.

Attractiveness and acceptance of infected plants by sharpshooter vectors are relevant for bacterial acquisition and disease spread. The yellowish coloration caused by infection of certain viruses and mollicutes can make host plants more attractive to vectors (Peterson 1973, Ajayi and Dewar 1983). A choice study revealed that the level of infection by *X. fastidiosa* influenced the selection of diseased citrus plants by sharpshooters. The vectors tend to reject severely infected plants with typical CVC symptoms, whereas symptomless infected plants are equally accepted in relation to healthy citrus (Marucci et al. 2005). This information supports the hypothesis that pathogenicity resulted from extensive vessel plugging is not a favorable outcome for vector acquisition and maintenance of *X. fastidiosa* in the environment (Newman et al. 2004).

Preference for young growth of shoots is a common behavior among various sharpshooters (Purcell 1975, Marucci et al. 2004) and may be important for inoculation success. In several grapevine cultivars, older foliage was more resistant to infection by *X. fastidiosa* after inoculation by sharpshooters than young leaves (Purcell 1981). However, the feeding preferences of *H. coagulata* for woody stems may allow the establishment of late season infections that are more likely to persist through the winter (Purcell and Feil 2001). It also was shown that *H. coagulata* can transmit *X. fastidiosa* to dormant vines during the winter (Almeida et al. 2005). This particular behavior of *H. coagulata* extends significantly the time frame during which grapevines are susceptible to chronic infections (Redak et al. 2004).

Vector Density and Mobility. It is reasonable to assume that probability of infection relates to vector abundance. However, it was noted that variables such as vector density and propensity to transmit *X. fastidiosa* are not adequate to explain the levels of PD incidence in California (Purcell 1985). A theoretical model based on estimates of sharpshooter density per plant, percentage of infective individuals, and transmission efficiency predicted much higher levels of infection than the disease incidence observed under the assumed conditions (Purcell 1981). More realistic levels of disease were obtained when date of inoculation was considered in this model. Because the sharpshooter *G. atropunctata* usually inoculates *X. fastidiosa* in the tips of grapevine shoots (Purcell 1975), infections that take place later than May are much less likely to move down to woody tissues and to persist after winter pruning (Feil et al. 2003). This shows that vectors must occur in sufficient numbers when conditions are favorable for establishment of chronic infections, which is during the spring. In central Florida, *Oncometopia nigricans* (Walker) is thought to be a more important vector than *H. coagulata* because it develops larger populations on grapevines in early spring (Adlerz and Hopkins 1979). Likewise, the ability to overwinter as adults in riparian woods adjacent to vineyards makes *G. atropunctata* a suitable vector to establish infections of *X. fastidiosa* in grapevines early in the spring in California (Purcell 1975). The apparent absence of diseases caused by *X. fastidiosa* in Europe may be because of the lack of vectors that overwinter as adults that could establish early season infections capable of surviving the following winter. Vector species that overwinter as eggs or nymphs would not be capable of spreading the bacterium among plants until after this critical spring period (Purcell 1997).

The effect of vector density on probability of infection is also dependent on characteristics of the crop system (Purcell 1985). For long-lived perennial crops, such as grapes and citrus that are planted in low densities, even low vector numbers should result in high levels of disease incidence after a few years of exposure. Disease levels are more likely to be related to vector densities for short-lived annual crops with high plant densities (Purcell 1985). For CVC, in which inefficient vectors are present at relatively low populations in citrus groves, it is likely that changes in vector density have a greater impact on probability of infection (Lopes 1999).

Vector dispersal capacity is an important factor in disease spread. It is a determinant of vector population density (Taylor and Taylor 1977) and pathogen distribution among host plants and vectors (Purcell 1985). There is little or no information available on dispersal abilities for many sharpshooter vectors. *H. coagulata*'s active movements among several habitats and host plants allow rapid spread of *X. fastidiosa* over a wide area, whereas movements of *G. atropunctata* are more limited to riparian vegetation and neighboring vineyards (Blua et al. 2001, Redak et al. 2004). Some spittlebugs are able to transmit the PD pathogen with relatively high efficiencies (12–65%) (Severin 1950). However, this group of vectors is not considered relevant for PD epidemics in California because it is not observed on grapevines under field conditions; usually does not disperse widely or rapidly (DeLong and Severin 1950; Redak et al. 2004), and overwinters in the egg stage, producing adults only after the time of year when chronic infection can be established.

