Review in Advance first posted online on April 18, 2008. (Minor changes may still occur before final publication online and in print.)

Living in two Worlds: The Plant and Insect Lifestyles of *Xylella Fastidiosa*

Subhadeep Chatterjee,[1] Rodrigo P. P. Almeida,[2] and Steven Lindow[1]

[1]Department of Plant and Microbial Biology, [2]Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720; email: schatt@nature.berkeley.edu, rodrigo@nature.berkeley.edu, icelab@berkeley.edu

Annu. Rev. Phytopathol. 2008. 46:243–71

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

This article's doi: 10.1146/annurev.phyto.45.062806.094342

0066-4286/08/0908/0243$20.00

Key Words

Pierce's disease of grape, sharpshooters, xylem vessels, cell-cell signaling, vector transmission, *Xanthomonas*

Abstract

Diseases caused by *Xylella fastidiosa* have attained great importance worldwide as the pathogen and its insect vectors have been disseminated. Since this is the first plant pathogenic bacterium for which a complete genome sequence was determined, much progress has been made in understanding the process by which it spreads within the xylem vessels of susceptible plants as well as the traits that contribute to its acquisition and transmission by sharpshooter vectors. Athough this pathogen shares many similarities with *Xanthomonas* species, such as its use of a small fatty acid signal molecule to coordinate virulence gene expression, the traits that it utilizes to cause disease and the manner in which they are regulated differ substantially from those of related plant pathogens. Its complex lifestyle as both a plant and insect colonist involves traits that are in conflict with these stages, thus apparently necessitating the use of a gene regulatory scheme that allows cells expressing different traits to co-occur in the plant.

INTRODUCTION

PD: Pierce's disease of grape

CVC: citrus variegated chlorosis

Strains of *Xylella fastidiosa* have been associated with a large number of diseases, many causing great economic losses. The most economically important diseases caused by *X. fastidiosa* are Pierce's disease of grape (PD) and citrus variegated chlorosis (CVC) of citrus species, both of which have received more attention by far than the many other diseases caused by this pathogen. Alfalfa, peach, plum, almond, elm, coffee, sycamore, oak, maple, pear are among the many other plant species for which diseases caused by *X. fastidiosa* are described, and the pathogen can be detected in hundreds of asymptomatic plant species (14, 27, 57, 59, 60, 62, 64, 65, 78, 83, 93, 125). This pathogen is obligately vector-transmitted from one plant to another by various xylem sap-feeding insects. The recent introduction of the glassy-winged sharpshooter (*Homalodisca vitripennis*) into southern regions of California in 1989 has transformed PD from a problem mostly associated with vineyards near riparian areas to one that potentially could involve grape production over most of the state. The blue-green sharpshooter, a native vector of the bacterial pathogen *X. fastidiosa*, has a much more limited range of flight and narrower host plant preferences than does the glassy-winged sharpshooter, and this new vector might put grapes at risk wherever it will spread, as it has in the southeastern United States where PD is a major limiting factor for grape production. For this reason, the study of PD has dramatically increased since the last reviews of this pathogen, which focused mostly on descriptions of symptom development and etiology of disease (64, 65, 93). Perhaps due to the increasing attention to this pathogen, as well as better means of detection and discrimination of its many strains, this pathogen is being detected in large numbers of new host plant species in California and elsewhere (27, 60, 78, 83); whether these numbers represent a broadening of its host range due to pathogen evolution or introduction of novel strains, or simply an appreciation of is exceptionally wide host range is unknown. Likewise, the outbreak of CVC in Brazil has also stimulated extensive study of the strains that cause that disease, and led to the complete sequencing of the genome of a CVC strain of *X. fastidiosa*, the first plant pathogenic bacterium to be sequenced (111). Together, there is a wealth of new knowledge of *X. fastidiosa* and its interactions with plants and insect vectors. The availability of genomic information on *X. fastidiosa* has led to numerous reports of proteins and other biochemical processes in this species (30, 35, 36, 45, 70, 84, 89, 103, 120), but these studies are not emphasized in this review. We also do not address the many studies of detection methods and strain variation (26, 39, 56, 58, 81). Instead, we emphasize the new insights into the disease process and the processes involved in insect vectoring of this pathogen gained from recent studies that have exploited new molecular techniques and the genome sequences of several *X. fastidiosa* strains. In addition, we focus on PD, as much recent work to understand the biology of this pathogen has used this system.

INTERACTIONS OF *X. FASTIDIOSA* AND PLANTS: THE DISEASE PROCESS

Unlike other plant pathogenic bacteria such as many species and pathovars of *Xanthomonas*, *Ralstonia solanacearum*, or *Pantoea stewartii* that can spread in plants via xylem vessels, *X. fastidiosa* appears to be exclusively xylem-limited, living only in xylem cells or tracheary elements (3, 51, 64, 65, 87, 119). As discussed in detail below, the virulence factors that *X. fastidiosa* apparently utilizes to cause disease differ from those of other vascular pathogens that also interact directly with living plant tissues.

The diseases caused by *X. fastidiosa* are typically a leaf-scorch associated with the extensive colonization of xylem vessels. Such symptoms usually appear only many weeks after inoculation with the pathogen and are most prominent in the field in late summer when maximum water demand by the plant is apparently not met by water supplied by the occluded vessels (77). The

development of disease depends on the ability of the pathogen to spread from the point of infection and ultimately to develop a population that is systemic in the infected plant. Presumably it is the difference in the ability of the pathogen to spread widely within susceptible host plants that distinguish them from the many plants in which spread is minimal and in which the pathogen exists as a relatively harmless endophyte (64, 65, 87, 93). In susceptible hosts such as grape, *X. fastidiosa* multiplies and spreads widely from the site of infection to colonize the xylem, a water transport network of vessels composed of dead, lignified cells. Vessels are interconnected by channels, called bordered pits, that allow the passage of xylem sap but block the passage of larger objects due to the presence of a pit membrane (118). Bacterial cells attach to the vessel walls and multiply, forming biofilm-like colonies that can, when sufficiently large, completely occlude xylem vessels, thereby blocking water transport. The systemic spread of the pathogen is limited by the pit membranes that separate one xylem water conduit from its neighbors, and perhaps by the production of tyloses and polysaccharide-rich gels by plants that block xylem cells following infection. It remains uncertain to what extent the disease symptoms are caused by direct bacterial blockage of vessels or by such plant-derived blockages, but it seems clear that the plant response is dependent on extensive bacterial colonization itself (51, 71). The movement of cells of *X. fastidiosa* is an active process and appears to be dependent on its ability to disrupt pit membranes. Because at least a few fluorescent spheres and bacterial cells can move throughout a severed grapevine immersed in suspensions of such particles, it appears that a few passages may enable bacterial cells to move passively within the length of a grapevine (21). However, when such spheres or cells are introduced as a point source into a grapevine little or no movement beyond the length of a typical vessel (ca. 10 cm) is seen (21), suggesting that natural passages (breaches in pit membranes?) sufficiently large to permit bacterial cells to move between xylem vessels are quite uncommon and that active mechanisms of movement through pit membranes are required for extensive movement of *X. fastidiosa* within the plant.

A prominent feature of symptomatic grape plants is the presence of many xylem vessels that are heavily colonized by *X. fastidiosa*; in many cases vessels would appear to be blocked by both the bacterial cells as well as gummy material of bacterial, or potentially also of plant origin. For this reason it has long been thought that the symptoms of PD, scorching of leaf margins and shriveling of grape berries, typically commencing in mid to late summer, are due to deficiencies of water delivery to the leaves and fruit of grape due to blockage of vessels. This conjecture has been difficult to verify since simple acute desiccation of grape plants by girdling or withholding of water does not reproduce these and other symptoms typically associated with PD (116). On the other hand, it is not uncommon to see symptoms typical of PD in healthy grapevines that are damaged in a way that would reduce water flow through the vines. Thus PD would seem to be a result of progressive and increasingly limited water flow through the vine that, when high temperatures and maximum water demand with a full canopy coincide in late summer, leads to progressive water stresses to the plant, resulting in such scorching symptoms. The patterns of gene expression in *X. fastidiosa*-infected grape have recently been compared with those in uninfected grape to determine host responses to infection (74). As such studies are in their infancy, it remains to be seen whether changes in gene expression in infected grape reflect primarily responses to localized water stress associated with pathogen-mediated blockage of vessels or evidence of other host defenses to the pathogen. Whatever the exact mechanism of pathogenesis, PD is seen only when plants become heavily colonized by *X. fastidiosa*, and thus a better understanding of the processes that lead to colonization of the xylem should help elucidate the disease process and facilitate possible methods of control.