Vector species not considered important in crops may still play a role in maintaining the infection cycle outside the affected crops (Purcell 1982). Outside

sources of inoculum seem to be particularly important when epidemics are generated mainly by primary spread, as it is observed in northern coastal areas and in the Central Valley of California, where adjacent riparian woods, alfalfa fields, and pastures serve as major reservoirs of *X. fastidiosa* for PD spread into vineyards (Hewitt et al. 1942, Purcell 1975). For example, some spittlebugs vectors not found on grapevines are fairly common on several herbs and shrubs that grow nearby vineyards and alfalfa fields in California (DeLong and Severin 1950); thus, they may contribute to maintain bacterium inoculum in weedy hosts.

Association of vectors with diverse habitats bordering affected crops allows colonization of a wider range of host plants that contribute to keep sufficient inoculum reservoirs for primary spread of the bacterium (Purcell 1985) and might allow survival of both vector and bacterium during unfavorable periods in the crop. Because *X. fastidiosa* interacts with diverse vector groups and colonizes a broad array of plant taxa (Hopkins 1989, Redak et al. 2004), there is probably selective pressure for the bacterium to maintain genes for attachment and colonization in the xylem of variable hosts as well as in the foregut of distinct vectors. The high diversity of species and polyphagy in Cicadellinae may increase the opportunities for the bacterium to adapt to new host plants and habitats and promote the evolution of new bacterial strains.

Development of *X. fastidiosa* Spread Control Strategies, a Case Study. Since Pierce (1892) first investigated what eventually came to be called PD, the main characteristics of PD epidemics in California have remained relatively unchanged. Exceptions to this include new outbreaks associated with plantings in new areas and the typical cyclical nature of PD (Hewitt et al. 1949). Yet, the major biological components in the system have remained static for >100 yr in California. PD epidemiology in California changed drastically in the mid-1990s with the introduction of a new vector, *H. coagulata* (Blua et al. 1999). *H. coagulata* is a well known and important vector of several *X. fastidiosa*-caused diseases in the southeastern United States, including PD, and precludes the successful culture of *Vitis vinifera* L. and *Vitis labrusca* L. in that region (Hopkins 1989). This leafhopper was first detected in southern California in 1989 (Sorensen and Gill 1996) and was implicated in the spread of a new *X. fastidiosa* disease of oleander in southern California, oleander leaf scorch, in the mid-1990s (Purcell et al. 1999).

In 1997, *H. coagulata* was first implicated in the spread of PD in the wine grape-growing area of Temecula in Riverside County of southern California (Blua et al. 1999). Initial observations of the epidemic indicated large differences from epidemics of PD present before in California. First, vector sources mainly consisted of citrus, not previously known to be important to PD vectors. Second, the distance with which disease was detected from vector sources was hundreds of meters from the edge of the vineyard (Perring et al. 2001) relative to native California vectors that spread disease mainly to grapevines within 100–200 m from the vineyard edge (Purcell 1974). Third, researchers noticed that the exponential increase of PD from year to year, which should occur where transmission from vine to vine, i.e., secondary spread, was important. In contrast, PD spread by native vectors was mainly from outside of vineyards into the vineyard (primary spread) but not among grapevines (Feil and Purcell 2001). Although the degree to which grapevines are infected as a result of primary versus secondary spread is not clear, secondary spread has the potential to occur much more rapidly than primary spread. Secondary spread generates a log-linear relationship between disease spread and time, whereas primary spread is relatively constant (linear) over time (Van der Plank 1963).

Importantly, recent studies showed that *H. coagulata* can inoculate dormant grapevines in the winter (Almeida et al. 2005), when these insects are commonly observed feeding on grape (Purcell and Feil 2001). *H. coagulata*'s ability to feed on and inoculate *X. fastidiosa* into woody grapevine tissue, on which native vectors do not feed, may be responsible for secondary spread if such infections during summer through fall establish chronic PD (Almeida and Purcell 2003c). Summer infections of grape by native vectors in California seldom cause infections that survive the following winter (Feil et al. 2003). The overwinter recovery of infections of summer-inoculated vines largely explains the lack of secondary spread in California vineyards (Hopkins and Purcell 2002). Given the evidence that secondary spread seems to be important where *H. coagulata* is present, a hypothesis worth testing is whether summer infections established by *H. coagulata* overwinter successfully.