Considerable evidence indicates that disease symptoms associated by *X. fastidiosa* colonization of plants is related to the proportion of vessels that harbors large cell aggregates and not simply to the number of vessels that are colonized. gfp-tagged cells of *X. fastidiosa* have recently been used to assess the process of colonization of xylem vessels in grape. This method is much less invasive than scanning electron microscopy (SEM) and other methods used previously. Cells expressing GFP (green fluorescent protein) can be visualized by confocal laser-scanning microscopy in situ in vessels that have not been ruptured by sectioning and without the need for fixatives, etc. In addition, large numbers of vessels can be easily examined by fluorescence microscopy for the presence of even a few marked cells, enabling cells in different-sized aggregates within vessels to be readily quantified. Such studies revealed that the fraction of colonized vessels was fivefold higher in symptomatic leaves than in asymptomatic leaves on the same plant (87). However, the differences in specific aspects of colonization, such as colony size distribution and vessel occlusion, between symptomatic and asymptomatic leaves was even more informative. For all infected leaves the colony size was variable, with vessels most commonly harboring colonies with relatively few cells (<100 cells/colony) (87). Although the number of vessels with small colonies were threefold higher in symptomatic leaves than in asymptomatic leaves, the number with large, occluding colonies (>1000 cells/colony) was 20-fold higher. This indicates that most cells in symptomatic leaves are in large colonies whereas this is not true in asymptomatic leaves. This shift to larger colonies in symptomatic leaves also suggests that large colonies have a more deleterious effect on the host than do small colonies. Small colonies in asymptomatic leaves apparently grow into large colonies over time; however, cells must also be dispersing to new vessels since symptomatic leaves also had many more vessels colonized. Therefore vessel blockage by bacterial colonization is apparently a critical variable in symptom expression. Since vessel plugging is a prerequisite for disease, it seems unlikely that bacterial toxins or plant-initiated vessel failure lead to disease symptoms. Although most attention has been directed to vessel plugging, the most obvious feature of *X. fastidiosa* colonization of xylem, only recently has the significance of the extensive colonization of vessels by small, nonplugging *X. fastidiosa* colonies been appreciated. On average only about 10% to 15% of the vessels colonized by *X. fastidiosa* are heavily colonized (87). As *X. fastidiosa* cells attached to vessel walls presumably obtain at least some of their needed nutrients and other resources such as oxygen from the xylem sap that would flow past the adhered cells, it would be expected that if the flow of xylem sap in the vessel were restricted the cells would suffer. Indeed, our microscopic examination of the viability of *X. fastidiosa* cells in the xylem of infected grape assessed by propidium iodide staining revealed that small colonies of *X. fastidiosa* cells contain exclusively live cells whereas cells in vessels that are heavily colonized are mostly dead (**Figure 1**). The combined observations that cells of *X. fastidiosa*

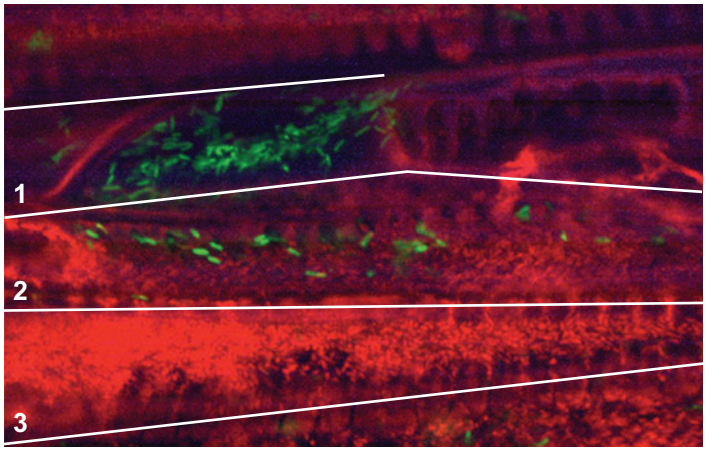

Figure 1

Viable and nonviable cells in grape xylem vessels colonized by a gfp-marked strain of *X. fastidiosa*. In the petioles of leaves showing very severe symptoms of Pierce's disease a high incidence of colonization of vessels is observed. In those vessels in which *X. fastidiosa* reaches very high concentrations and blocks vessels (vessels 2 and 3), most of the cells are dead since they stain red with propidium iodide whereas in those vessels in which the number of *X. fastidiosa* cells is lower (vessel 1), most cells remain alive (*green cells*). Image courtesy of K. Newman.

usually do not block xylem vessels of grape, and that the pathogen suffers when they do block xylem vessels, suggest that it has evolved mechanisms to avoid overzealous colonization of grape xylem. The finding that most vessels are only sparsely colonized by *X. fastidiosa* would be expected if it was primarily an endophytic colonist of plants that only accidentally caused blockage of vessels sufficient to induce water stress to plants under certain conditions. *X. fastidiosa* is also apparently very efficient in movment between vessels since only relatively few vessels in a plant are occluded while many more contain cells of the pathogen. Although such dispersal within plants would clearly benefit a commensal endophytic interaction with the plant, only when colonization becomes excessive in plant species such as grape and citrus would such an interaction become pathogenic. As developed later in this review, the larger aggregate states that the cells attain in vessels might well contribute to its other necessary life state: association with insect vectors.

COMPARISON OF THE DISEASE PROCESS IN *X. FASTIDIOSA* AND *XANTHOMONAS* SPECIES: INSIGHTS FROM GENOME SEQUENCES

As *X. fastidiosa* is most closely related to various *Xanthomonas* species, and since both groups can colonize the xylem, it is instructive to consider the similarities of and differences between these two groups. In addition, due their relative ease of study compared to *X. fastidiosa*, Xanthomonad pathogens have historically received much more research attention than *X. fastidiosa*. The availability of complete genome sequences for several *X. fastidiosa* strains as well as different *Xanthomonas* species makes possible comparison of these species. The CVC strain of *X. fastidiosa* was the first to be sequenced (111), followed by publication of draft genome sequences of a strain from almond (strain Dixon) and from oleander (strain Ann1) (15, 16) and the complete sequence of the genome of a strain causing PD of grape (strain Temecula-1) (121). Genome analysis of the different *X. fasitidiosa* strains revealed interesting similarities with and differences from the closely related plant pathogens *Xanthomonas campestris* pv. *campestris*, a pathogen of crucifer, and *Xanthomonas oryzae* pv. *oryzae*, a pathogen of rice, both of which are vascular pathogens that enter the plant through hydathodes, terminal extensions of the xylem located at the leaf margin at the end of the veins (**Figure 2**). The bacteria enter through the hydathodes and then migrate to the lateral veins where they proliferate. The bacteria can subsequently invade the surrounding mesophyll tissues resulting in severe disease symptoms. In contrast, *X. fastidiosa* is strictly xylem limited and is delivered in the xylem vessel by xylem sap-feeding insect vectors. Once inside the xylem vessel, *X. fastidiosa* proliferates as a biofilm only along the xylem wall. Its spread in the plant is limited to the xylem vessels, but it apparently spreads extensively from the vessels into which it is transmitted by insect vectors to adjacent uncolonized xylem vessels, probably by the dissolution of the pit membrane (**Figure 2**). Many genes have been implicated in the virulence of *Xanthomonas* species, and many of these homologs are present in *X. fastidiosa*. We discuss the similarities and differences in the presence of these virulence genes in *Xanthomonas* and *X. fastidiosa*, and relate how these traits are consistent with the different lifestyles of these important plant pathogens (**Figure 2**).

Absence of a Type III Secretion System: Does *X. Fastidiosa* Secrete Virulence Factors by a Type I Secretion System?

Genome analysis of several different *X. fastidiosa* strains revealed the absence of genes encoding conserved Type III secretion system machinery as well as the lack of apparent Type III secretion effectors (15, 16, 41, 111, 121). The Type III secretion effectors are highly diverse in different plant pathogens. *X. fastidosa* may encode Type III-like effectors that do not show homology to known Type III effectors in other

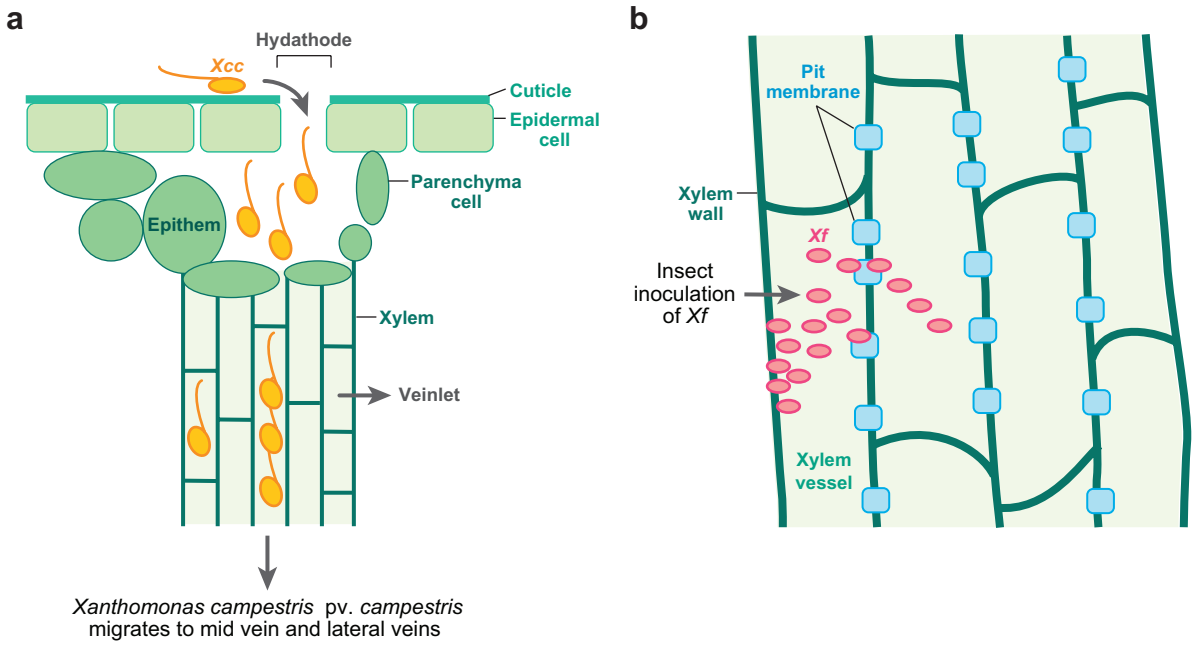

Figure 2

Schematic representation of the mode of entry and growth of *Xanthomonas campestris* and *Xylella fastidiosa*. (*a*) *X. campestris* pv. *campestris* enter through openings called hydathodes, which are located on the leaf margin, possibly by flagellar-driven chemotaxis. (*b*) *X. fastidiosa* is delivered directly into the xylem vessels by xylem sap-feeding insect vectors. *X. fastidiosa* is xylem-limited and escapes only to the neighboring xylem vessel through dissolution of pit membranes (PM). *X. campestris* moves from veinlets to the mid and lateral veins, likely through the action of its extracellular enzymes that may contribute to digestion of xylem tissues. Excessive growth and tissue damage can also lead to escape of the pathogen to the spaces between living cells such as the surrounding mesophyll cells.

plant pathogens. However, the absence of a conserved Type III secretion apparatus strongly suggests that *X. fastidiosa* is devoid of Type III effectors. A major role of the Type III secretion system in plant pathogens is to suppress host plant defense responses, which are initiated by the plant in response to detection of microbial PAMP (pathogen-associated molecular patterns; sometimes also called MAMP, microbe-associated molecular patterns) or in response to detection of pathogen effector molecules themselves (2, 24). The lack of a Type III secretion system in *X. fastidiosa* may reflect the fact that it may seldom encounter living plant cells while colonizing xylem vessels which consist of dead cells. There is no evidence of its colonization of adjacent living parenchymal cells. *X. fastidiosa* harbors many genes similar to those encoding various hemolysins and components of the Type I secretion system (111, 121). Meidanis and coworkers (79) performed a whole genome analysis of various putative transporters that would be encoded by the *X. fastidiosa* genome. The study revealed that *X. fastidiosa* has at least 23 systems comprising 46 proteins belonging to the ABC (ATP binding cassette) superfamily. The Type I secretion system (which consists of ABC-type transporters) is involved in processes such as drug resistance via multidrug efflux pumps, secretion of hemolysins, and even elicitation of resistance responses to rice by *X. oryzae* pv. *oryzae* (31, 75). In general, bacterial Type I secretion systems consists of two paired proteins that are localized in the inner membrane and a third component that is usually encoded by TolC, which spans the inner and outer membranes (31, 75). *X. fastidiosa* has only one TolC homolog. A recent study by Reddy and coworkers (100) indicated that mutation of *tolC* in *X. fastidiosa*

ABC: ATP binding cassette

causes a severe loss of virulence in grape. Furthermore, viable cells of *tolC* mutants were not recovered after inoculation into grape xylem, strongly indicating that an efflux pump in which TolC is involved is critical to the survival of this pathogen in the xylem. Significantly, the *tolC* mutant of *X. fastidiosa* was more sensitive to toxic phytochemicals such as berberine and rhein, the wetting agent Silwet L-77, and crude plant homogenates in culture than is the wild-type strain (100). However, the *tolC* mutant exhibited similar survival as the wild-type strain in isolated xylem sap, which calls into question whether toxic compounds circulate in the xylem sap in intact plants. Since *X. fastidiosa* is strictly xylem-limited, it remains unclear whether it might trigger plant innate defense responses in response to PAMPS such as lipopolysaccharide, elongation factor, etc., which it apparently produces. Further analysis of host responses to *X. fastidiosa* infection by transcriptional profiling and other methods should clarify if such responses characterize interactions with *X. fastidiosa*. Still to be determined is whether *X. fastidiosa* utilizes these different Type I secretion systems to secrete effectors or toxins to elicit disease symptoms and modulate plant defense responses or whether they may be merely necessary to overcome the plant's preformed chemical defenses.

Type II Secretion System of *X. fastidiosa* and its Effectors

The remarkable similarity in the Type II secretion system in *Xanthomonas* and in *X. fastidiosa* suggests that this system may play a role in virulence of *X. fastidiosa* as it does in other pathogens. The Type II secretion system is important for the virulence of a number of plant pathogenic bacteria including *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae* (43, 66, 98). The Type II secretion system in *Xanthomonas* species (often called *Xps*, *Xanthomonas* protein secretion) is largely involved in the secretion of extracellular enzymes required for the hydrolysis of different component of the plant cell wall. A variety of such extracellular enzymes are secreted by *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae*, including polygalacturonate lyase, endoglucanase, protease, α amylase, lipase/esterase, and cellobiosidase [for detailed review see (68)]. Comparison of the genome of *X. fastidiosa* with that in *Xanthomonas* pathovars revealed that it has all of the essential genes required for Type II secretion (68, 111, 121). Mutation of individual genes encoding different Type II-secreted effectors in *X. oryzae* pv. *oryzae* does not result in a substantial reduction in virulence compared to the wild-type strain whereas mutation of multiple Type II effectors is additive, and results in a much greater reduction in virulence (68, 96). This reduction in virulence may be due to the functional redundancy of these secreted extracellular enzymes in degrading different plant cell wall components. *X. fastidiosa* has a complete set of *Xps* homologs and also harbors a number of genes capable of encoding various extracellular enzymes such as several β-1,4 endoglucanases, xylanases, xylosidases, and one polygalacturonase (111, 121). These putative Type II-secreted effectors are likely involved in degradation of different components of pit membranes in a synergistic manner. Mutation of components of the Type II secretion system of *Xanthomonas* pathovars affects their virulence and growth in plants since it results in severely reduced secretion of extracellular cell wall-degrading enzymes. Whether mutation in the *xps* homologs of *X. fastidiosa* has any effect on its virulence and fitness is still to be determined. However, a polygalacturonase (*pglA*) mutant of a PD strain of *X. fastidiosa* was recently shown to be greatly reduced colonization and pathogenicity in grapevines (102). This may not be surprising as *X fastidiosa* has only one *pglA* ortholog in its genome, hence mutation in the single *pglA* gene would be expected to lead to a major virulence deficiency if polygalacturonase was central to virulence of this species. The *pglA* mutant was impeded in long-distance movement along the grapevine xylem vessel. This deficiency in movement might be attributable to inability of the cells of the *pglA* mutant to degrade pit membranes that restrict both lateral and longitudinal movement of the

pathogen between xylem vessels. It has been hypothesized that *X. fastidiosa* requires such enzymes to enable intervessel migration, as the pore size of intact pit membranes of grape are too small to allow passive movement of the bacteria (114). Analysis of the population size in xylem vessels indicated that the *pglA* mutant was deficient in growth inside the grape xylem vessels. As *X. fastidiosa* must digest pectin in pit membranes to enable its spread through the plant, the digested pectin is also likely used as nutrient source that would supplement the low amounts of nutrients available in the xylem sap (102). The digestion of pectin may also expose other cell wall polysaccharides that could then be digested by other extracellular enzymes such as glucanases and xylanases. The reduced virulence of *X. fastidiosa* in grape harboring a cloned polygalacturonase-inhibiting protein from pear (1) is consistent with the concept that pit membrane digestion is central to the movement of the pathogen. The polygalacturonase (PG) ortholog in the CVC strain and in some coffee strains of *X. fastidiosa* has a frameshift mutation (121). It has been hypothesized that the apparent lack of PG (polygalacturanase) in the *X. fastidiosa* CVC strain might be responsible for its less aggressive nature as compared with grape strains (121).

A detailed analysis of the genome sequence of different *X. fastidiosa* strains revealed that this pathogen has limited and simple aerobic as well as anaerobic respiratory pathways (15, 16). Based on the genomic analysis, it was suggested that *X. fastidiosa* is able to use only a few sugars (glucose, fructose, mannose, ribose, N-acetylglucosamine), cellulose, as well as glycerol. Further analysis of its genome indicated that this bacterium is unable to degrade organic acids such as D- or L-lactate, malonate, propionate, etc., and these predictions have been verified in studies of its growth in minimal media (6, 20). However, the organic acids that enter the tricarboxylic acid cycle, such as malate, oxaloacetate, citrate, or fumarate, can be degraded by this bacterium. Genome analysis also supported the idea that *X. fastidiosa* can utilize cell wall breakdown products as a possible carbon source; it contains genes that putatively encode enzymes to degrade polysaccharides such as cellulose and galacturonans such as xylan, rhamnan, and arabinan. The genome of *X. fastidiosa* also encodes putative sugar transport systems (16, 79, 121), which further support the concept that cell wall-derived sugars are consumed by this pathogen.

Motility and Virulence

The processes by which *Xanthomonas* pathovars and *X. fastidiosa* move within plants differ substantially. Flagellar-driven chemotaxis and motility have been implicated in the colonization in *X. oryzae* pv. *oryzae* (107). The flagellar mutant (FlgF$^-$) of *X. oryzae* pv. *oryzae* was, however, not deficient in virulence when applied topically or by wound inoculation of rice leaves. This *flgF* mutant was also not completely deficient in motility, which may account for its retention of virulence? This may be due to the presence of other motility mechanisms such as twitching, which would compensate for the loss of flagella driven-motility within the plant. Although *X. fastidiosa* is a nonflagellated bacterium, it is efficient in the colonization of plants and even upstream movement in the xylem of grape. Genome analysis of *X. fastidiosa* revealed the presence of several gene orthologs that may encode proteins involved in biogenesis and function of Type IV pili (111, 121). Recently it has been shown that grape strains of *X. fastidiosa* have functional Type IV pili that are located only at one pole of the cell (80). These long pili are primarily implicated in twitching motility and migration. Mutation in the Type IV pili genes *pilB* and *pilQ* of *X. fastidiosa* causes loss of twitching motility and inhibition of basipetal movement in planta. In addition to long pili, *X. fastidiosa* also possess short pili (Type I pili), which are involved in attachment and biofilm formation. The Type IV and Type I pili appear to have opposite effects on the movement and biofilm formation of *X. fastidiosa*, indicating that these processes oppose each other. A *fimA* (Type I pilus) mutant of *X. fastidiosa* exhibited twitching

motility that was even more active than that of the wild-type strain but the mutant was unable to form a biofilm in vitro. In contrast, the Type IV pili mutants (*pilB* and *pilQ*) were more proficient in forming biofilm then the wild type. The presence of both long (Type IV) and short (Type I) pili on the wild-type *X. fastidiosa* indicates distinct functional roles for the two different classes of pili. These results further indicate that short pili are important for attachment and biofilm formation. *X. fastidiosa* may possibly maintain a fine balance of the distribution of long and short pili, depending on different stages of its colonization in the plant host or the insect vector. For example, initial attachment of *X. fastidiosa* to the insect vector may require strong attachment contributed by Type I pili to counter the rapid xylem flow through the mouthparts during insect feeding. In contrast, cells must be free to move as they multiply in the plant and utilize long pili for vessel-to-vessel spread by twitching motility; Type I pili would be expected to inhibit this process. A FimA mutant of *X. fastidiosa* exhibited enhanced basipetal movement from the point of inoculation in grape compared to the wild-type strain (80).