A necessary extension of PD research is to use new information in conjunction with knowledge of cropping systems to generate economically sound disease management strategies. Under development are novel disease management tactics for deployment at landscape, vineyard, and grapevine scales. These tactics focus on reducing the numbers of *H. coagulata* over a large area, to keep them out of vineyards, manipulating their interactions with grapevines, and manipulating the interactions between grapevines and *X. fastidiosa.*

Ecology of *X. fastidiosa* Spread to Grapevines by *H. coagulata* in California. Understanding the details of the spread of *X. fastidiosa* by *H. coagulata* to grapevines is useful to identify research needs, mathematically model epidemiology, and develop control methods and an overall PD management strategy. It is important to consider not only the interacting organisms in the crop (i.e., grapevines, *X. fastidiosa*, and *H. coagulata*) but also the effects of the environment, including the larger agricultural community. This is particularly important because of the large reproductive host range (Mizell and French 1987, Turner and Pollard 1959) and wide dispersion potential of *H. coagulata* (Blua and Morgan 2003).

The larger agricultural community provides plant hosts of *X. fastidiosa* and *H. coagulata.* Plant hosts of PD strains of *X. fastidiosa* are important to the epi-

demic in proportion to their use as feeding hosts for vectors. Pathogen host plants include annuals, perennials, herbaceous, and woody plants as well as native, feral, agricultural, and ornamental plants (Freitag 1951, Purcell and Saunders 1999). Likewise, this agricultural community also provides hosts for *H. coagulata.* As with pathogen hosts, hosts for the vector are best considered a continuum from plants that are not fed on, such as *Eriogonum fasciculatum* (Benth.) (buckwheat), a common native plant, to citrus, an excellent feeding and overwintering host on which *H. coagulata* reproduces (Adlerz 1980, Mizell and French 1987). Citrus seems to be the single most important host that contributes to high population densities of *H. coagulata* in southern California.

Plants that have access to water during the summer, most typically via irrigation in California, seem to be the most important for *H. coagulata.* Natural plant communities in southern California provide hosts during the winter rainy season and in riparian areas. Some nonriparian native plants, such as Toyon, *Heteromeles arbutifolia* (Lindl.) M. Roemer, allow feeding and reproduction (M.J.B., unpublished data). Riparian native plants as a group, however, are generally in a more hydrated state than nonriparian natives and are likely more acceptable as hosts for *H. coagulata.*

Residential landscaping provides a plethora of irrigated feeding and reproductive hosts that can serve as reservoirs of *H. coagulata* and *X. fastidiosa.* The grape growing-area of Ontario, CA, that has recently suffered significant outbreaks of PD spread by *H. coagulata* in the first time in their >80-yr-old history of wine grape-growing (M.J.B., unpublished data), has no large blocks of citrus like the other areas. Rather, it has numerous residences with various reproductive and feeding hosts of *H. coagulata* surrounding the vineyards. The larger agricultural community provides an opportunity for interactions with other vectors that may be important to the spread of *X. fastidiosa* in grapevines. An otherwise small proportion of infected grapevines in a vineyard generated by native vectors would be a key source from which the pathogen is spread by *H. coagulata* among grapevines in the vineyard. The main interacting characters involved in the new PD epidemic in southern California are grapevine, *X. fastidiosa,* and *H. coagulata. V. vinifera* is an excellent host of *X. fastidiosa* that supports the growth of a high density of bacterial cells. For 2–4 yr, or until the vine declines severely, such plants can be an excellent acquisition host for vector transmission.

Current Management of PD Spread by *H. coagulata.* PD epidemics can be managed based on disruption of the interactions among the main characters involved, including the agricultural community. Absolute disruption of any single interaction would completely diminish disease spread. Yet, absolute disruption of any interaction is unlikely, so combining multiple tactics that partially interrupt more than one interaction is the current management strategy.