A mutation in the *cheA* gene of *Pseudomonas fluorescens*, which is required for flagella-driven chemotaxis, affects its colonization of different parts of the root system of plants (124). Recent genome and functional analysis of *X. fastidiosa* revealed that the bacterium also contains a complex chemosensory system that may control Type IV pilus–driven twitching motility. *X. fastidiosa* twitching motility was shown to be controlled by a signal transduction pathway consisting of the *pilG-chpC* cluster, which is highly homologous to chemosensory systems controlling flagella rotation in several other bacteria including *Pseudomonas aeruginosa* (19). Two of the chemosensory signal transduction pathway genes (*pilL* and *cheY2*) in *X. fastidiosa* have been recently characterized (19). Both *cheY2* and *pilL* are predicted to be part of a putative chemosensory system regulating twitching motility in *X. fastidiosa*. The *pilL* mutant of *X. fastidiosa* lacks twitching motility, and the *cheY2* mutant exhibited an aggregated pattern of motility in the form of large clumps that moved more slowly than the wild-type cells. The *cheY2* mutant was also less efficient in forming a biofilm in vitro than was the wild-type strain. Both Type I and Type IV pili were observed on the *cheY2* mutant of *X. fastidiosa*, indicating that the lack of twitching of the mutant is due to deficiency in initiating the signal transduction cascade associated with pilus retraction (19). While these regulators are apparently required for motility per se, whether *X. fastidiosa* exhibited true chemotactic movement toward particular compounds remains to be shown.

EPS: extracellular polysaccharides

Extracellular Polysaccharide Genes

Extracellular polysaccharides (EPS) play important roles in the virulence and biofilm formation of several plant pathogenic bacteria (37). In the *Xanthomonas* group of plant pathogens such as *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae*, the extracellular polysaccharide is called xanthan gum and the genes encoding enzymes required for its biosynthesis are designated as *gum* genes. A cluster of 12 genes (*gumB* to *gumM*) encodes enzymes involved in the synthesis and polymerization of xanthan gum (28, 72). Mutations in many genes in the gum operon of *Xanthomonas* cause a loss of virulence on different hosts (25, 38). *X. fastidosa* contains homologs of 9 of the 12 *gum* genes in different *Xanthomonas* strains, but does not harbor *gumI*, *gumJ*, and *gumL* homologs, which are required for addition of terminal mannosyl residues to xanthan gum in *Xanthomonas* (111, 121). Thus it has been predicted that *X. fastidiosa* makes a xanthan EPS similar to *Xanthomonas*, but one lacking the terminal mannosyl residue (29). Recently, Roper and coworkers (101) demonstrated that a PD strain of *X. fastidiosa* produces such an exopolysaccharide both in vitro and in planta, using antibodies specific to the EPS isolated from a *gumI* mutant of *X. campestris* pv. *campestris* that was expected to produce an EPS similar to that produced by *X. fastidiosa*. However, wild-type *X. fastidiosa*

strains produced very little EPS as compared to *X. campestris* (which produces copious amounts of EPS). The low levels of production were suggested to be associated with the fastidious nature of the bacterium when grown in rich complex medium (101). This group observed that EPS of *X. fastidiosa* was usually closely associated (colocalized) with cell aggregates found in the xylem vessels of grape plants. However, they also occasionally observed that *X. fastidiosa* EPS was found in the xylem vessels but not associated with GFP-producing bacterial cells. They speculated that this may be due to EPS being carried away from the biofilm by the xylem sap. It is also possible that cells of *X. fastidiosa* may die and subsequently lyse, thus releasing the GFP marker proteins in heavily colonized vessels (see **Figure 1**), and thereby obscuring the fact that the EPS may have been associated with dead cells. As *X. fastidiosa* produces enzymes like endoglucanases, it is also possible that they could be involved in the degradation of the β-1,4-glucan backbone of its own EPS. In *X. axonopodis* pv. *citri* EPS plays an important role in forming a structured biofilm on leaves (117). There is no report of mutational studies of the *gum* genes in grape strains of *X. fastidiosa* (108), whereas *gumB* and *gumF* mutants have been constructed in the CVC strain (113). These *gumB* and *gumF* mutants were reduced in capacity to form a biofilm in culture but were still able to attach to surfaces, indicating that EPS is involved in biofilm maturation rather than in initial attachment. It will be interesting to see the behavior of EPS-deficient mutants of *X. fastidiosa* in pathogenicity, and colonization of insect vectors.

Adhesins and Hemagglutinins

Analysis of the genome sequences of several *X. fastidiosa* strains reveals that this pathogen has a surprisingly large numbers of adhesins and hemagglutinin-encoding genes. This may reflect the pathogen's complex lifestyle in both the plant host and in insect vectors, where different adhesins may be required for interaction with different substrates. Interaction with a different cell surface might be a crucial initial step in biofilm formation. These adhesins may also contribute to cell-to-cell attachment, also an important component of biofilm development. Genome analysis of the grape strain Temecula of *X. fastidiosa* indicated that it has at least seven ORFs (PD0986, PD0988, PD1246, PD1792, PD2110, PD2116, and PD2118 with the potential to encode hemagglutinins (121), although several of these ORFS are apparently truncated and may not be functional. In a screen for *X. fastidiosa* mutants altered in virulence, Guilhabert & Kirkpatrick (52) identified several mutants that were more virulent than the parental strain. Of these one was a mutant in PD2118 (*hxfA*). Insertional disruption of the other hemagglutinin gene PD1792 (*hxfB*) also resulted in a hypervirulent phenotype. Both *hxfA* and *hxfB* mutants were altered in cell-cell aggregation, and also moved further in grape xylem vessels than the wild-type strain. Study of their attachment to glass surfaces revealed that the *hxfA* mutant was only slightly reduced in attachment compared to the wild-type strain, because another hemagglutinin such as *hxfB* may have a redundant function to that of *hxfA*. Loss of stickiness has been postulated as a possible reason for their hypermovement and increased spread along the xylem vessel and ability to exhibit more disease than the wild-type strain. It has been hypothesized that both *hxfA*- and *hxfB*-encoded hemagglutinins facilitate cell-cell aggregation as well as cell-surface interactions. As such, the expression of these adhesins might be expected to attenuate the virulence of *X. fastidiosa* by enhancing its attachment to surfaces, thus limiting its colonization abilities, which, in turn, reduces the number of xylem vessels that might be colonized. It would be interesting to know the role of the other hemagglutinins of *X. fastidiosa* in virulence to grape, as well as their contribution to insect acquisition and transmission to the new host plant as adhesion to surfaces is probably important in both processes.

X. fastidiosa produces several other fimbrial and afimbrial adhesins besides hemagglutinins. Expression analysis has revealed that *fimA*,

which encodes a fimbrial adhesin, was upregulated in the CVC strain of *X. fastidiosa* during biofilm formation (35). A recent study (47) revealed that both *fimA* and *fimF* mutants (encoding Type I fimbrial adhesins) of *X. fastidiosa* adhered to glass surfaces less efficiently than the wild-type strain and that these cells occurred primarily as solitary cells and not as cell aggregates, unlike the wild-type strain. This suggested that the Type I pili have a particularly important role in cell-to-cell aggregation. In *Xanthomonas* species, a non-fimbrial adhesion called *xadA* plays an important role in virulence and colonization of rice plants (97). The *Xanthomonas xadA* was also shown to be expressed in minimal medium that closely mimics the environment inside xylem vessels. A *xadA* mutant exhibited an opaque colony morphology, which suggested that it may be involved in the interaction with extracellular polysaccharides. *Xanthomonas* species also have a homolog of an afimbrial adhesion called *xadB*, whose function is not yet known. The attachment to surfaces appears to involve the coordinated contribution of several adhesins. For example, *xadA* and the *hxfB*-afimbrial mutants both exhibited a much lower frequency of adherence to glass surfaces than did the wild-type strain (47). Although *fimA* and *fimF* mutants did not form cell aggregates on glass surfaces when incubated as individual strains, aggregates containing *fimA* or *fimF* mutants were observed when coinoculated with either *xadA* or *hxfB* mutants, respectively (47). These results indicate that FimA and FimF are involved preferentially in cell-cell aggregate formation whereas the afimbrial adhesions such as XadA and HxfB contribute to initial cell binding to surfaces.