Tactics used to reduce the spread of PD by *H. coagulata* on a local level target vectors primarily. These tactics include the use of biocontrol agents in the larger agricultural community and biocontrol and insecticides to reduce *H. coagulata* in citrus and vineyards (Wendel et al. 2002, Hix et al. 2003). Systemic insecticides, especially neonicotinoids, and repellents, such as kaolin, a formulation of aluminum silicate, are used in vineyards. Besides impinging on interactions between *H. coagulata* and grapevines, they disrupt the *H. coagulata–X. fastidiosa* interface by impacting vector orientation, host determination, and feeding behavior (Puterka 2002, Tubajika et al. 2003). Another tactic gaining interest to impact the *H. coagulata–X. fastidiosa* interface is the removal of diseased grapevines (Hashim and Hill 2003). Currently, there are no tactics available to interrupt the grapevine–*X. fastidiosa* interface.

In the *H. coagulata–X. fastidiosa* system, tactics used to manage PD spread by *H. coagulata* are costly, not completely effective, and not all are sustainable. Because grapevines are perennial, the use of these tactics to "hold back the tide" may eventually prove to be ineffective. With current tactics, an important challenge looms in sustaining the management of *H. coagulata* in citrus, where its economic threshold far exceeds that of grapevines. Inoculative releases of parasitoids (e.g., *Gonatocerus* sp.) of *H. coagulata* eggs likely can maintain sharpshooter populations below the economic threshold for citrus yet allow citrus to produce population densities that are dangerous to nearby vineyards. A major problem for effective biological control with these egg parasitoids is that their populations decline precipitously from September through the winter, when egg production by *H. coagulata* is low. Thus, the parasites have a difficult time surviving winters, and only a small proportion of first generation (spring) sharpshooter eggs are parasitized. In the second generation, rates of parasitization frequently were >90% in some areas (Morgan et al. 2001). Difficulties in rearing *H. coagulata,* and consequently rearing egg parasites, make inundative releases implausible.

An Overview of PD Management Tactics under Development. One likely consequence of the current *H. coagulata*–PD management system is that it will allow growers to maintain vineyards until more effective or sustainable tactics can be developed. The discussion below clarifies management tactics for PD spread by *H. coagulata* on the basis of interrupting two-way interactions related to pathogen transmission and disease spread: vector–pathogen and vector–plant. Some of the tactics under development may impinge on plant-pathogen interactions as well.

Interrupting the H. coagulata*–Grapevine Interaction.* Improvements in traditional tactics and novel approaches are under development. They include the use of improved insecticides, especially soil-applied systemics and insect growth regulators, that are compatible with biocontrol and do not affect most nontarget species because of their method of application or activity (Toscano and Castle 2002, Akey et al. 2002, Redak and Bethke 2003). Further studies are optimizing the deployment of insecticides in vineyards and key crops that are *H. coagulata* reproductive hosts

(e.g., citrus) to reduce its population densities in the agricultural community that can impact vineyards (Akey et al. 2002, Toscano and Castle 2002). Research continues to improve biocontrol by searching for and collecting additional *H. coagulata* egg parasitoids (Hoddle and Triapitsyn 2003) and examining the impact of entomopathogenic fungi, particularly to target overwintering *H. coagulata* adults in citrus (Kaya 2003).

A screen barrier is being developed as a tactic to impede the movement of *H. coagulata* into vineyards or plant nurseries (Blua and Redak 2003). Investigations of *H. coagulata* flight height revealed a potential of a 5-m-high screen barrier to reduce their movement from citrus by 97–99% (Blua and Morgan 2003, Blua and Redak 2003). Further studies indicate that 70% of *H. coagulata* placed on a platform near the barrier flew away from it. Only 6% of individuals placed on the barrier flew over it, and none walked over it or more than a few centimeters (Blua and Redak 2003). The disadvantages to a barrier tactic include the high cost of materials and installation, but the barriers should last at least 10 yr. The greatest advantage to a barrier is its compatibility with other tactics such as insecticides and biocontrol.