DSF: diffusible signaling factor

Cell-Cell Signaling Mediated by Small Molecules

X. fastidiosa shares with several other plant pathogenic bacteria the ability to coordinate gene expression in a cell density–dependent fashion via the production of small signal molecules. In *Xanthomonas* species such as *X. campestrsis* pv. *campestris* and *X. oryzae* pv. *oryzae*, cell-to-cell signaling is mediated by a small diffusible signaling molecule called DSF (Diffusible Signaling Factor). DSF is synthesized by RpfF, which exhibits similarities to long-chain fatty acyl CoA ligases (13, 22, 49). The *rpfF* gene in *X. campestris* pv. *campestris* is within a cluster of nine genes (*rpfA* to *rpfI*). Analysis of genome sequences of different *X. fastidiosa* strains (111, 121) revealed the presence of remarkable synteny and similarity of genes in the rpf cluster to those of *Xanthomonas* strains (28, 72) (**Figure 3**). The most striking difference between the rpf cluster of *Xanthomonas* and *X. fastidiosa* is the absence of

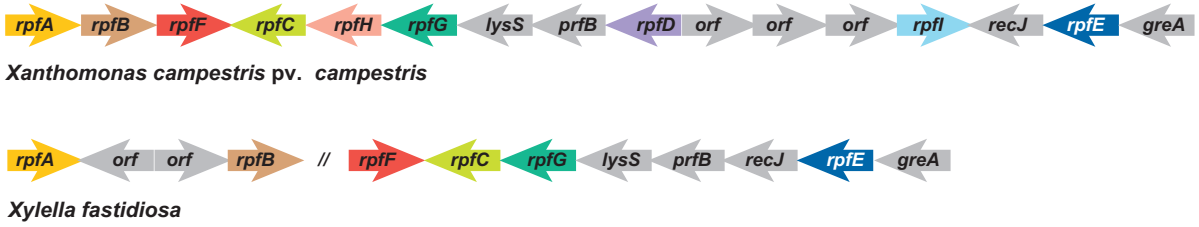

Figure 3

Schematic representation of synteny between the *rpf* gene cluster of *Xanthomonas campestris* pv. *campestris* and *Xylella fastidiosa*. The homologous genes in the *rpf* clusters of *X. fastidiosa* and *X. campestris* pv. *campestris* are indicated in identical colors. Unlike in *X. campestris* pv. *campestris*, *rpfA* and *rpfB* are located at different genomic loci separate from the main *rpf* cluster. *rpfF*, *rpfC*, *rpfG*, and *rpfE* have a similar organization as in *X. campestris* except that *X. fastidiosa* lacks *rpfH*, *rpfD*, and *rpfI*. *rpfF* encodes for the DSF synthase. *rpfB* exhibits homology to long-chain fatty acyl CoA ligase and may be involved in DSF synthesis (13). *rpfC* encodes for the hybrid two-component sensors, which are predicted to participate with RpfG (response regulator), which is part of the two-component DSF signal transduction cascade. *rpfF* encodes for an aconitase, which also influences extracellular enzyme and EPS production in *X. campestris* pv. *campestris*.

rpfH, *rpfD* and *rpfI* in the latter. Also, *rpfB* and *rpfA*, which are within the *rpf* cluster of different *Xanthomonas* strains (28, 41, 111, 121), are located in a separate region of the *X. fastidiosa* genome (**Figure 3**).

The structure of the DSF made by a citrus strain of *X. fastidiosa* has been proposed to be 12-methyl-tetradecanoic acid (110), whereas that of *X. campestris* pv. *campestris* strain XC1 was determined to be *cis*-11-methyl-2-dodecenoic acid (122). Although the proposed structure in *X. campestris* pv. *campestris* has been verified by complementation of different DSF-dependent phenotypes with the chemically synthesized compound, the proposed DSF structure from the CVC strain of *X. fastidiosa* has yet to be functionally verified with a chemically synthesized analog. Nor is it known whether the DSF produced by different strains of *X. fastidiosa* is the same, but the DSF made by grape strains of *X. fastidiosa* appears to be different from that made by *X. campestris* pv. *campestris* (88).

Many genes in the *rpf* signaling system are common to several plant pathogenic bacterial species, although the patterns of gene expression that they mediate and the traits that they control differ substantially among taxa. Disruption of *rpfF* (encoding DSF synthase) and *rpfC* (hybrid two-component DSF sensor) leads to different phenotypes in different *Xanthomonas* species as well as in *Stenotrophomonas*, an animal pathogen (**Table 1**). This strongly suggests that although different closely related bacteria share a common *rpf*-mediated signaling system, they regulate different phenotypes, consistent with their varied lifestyles. Studies of the role of DSF in virulence of *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae* indicated that although the genes in the *rpf* cluster shared significant homology and organizational similarity, they differ in their role in virulence. DSF is involved in regulating production of virulence-associated functions such as EPS and extracellular enzymes in *X. campestris* pv. *campestris* whereas in *X. oryzae* pv. *oryzae*, it is involved in iron uptake (**Table 1**). *rpfF* and *rpfC* mutants of *X. axonopodis* pv. *citri* exhibit differential virulence phenotypes, with the *rpfC* mutation having a more pronounced effect on virulence than that of the *rpfF* mutation (**Table 1**) (109). Cell-cell signaling–deficient *rpfF* mutants of *X. fastidiosa* were deficient in colonizing insect vectors and therefore were not transmitted to new hosts (88). However, the *rpfF* mutant of *X. fastidiosa* exhibited hypervirulence when needle-inoculated in grapevines (88), producing more extreme and rapid symptoms. A recent study (S. Chatterjee & S.E. Lindow, unpublished information) indicated that an *rpfF* mutant of *X. fastidiosa* colonized over threefold more xylem vessels than the wild-type strain. Furthermore, the colonized vessels were much more likely to be blocked by cells of the *rpfF* mutant than the wild-type strain; over fourfold more vessels were blocked by the *rpfF* mutant than in the wild-type strain. This likely explains the hypervirulence phenotype of the *rpfF* mutant as it has been previously reported that vessels are blocked by *X. fastidiosa* at a higher frequency in symptomatic leaves exhibiting typical PD symptoms than in asymptomatic leaves (87).

Mutants of *X. fastidiosa* blocked in expression of the DSF sensor *rpfC* exhibit a phenotype opposite to that of DSF-deficient *rpfF* mutants (**Table 1**) (23, 88). The *rpfC* mutants of *X. fastidiosa* were deficient in virulence as well as in longitudinal migration along the xylem vessel. Such strains thus attained much lower population sizes in grape stems at a given distance from the point of inoculation than the wild-type strain, which in turn, had lower population sizes than the hypervirulent *rpfF* mutants. Furthermore, the *rpfC* mutants could be acquired by insects upon feeding on infected plants but were somewhat deficient in insect transmission (**Table 1**) (23).

Studies using mutational and biochemical analyses and computational modeling have enabled a pathway for the DSF-dependent signal transduction in *Xanthomonas* to be proposed (10, 40, 53–55, 112). The DSF synthase protein RpfF is believed to be involved in the synthesis of DSF as well as to interact with RpfC, the putative DSF sensor. Binding of DSF to the signal receiver domain of RpfC mediates a conformational change in this sensor, causing release of

Table 1 Phenotypes associated with mutation in different *rpf* components of various DSF-producing bacteria

DSF producing strains	Phenotypes due to mutations in different *rpf* genes			Reference
	rpfF	*rpfC*	*rpfG*	
Xanthomonas campestris pv. *campestris* (*Xcc*)	Virulence deficient	Virulence deficient	Virulence deficient	13, 112, 117
	Extracellular polysaccharide (EPS) deficient	EPS deficient	EPS deficient	
	Deficient in Extracellular enzymes-endoclucanase, polygalacturonase	Deficient in Extracellular enzymes and EPS	Deficient in extracellular enzymes and EPS	
Xanthomonas oryzae pv. *oryzae*	Deficient in virulence	Proficient in in planta growth but deficient in symptom development	n.d	22, 115
	EPS proficient	EPS deficient	n.d	
	Proficient in extracellular enzymes such as xylanase	Proficient in extracellular enzyme production	n.d	
	Deficient in iron uptake	n.d	n.d	
Xanthomonas axonopodis pv. *citri*	Deficient in virulence	Deficient in virulence; deficiency more severe compared to the *rpfF* mutant	n.d	109
	Deficient in EPS production	Deficient in EPS production	n.d	
	Deficient in extracellular enzymes such as endoglucanase and protease	Deficient in extracellular enzymes such as endoglucanase and protease	n.d	
Xylella fastidiosa (PD strain-Temecula)	Hyper virulent on grape	Reduced virulence on grape	n.d	23, 88
	Deficient in insect colonization and transmission	Able to colonize insect but deficient in transmission	n.d	
	Deficient in biofilm formation and attachment compare to the wild type strain	Proficient in in vitro attachment and biofilm formation then the wild type strain	n.d	
Stenotrophomonas maltophilia	Effected in iron uptake and motility	n.d	n.d	49, 67
Strain WR-C	Proficient in extracellular protease production	n.d	n.d	
	Proficient in biofilm production	n.d	n.d	
	Reduced motility and production of extracellular protease	n.d	n.d	
Strain K279a	Reduced tolerance to antibiotics and heavy metals	n.d	n.d	
	Deficient in microcolony formation (biofilm)	n.d	n.d	

*n.d indicates not determined.

RpfF. This also initiates phosphorylation and phosphor-transfer to the cognate response regulator RpfG, which in turn positively regulates expression of virulence-associated genes such as those responsible for extracellular polysaccharide synthesis, endoglucanase, protease, etc. While sequence analysis of the RpfC homolog of *X. fastidiosa Temecula* revealed interesting differences from that in *Xanthomonas* species and other closely related *X. fastidiosa* strains, the significance of these differences is not clear. The RpfC of *X. fastidiosa* (Temecula strain), like that of citrus strain 9a5c, appears to be truncated at the N-terminus (23). Recently, three protein domains (GGDEF, EAL, and HD-GYP) have been implicated in the modulation of cyclic di-GMP, which acts as an intracellular signaling molecule (33, 42, 67, 104). These refer to conserved domains based on the presence of the conserved consensus sequences GGDEF (G, glycine; D, aspartic acid; E, glutamic acid; F, phenyl alanine), EAL (E, glutamic acid; A, alanine; L, leucine), and HD-GYP (H, histidine; D, asparic acid; G, lycine). In *Xanthomonas* it has been shown that RpfC-RpfG two-component systems interact with other two-component systems such as NtrBC as well as with several other GGDEF domain proteins (10). The RpfC protein of *X. campestris* pv. *campestris* also has a functional HD-GYP domain involved in cyclic di-GMP degradation (104).