Interrupting the H. coagulata–X. fastidiosa *Interaction.* The advent of new systemic insecticides, especially neonicotinoids, has spurred research focused on developing tactics directed against the *H. coagulata–X. fastidiosa* interface. Breaking this interaction involves altering the behavior of the vector related to *X. fastidiosa* transmission or altering surface sites at the cellular level that allow bacterial adhesion to the vector's foregut.

Field studies showed that application of a neonicotinoid insecticide slowed the rate of PD spread even in the presence of a high *H. coagulata* infestation (Krewer et al. 2002), suggesting that the insecticide was operating on the transmission process and not just decreasing sharpshooter population density. Currently in California, restrictions on the amount of neonicotinoids used per unit area do not allow high enough levels to ensure high *H. coagulata* mortality throughout the year. But sublethal effects still may be important to disease transmission if they impact *H. coagulata* feeding behavior. Field studies by M.J.B. (unpublished data) reveal that three different neonicotinoids in grapevines blocked feeding by *H. coagulata*, as measured by the production of excreta, for 6 wk after application. For one neonicotinoid examined further, a substantial decrease in feeding was observed 11 mo after treatment on Chardonnay grapevines, whereas feeding was blocked on Cabernet Sauvignon grapevines. Interestingly, grapevine variety also affected feeding, with individuals on Chardonnay producing ≈4 times the excreta as individuals on Cabernet Sauvignon in the same period. It is not known whether decreasing *H. coagulata* feeding may reduce their ability to acquire *X. fastidiosa* from infected grapevines that are treated or inoculate healthy treated grapevines.

However, this neonicotinoid-induced reduction in sharpshooter feeding does not necessarily translate into a similar reduction in disease spread. Field studies examining the impact of neonicotinoids on the spread of PD in a 1000-grapevine experimental vineyard showed a reduction in disease because of neonicotinoid treatment by ≈36% relative to untreated controls (Redak and Blua 2002). Vector pressure in this experimental vineyard was large relative to outbreaks in commercial vineyards. After one season of exposure, 69% of the untreated control grapevines expressed symptoms of PD, indicating a large primary spread event. After the next season, all treatments had high levels of PD, and there were no statistical differences. It is likely that neonicotinoid treatment would have a larger impact on secondary spread than primary spread, because vectors would be exposed to treated vines twice; once during feeding for acquisition and once during feeding for inoculation. For primary spread, exposure to treated grapevines occurs only during inoculation.

Another treatment examined in the experiment described above is applications of kaolin to grapevines, creating a "particle film" that disrupts insect behavior (Puterka 2002, Tubajika et al. 2003). The overall effect of kaolin is to reduce acquisition of *X. fastidiosa* from infected grapevines and the ability of *H. coagulata* to inoculate noninfected vines. Treatments with kaolin, either alone or in combination with neonicotinoids, had 50–57% less PD than untreated controls. Civerolo (2001) reported similar results. Part of the impact of kaolin likely involves masking visual cues that *H. coagulata* relies on in host finding, because treatments make grapevines look white. Studies show that through a season ≈50% fewer *H. coagulata* were caught in kaolin-treated grapevines compared with control plots (Redak and Blua 2002). This does not likely account for the reduction in PD observed, so other factors are likely significant, including plant surface interactions that mask host tactile cues and direct effects of kaolin that irritate sharpshooters.

Logically, removing infected grapevines should reduce secondary (vine-to-vine) spread of *X. fastidiosa.* However, this tactic is only worthwhile if secondary spread of the pathogen is important. Removing infected grapevines when PD epidemics involved native California vectors did not significantly reduce disease (Hewitt et al. 1949). The explanation for the ineffectiveness of rouging diseased vines is that in traditional PD, only primary spread by vectors entering the vineyard from outside is important in establishing chronic disease. Disease removal is currently being reinvestigated as a PD management tactic where *H. coagulata* is present, however, because of the possible importance of secondary spread of chronic PD by this vector (Hashim and Hill 2003). Unfortunately, by the time grapevines show disease symptoms, they may have been point sources for the spread of *X. fastidiosa* for months. Research involving a strain of *X. fastidiosa* that causes CVC in Brazil revealed that vectors prefer plants that do not show disease symptoms (Marucci et al. 2005). Also, *H. coagulata* abundance was lower on peach trees showing symptoms of phony peach dis-

ease compared with control trees (Mizell and French 1987). Thus, the greatest benefit of the removal of diseased plants would be achieved if infection could be determined soon after inoculation or at least before symptom production.

Genetically induced interruption of the *H. coagulata–X. fastidiosa* interface is being investigated by manipulating endophytic bacteria to block transmission. One line of investigation is focusing on genetically altering *Acaligenes xylosoxidans denitrificans*, an *H. coagulata* gut bacterium that also is found in the xylem of grapevines and other plants (Lauzon and Miller 2002). The objective is to insert genes coding for antibody fragment that interacts with the surface of *X. fastidiosa* cells, rendering them incapable of adhering to the *H. coagulata* mouthparts/foregut (Lampe and Miller 2003). This tactic would tend to block secondary transmission, but it might be ineffective for primary spread because primary spread involves acquisition from nongrapevine sources that would not be treated with the transgenic bacterium.

Integrating Management Tactics. The most robust and sustainable management strategy to combat PD associated with *H. coagulata* should use tactics that disrupt multiple interactions, without reducing the maximum efficacy of each. Reliance on a single tactic that is adequate to reduce disease spread below the economic threshold may be worthwhile and cost-effective, but at a greater risk. At the foundation of current management for PD spread by *H. coagulata* is control of the spread of this new vector to areas of California that are not infested. The California Department of Food and Agriculture (CDFA 2004) imposed strict regulations on the nursery industry in California to curtail movement of *H. coagulata* via nursery stock transported to noninfested counties. CDFA also traps to monitor *H. coagulata* movement throughout the state and attempts to eradicate new, localized infestations by repeated insecticide treatments.

Even if a solution to PD is developed, diseases induced in other plants by different strains of the pathogen can create new problems. Of particular concern is the spread of *X. fastidiosa*-induced diseases other than PD, such as almond leaf scorch, alfalfa dwarf, and oleander leaf scorch. Recent studies show that various ornamentals are currently supporting different strains of *X. fastidiosa* in California, some of which are associated with disease symptoms (Wong et al. 2004). These studies also have confirmed the presence of mulberry leaf scorch (Kostka et al. 1986), a disease not previously identified in the state (Wong et al. 2004). Concerns are high that other diseases caused by different strains of *X. fastidiosa*, such as those causing CVC (Lee et al. 1991) and phony peach disease (Turner and Pollard 1959), may spread to new regions.

The California epidemic of PD in grapevines spread by *H. coagulata* has several aspects that make it particularly difficult to manage. From an economic perspective, at some proportion of infected vines, maintaining the vineyard or orchard is not profitable. An economic threshold for *H. coagulata*-spread PD is a complex issue dependent on, among other factors, grape variety, yield, region, year, relative contribution of primary and secondary spread for the vineyard in question, and for wine grapes, whether a vineyard is owned and operated by a winery. Second, *X. fastidiosa* is a pathogen of a few plants but a commensal of many. Thus, many plants, including wild plants, ornamentals, and crops may be long-term point sources from which the pathogen is acquired by vectors. Third, *H. coagulata* is relatively long-lived, and once it acquires *X. fastidiosa* as an adult, it maintains the capacity to inoculate plants through the remainder of its life. This vector has a large host range (Turner and Pollard 1959, Mizell and French 1987) and disperses widely in short hopping flights that tend to enhance the spread of *X. fastidiosa* (Blua and Morgan 2003). *H. coagulata* also overwinters as adults and is frequently observed feeding on dormant grapevines that Almeida et al. (2005) showed may be at risk for inoculation. Finally, *H. coagulata* seems to spread *X. fastidiosa* from vine to vine, thus causing a very rapid epidemic once pathogen sources are in the vineyard, but this is a hypothesis that needs to be tested rigorously because of its disease control implications. Transgenic techniques hold great promise to produce future PD control tactics. But gene-conferred protection should not have specificity that would allow protection to break down if a closely related strain of *X. fastidiosa* challenged the system. The issue of regulatory and public acceptability of transgenic tactics, however, will be a key as to whether successful methods could ever be applied.

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