Expression analysis of selected virulence genes such as *tolC*, *pglA*, and various fimbrial and afimbrial adhesins (36, 52, 100, 102, 108) in *X. fastidosa* revealed that DSF-dependent gene regulation is more distinct in this species than in *Xanthomonas* strains (23, 115). This finding explains why *rpfF* and *rpfC* mutants of *X. fastidiosa* have opposite phenotypes. The collection of fimbrial and afimbrial genes required for cell-cell attachment and attachment to surfaces are positively regulated by DSF (23, 123) (**Figure 4**). These results are consistent with those from studies of DSF-deficient *rpfF* mutants and DSF-overproducing *rpfC* mutants, suggesting that DSF accumulation promotes biofilm formation in *X. fastidiosa*. The proportion of the *X. fastidiosa* population that formed a biofilm in cultured cells was higher in *rpfC* mutants than for wild-type cells but lower for *rpfF* mutants than for wild-type cells (23). Such results are consistent with the observation that *rpfF* mutants of *X. fastidiosa* did not form a biofilm in sharpshooter vectors after acquisition from infected plants (88). On the other hand, the expression of genes such as *tolC* and *pglA* is dependent on the presence of both RpfF and RpfC and a putative repressor protein. DSF can be sensed by an intracellular or a low-affinity membrane-localized sensor and can positively regulate the synthesis of different adhesins (**Figure 4**). Analysis of the genome sequence of *X. fastidiosa* indicated the presence of several GGDEF- EAL domain proteins (PD1994 and PD1617). PD0279 has a GGDEF domain but not an EAL domain, indicating that it may have a dedicated function for cyclic di-GMP synthesis. Expression analysis indicated that the production of this protein is strongly regulated by DSF (23). It seems reasonable to conclude that DSF-dependent signaling involves the modulation of cyclic di-GMP levels in *X. fastidiosa* (**Figure 3**). Preliminary results of studies of mutants blocked in production of this GGDEF domain protein indicate that it is involved in attachment and biofilm formation (S. Chatterjee & S.E. Lindow, unpublished information). DSF likely couples cyclic di-GMP levels by regulating the expression and/or interaction of various GGDEF, EAL, and HD-GYP domain proteins with different RPF components, in controlling various steps of the *X. fastidiosa* colonization in insects as well as plants (**Figure 4**).

INSECT VECTOR BIOLOGY AND TRANSMISSION OF *X. FASTIDIOSA*

Traditionally, insect-borne plant pathogens are grouped based on the results of transmission bioassays that determine temporal aspects of vector-pathogen associations (see 86 for review). The transmission of *X. fastidiosa* is unique among plant pathogensin that it multiplies within vectors (61) but is transmitted without

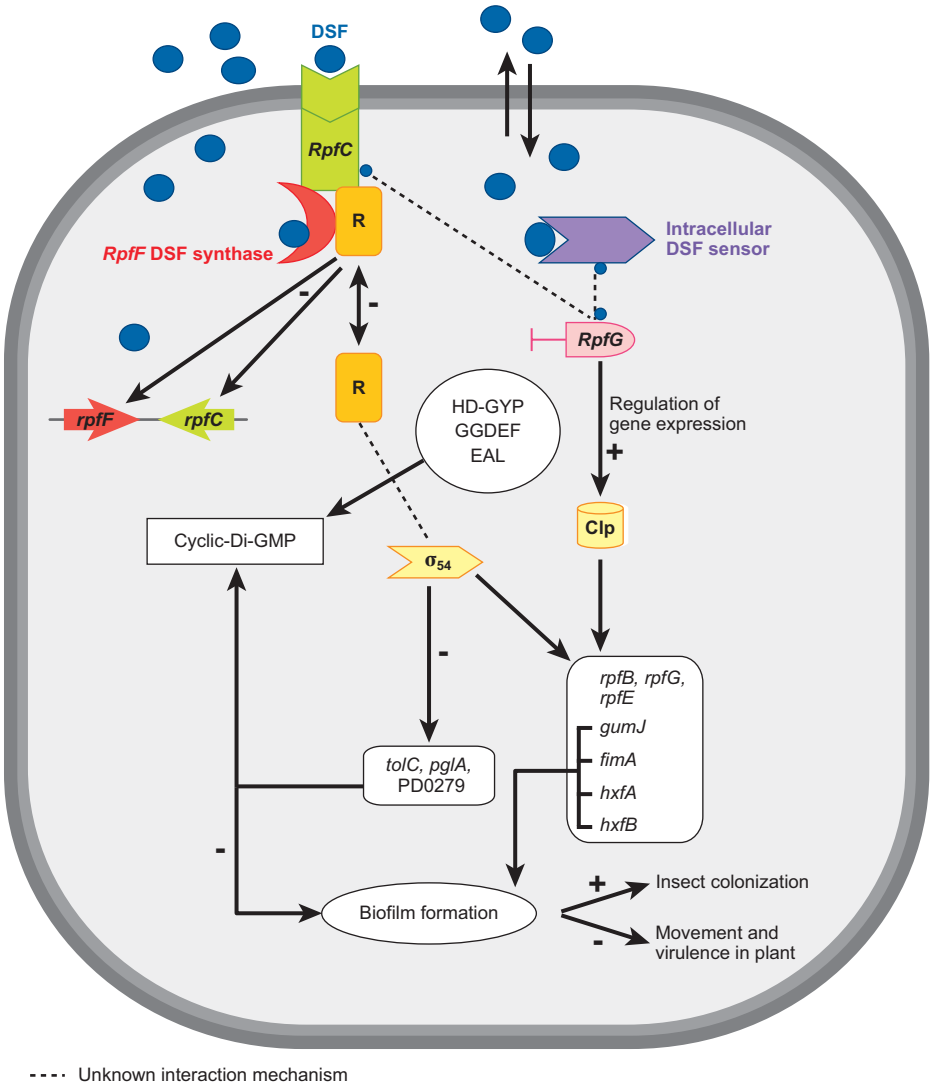

Figure 4

A proposed model for DFS-mediated cell-cell signaling in *Xylella fastidiosa*. *rpfF* encodes the DSF synthase. *rpfC* encodes a hybrid two-component sensor of DSF. DSF levels negatively regulate the expression of *rpfF* by negative feedback. DSF binding to a two-component intracellular DSF sensor leads to autophosphorylation and phosphorelay to a two-component response regulator, likely RpfG, which positively regulates genes required for attachment and biofilm formation (*hxfA*, *hxfB*, *fimA*, and *gumJ*) by modulating transcriptional regulators like-sigma 54 or Clp (10, 23, 54, 55). As in *Xanthomonas*, RpfC likely interacts with RpfF while both RpfF and RpfC may interact with a putative intracellular repressor "R" (which might be also an anti sigma factor) and also with several proteins with GGDEF, EAL, and HD-GYP domains (10). DSF binding to the RpfC or to the intracellular DSF sensor influences interaction with different cyclic-Di GMP modulating proteins containing GGDEF, EAL, or HD-GYP domains. The amount of free "R" would be dependent on the levels of RpfF, RpfC, and cyclic Di GMP as well as DSF. Free repressor would repress virulence genes such as *tolC*, *pglA*, and PD0279 (encoding GGDEF domain protein), which are required for extensive colonization and movement of *X. fastidiosa* in the plant.

the requirement of a latent period (91). Important breakthroughs in our understanding of *X. fastidiosa* transmission occurred in the 1940s, when xylem sap-feeding insect specialists were shown to transmit the pathogen; those insects include sharpshooter leafhoppers (Hemiptera, Cicadellidae) and spittlebugs (Hemiptera, Cercopidae) (105, 106). Other leafhopper subfamilies and hemipteran families not specialized in xylem sap have been tested but never shown to transmit *X. fastidiosa* (95, 105). This is an intriguing aspect of *X. fastidiosa* transmission biology, as insects in those groups occasionally feed on xylem sap (85). No studies have determined if *X. fastidiosa* colonizes these insects, but comparative studies on insect probing behaviors and morphology and pathogen colonization patterns may yield valuable information on *X. fastidiosa* transmission. The ecology of *X. fastidiosa* sharpshooter vectors and its transmission biology have been recently reviewed (5, 99).

Unlike most vector-borne plant pathogens, the lack of vector species-*X. fastidiosa* strain specificity is a remarkable characteristic of this system (5, 50). Essentially, all sharpshooter species are likely capable of transmitting all *X. fastidiosa* strains, albeit with variable transmission efficiencies. Efficiency seems to be associated more with bacterial populations within plants and vector-plant interactions than with vector-*X. fastidiosa* interactions (63). Work on the transmission of a South American isolate of *X. fastidiosa* by a North American vector is an example of this aspect of transmission (32). This strongly suggests that the mechanisms of transmission, yet to be determined, are highly conserved across vector species and strains of *X. fastidiosa*.

There are three essential steps on *X. fastidiosa*'s vector transmission to plants. First, the pathogen must be acquired from an infected plant. Retention is the second step, in which *X. fastidiosa* must attach to the cuticle of insects, followed by colonization of that surface. Lastly, vectors inoculate the pathogen into a susceptible host generating a new infection. Vectors can acquire *X. fastidiosa* from an infected plant and immediately inoculate it into a new host (91); thus colonization is not a requirement for transmission and there is no latent period. In addition, infectivity is lost after nymphal stages of vector molt. Since the cuticular lining is shed during each molt, the site of *X. fastidiosa*'s attachment and vector colonization is likely inthe foregut (7, 91). Infectivity is not lost in adults (which do not molt), and because *X. fastidiosa* multiplies within its vectors, the insects are able to transmit the pathogen to plants for months after acquisition from an infected plant (61, 105).

The foregut of leafhoppers is composed of modified mouthparts that, among many things, form a syringe-like apparatus (food canal) to suck sap from xylem vessels (73). Sap is pumped into a chamber (cibarium) through the food canal when insects contract muscles attached to a membrane sealing it, creating a low pressure environment that allows fluid to flow into the cibarium (92). Connecting the food canal to the cibarium is a narrow canal named the precibarium; in this canal a small valve (precibarial valve) is present (11). Once the cibarium is full with sap, the cibarial muscles relax and the membrane collapses pushing fluid into the midgut. The precibarial valve likely functions as a check-valve preventing sap from flowing back into plants. Muscle contraction and pumping seem to occur once every few seconds while insects are feeding, ensuring a very turbulent environment. *X. fastidiosa* has been observed in the precibarium and cibarium of vectors (4, 8, 18, 87, 88, 92). Although probing behaviors and the mechanism of *X. fastidiosa* inoculation are yet to be determined, correlation-based experiments indicate that cells are inoculated into plants from the precibarium (8). Indirect evidence from transmission experiments suggests that an insect-associated probing behavior is responsible for inoculation events (5, 9).

The precibarium is a canal of approximately 20 μm in diameter and 150 μm in length in the case of sharpshooters, although dimensions are variable and species dependent (12). Therefore, there is a physical limitation to the number of cells that can be present at the inoculum

site in the foregut. Flow of sap in the precibarium has been estimated to be >5 cm/s (91), suggesting that cells must be well attached to the cuticle in an environment of extreme velocity. As sharpshooters can ingest over a hundred times their body weight per day (82), attachment is not a trivial process. Nevertheless, *X. fastidiosa* seems to have the machinery necessary to colonize such an environment, as it has been suggested that its Type I pili may serve as an anchor-like system to permit attachment in fast fluid flow conditions (34). Precibarium colonization seems to occur in a stepwise fashion, with early colonizers attached sideways and late colonizers, which have multiplied in the foregut, attached polarly and forming a monolayer (8). These observations suggest that *X. fastidiosa* colonizes vectors similarly to other biofilms, although details are lacking. Polar attachment may be a strategy allowing a larger number of cells to colonize the precibarium and increase the cell surface area available for nutrient uptake; cells must be able to acquire nutrients from an extremely dilute solution under what are likely very turbulent and fast flow conditions.

The number of bacteria in an inoculum is an important factor for successful host infection for many plant pathogens. Mechanical inoculation of plants with *X. fastidiosa* increases in efficiency with increases in the number of cells in inoculated bacterial suspensions (90). However, mechanical inoculation is dramatically different from vector-borne processes, because high concentrations of bacteria are used in the inoculum and the delivery process destroys plant tissue. Furthermore, although vessel embolism occurs during mechanical inoculation, its occurrence during the insect's probing is yet undetermined. Because there is no latent period for *X. fastidiosa* transmission, number of bacteria within insects is thought not to be associated with transmission efficiency. The fact that the number of cells within vectors was not associated with plant infection supports this hypothesis (M. Daugherty & R.P.P. Almeida, unpublished information). However, the number of infective individuals was significantly related to plant infection and bacterial colonization rates, suggesting that a potentially larger number of inoculation events generate larger numbers of infections that develop faster than those based on single individuals or large inoculum (M. Daugherty & R.P.P. Almeida, unpublished information).

The lack of vector-strain specificity suggests that sharpshooters do not benefit from this association. For *X. fastidiosa*, which has a wide host range, it is beneficial to be associated with polyphagous insects that visit many plants during their lifetime. On the other hand, colonization of the precibarium may affect vector feeding. Vectors also avoid plants with symptoms of *X. fastidiosa* infection (76), indicating that those are not preferred hosts. In certain cases of other insect-borne plant pathogens, the insects either directly benefit from carrying the pathogen (44) or are attracted to and have fitness benefits provided by symptomatic plants (17, 48).

TRAITS OF *X. FASTIDIOSA* THAT IMPACT ITS TRANSMISSION TO PLANTS BY VECTORS

Our understanding of the biology of *X. fastidiosa* is hampered by lack of knowledge on *X. fastidiosa*–vector interactions. Biological evidence suggests this is a complex process conserved among taxa. Although much information on how *X. fastidiosa* colonizes plants could be inferred from genome sequences, little information was generated that improved our understanding of how it colonizes insects. Based on those sequences, initial attention was directed to fimbrial and afimbrial adhesins and gum (111) because of the adhesion-related phenotypes or orthologs in other organisms. All such candidates could, and have been, shown to be associated with plant colonization (as discussed above). The availability of microarray gene expression data and the demonstration that DSF controls *X. fastidiosa* attachment to the foregut of vectors and subsequent transmission to plants provided indirect information to develop hypotheses on which genes may be associated with transmission (88). Specifically, genes upregulated by DSF in plants are of

putative importance for transmission, as are genes upregulated in plants compared to rich solid medium (23, 88, 123). *X. fastidiosa* grown on rich solid media is acquired by vectors through artificial feeding systems but is not transmitted to plants (R. P.P. Almeida, unpublished information). Following this approach, Type IV pili were putatively identified as not essential for transmission, whereas hemagglutinin-like proteins and gum were identified as candidates for adhesion to vectors. Further support for these hypotheses was obtained through comparative experiments conducted with *rpfF*- and *rpfC*-mutants of *X. fastidiosa*. *rpfF*-mutants are not colonizers of insects and are not transmitted by vectors, and *rpfC*-mutants colonize vectors but are limited in their ability to be detached from the cuticle (23, 88). However, the use of *rpf* mutants does not allow for the testing of specific genes for transmission, as DSF regulates several pathways and bacterial colonization of vectors is probably similar to the formation of biofilms. Testing of specific *X. fastidiosa* mutants for vector transmission may be difficult, as some mutants do not colonize host plants well (e.g., Type IV pilus mutant, 80) and transmission efficiency can be affected as bacterial numbers (or population size?) within plants are associated with vector efficiency (63). Recentbiochemical approaches have focused on *X. fastidiosa* attachment to polysaccharides (69), and have shown that *X. fastidiosa* surface proteins and not gum are associated with attachment to sugars. Similar tests with knockout mutants of fimbrial and afimbrial adhesins, in addition to DSF mutants, identified hemagglutinin-like proteins as important for initial attachment of cells to vectors (N. Killiny & R.P.P. Almeida, unpublished information). The identification of hemagglutinin-like proteins as an important surface protein for transmission is tentative, however, as *X. fastidiosa* has two copies of the gene and it is as yet difficult to produce double knockout mutations in the same isolate of this pathogen (52). Better characterization of *X. fastidiosa* hemagglutinin-genes is warranted because of the strong phenotypic changes associated with their blockage in plants and insects. Furthermore as and because the copies are orthologs to a *Xanthomonas* adhesion protein (Xad) and have little homology to hemagglutinins they might have other unexpected features that contribute to the behavior of *X. fastidiosa*.

Different genes may be of importance for different steps in the cuticle colonization process. In this hypothesis, three steps for colonization occur: (*i*) initial attachment and early colonization, (*ii*) formation of mature biofilm, and (*iii*) detachment. The formation of a mature biofilm is not required for cell detachment (*X. fastidiosa* exhibits no latent period for transmission by sharpshooters), and thus the turbulent environment within the precibarium may result in occasional detachment of cells at any stage of colonization. However, there is evidence for early and late stages of colonization (8). Cells are initially attached sideways in relation to the surface, often embedded in an extracellular matrix. Cells at higher densities are polarly attached and the matrix, which is certainly present, does not seem to occur in large amounts as in early stages on colonization. In this scenario, hemagglutinin-like proteins could be more important for early colonization and polar fimbrial adhesins may facilitate late colonization and formation of a mature biofilm as in in vitro biofilms. Type I and Type IV pili are associated with *X. fastidiosa* movement on and attachment to surfaces (34), and they may have a role on biofilm formation and/or maturation within insects after initial attachment mediated by other proteins. Testing these hypotheses in situ will be challenging, but we predict that results will show that vector colonization is a complex stepwise process mediated by cell-cell signaling as cell densities increase and biofilms change morphology. However, the role of DSF and attachment proteins has not been studied in situ and may differ from in vitro and in planta observations, as environmental conditions are dramatically different within leafhoppers. The development of artificial feeding systems that allow for *X. fastidiosa* to acquired by the insect in vitro would permit

better understanding of these interactions by eliminating the need for infected plants as a source of inoculum as mutants often differ from the wild type in their colonization patterns, impacting initial acquisition by the vector.

A MODEL OF DISEASE CAUSATION AND INSECT TRANSMISSION OF *X. FASTIDIOSA*

Simplistically, the virulence of *X. fastidiosa* in plants appears to be influenced most strongly by three major factors: (*i*) fimbrial and afimbrial adhesins that are involved in attachment of *X. fastidiosa* to surfaces and to each other to form a biofilm, (*ii*) Type IV pili that are involved in twitching motility that is apparently important in the movement of *X. fastidiosa* along and perhaps between xylem vessels, and (*iii*) extracellular enzymes such as polyglacturonases and endoglucanases that are required for degradation of pit membranes to enable *X. fastidiosa* to spread from one xylem vessel to another. Importantly, these traits are all controlled by the accumulation of DSF, and hence exhibit a population size dependency. Curiously, these traits are oppositely regulated by DSF, with adhesins, as a group, induced in the presence of DSF and production of Type IV pili and extracellular enzymes suppressed with increasing DSF production (**Figure 5**). Given that DSF-deficient

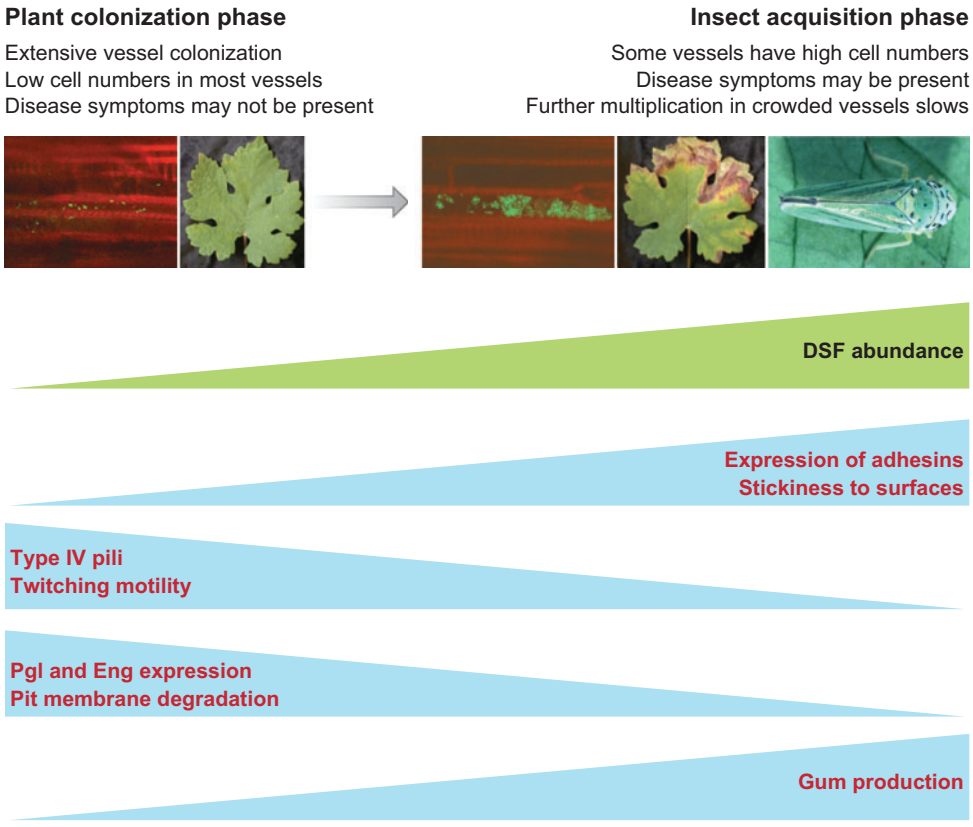

Figure 5

A conceptual model illustrating that plant colonization by *X. fastidiosa* and its transmission by insects are largely mutually exclusive processes that are controlled in large part by the extent of vessel colonization. The model emphasizes the fact that cells exhibit different phenotypes (shown in *red*) depending on their density within xylem vessels.

mutants of *X. fastidiosa* are far more virulent to plants but severely compromised in insect transmission, the traits that maximize plant colonization and those that optimize insect transmission would appear to be in conflict. That is, plant colonization and thus virulence would seem to be optimum in cells that did not express adhesins and hence were not "sticky," enabling them to move freely through the plant. Likewise, cells that expressed Type IV pili and extracellular enzymes would be able to degrade pit membranes and move through the openings made in the vessels to adjacent xylem vessels. As DSF levels would be expected to be low in vessels lacking large colonies of *X. fastidiosa*, the cells should have low expression of adhesins but high expression of Type IV pili and extracellular enzyme production. Such cells would be in an "exploratory phase" of colonization of the plant where frequent movement to new vessels would be expected (**Figure 5**). In contrast, in those vessels in which large colonies might form, DSF levels would increase, thereby suppressing further enzyme production. Cells would also become more "sticky" with the enhanced production of various adhesins, making movement more difficult. Type IV pili production would also decrease, perhaps reflecting the futility of attempted movement. As the pathogen apparently acquires substantial nutrition from the degradation products of the pit membranes, DSF thus suppresses the multiplication in vessels as cell numbers, and hence DSF, accumulate. This would tend to act as a negative feedback loop to prevent excessive grown of *X. fastidiosa* in vessels, which apparently is detrimental if it leads to vessel blockage. The "sticky" cells in those vessels in which cell numbers had increased, however, would most likely be acquired by insect vectors (**Figure 5**). *X. fastidiosa* thus apparently must express traits involved in attachment differently in different phases of its lifestyle. Our model predicts that *X. fastidiosa* coordinates its behavior in a plant to have first an "exploratory" phase for systemic spread in the plant but limited acquisition by insect vectors; the exploratory phase which occurs until cells start to become locally abundant. This phase is followed by an "acquisition phase" in a subset of the cells that are maximally transmitted by insects. Thus, because the plant lifestyle (as an endophyte) conflicts with its ability to adhere to insects and be transmitted, the pathogen apparently takes on a "bipolar" lifestyle of two different physiologies that are adapted for plant invasion and insect transmission, respectively. DSF serves as the switch to coordinate the plant lifestyle and convert cells into the insect acquisition phase.

Based on this model, *X. fastidiosa* produces a mixed population of cells that may be spatially or temporally segregated in the plant in a manner that involves DSF production that participates differentially in plant colonization and insect transmission. Clearly, as more is learned of other factors that contribute to virulence and insect transmission and how such traits are regulated by endogenous cues such as DSF or exogenous cues from the plant or insect, a more complex model of behavior of *X. fastidiosa* will emerge. However, the complex lifestyle of *X. fastidiosa*, with more than one host, will require it to coordinate its behavior with signaling molecules whose perception and function is fine-tuned to suit specific needs for colonizing these different hosts.

SUMMARY POINTS

1. While *X. fastidiosa* has long been associated with several diseases such as PD of grape and diseases of other horticultural crops, important new diseases of citrus and the spread of vectors of the pathogen have greatly increased the economic repercussions of this pathogen.

2. Molecular tools such as the marking of strains with GFP show that the pathogen occurs most commonly in relatively small colonies in colonized xylem vessels and much less frequently blocks vessels, suggesting that excessive multiplication in vessels is detrimental to pathogen survival.
3. The complete genome sequence for several *X. fastidiosa* strains revealed that, while this pathogen harbors many of the same virulence traits and regulatory systems as Xanthomonas species, it lacks many traits typical of pathogens that interact with living plant cells.
4. The coordinated regulation of a variety of virulence factors such as adhesins, Type IV pili, and extracellular cell wall-degrading enzyme by a small diffusible signal molecule similar to, but different from that used in *Xanthomonas* species, is a prominent feature of the behavior of *X. fastidiosa*.
5. The colonization of insect vectors occurs in discrete sites in the insect and traits such as adhesins are apparently important in colonization, given the high turbulence of fluids in the regions where attachment and biofilm formation occur.
6. The traits that maximize plant colonization and those that optimize insect transmission are in conflict, suggesting that the expression of these traits by *X. fastidiosa* in plants is spatially or temporally heterogeneous, and regulated by diffusible signal molecules to enable plant colonization and insect transmission to occur simultaneously in the same plant, but probably not be the same cells.

FUTURE ISSUES

1. While the roles of many putative virulence factors used by *X. fastidiosa* have been identified from analysis of genomic sequences, and some have even been studied by mutational analysis, many more such traits presumably exist and more research is needed on their patterns of expression in plants and insects.
2. As plant virulence traits in *X. fastidiosa* are down-regulated upon the accumulation of a small diffusible signal molecule, disease control by a process of "pathogen confusion," involving elevation of such signal molecules in plants, might provide a novel means of disease control. More research on how such a process could be implemented and its effect of the disease process is needed.
3. The genome sequences of different strains of *X. fastidiosa* reveal remarkable similarities to each other even though the strains have different host ranges. However, the traits that determine the host range of this pathogen have not yet been resolved.
4. As a small endogenous signal molecule greatly affects the interactions of *X. fastidiosa* with plants and insects, the pathogen likely perceives other signals of plant or insect origin to coordinate behaviors in a host-appropriate manner. The environmental cues for pathogen behavior need to be better understood.

5. The study of traits in *X. fastidiosa* that contribute to insect colonization and transmission can now be addressed using appropriate mutant strains. However, progress in this area will be dependent on developing artificial feeding systems for insect acquisition of the pathogen since the traits involved in insect acquisition may be negatively related to plant colonization.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to the many colleagues who have influenced our thinking through work that was not explicitly cited. We appreciate the many insightful discussions with Dr. Sandy Purcell who has long considered the issues raised in this review. We would like to acknowledge the support for research in our labs by the Pierce's Disease and Glassy-winged Sharpshooter Board, and the University of California Pierce's Disease Grants Program that has enabled us to address some of the issues raised here.

LITERATURE CITED

1. Aguero CB, Uratsu SL, Greve C, Powell ALT, Labavitch JM, et al. 2005. Evaluation of tolerance to Pierce's disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. *Mol. Plant Pathol.* 6:43–51
2. Alfano J, Colmer A. 1997. The typeIII (hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, avr proteins, and death. *J. Bacteriol.* 179:5655–62
3. Alves E, Marucci CR, Lopes JRS, Leite B. 2004. Leaf symptoms on plum, coffe, and citrus and the relationship with the extent of xylem vessels colonized by *Xylella fastidosa*. *J. Phytopathol.* 152:291–97
4. Almeida RPP, Backus EA. 2004. Stylet penetration behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae): EPG waveform characterization and quantification. *Ann. Entomol. Soc. Am.* 97:838–51
5. Almeida RPP, Blua MJ, Lopes JRS, Purcell AH. 2005. Vector transmission of *Xylella fastidiosa*: applying fundamental knowledge to generate disease management strategies. *Ann. Entomol. Soc. Am.* 98:775–86
6. Almeida RPP, Mann R, Purcell AH. 2004. *Xylella fastidiosa* cultivation on a minimal solid defined medium. *Curr. Microbiol.* 48:368–72
7. Almeida RPP, Purcell AH. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). *J. Econ. Entomol.* 96:264–71
8. Almeida RPP, Purcell AH. 2006. Patterns of *Xylella fastidiosa* colonization on the precibarium of sharpshooter vectors relative to transmission to plants. *Ann. Entomol. Soc. Am.* 99:884–90
9. Almeida RPP, Wistrom C, Hill BL, Hashim J, Purcell AH. 2005. Vector transmission of *Xylella fastidiosa* to dormant grape. *Plant Dis.* 89:419–24
10. Andrade MO, Alegria MC, Guzzo CR, Docena C, Rosa MCP, et al. 2006. The HD-GYP domain of RpfG mediates a direct linkage between the Rpf quorum-sensing pathway and a

subset of diguanylate cyclase proteins in the phytopathogen *Xanthomonas axonopodis* pv *citri*. *Mol. Microbiol.* 62:537–51
11. Backus EA, McLean DL. 1982. The sensory systems and feeding behavior of leafhoppers. I. The aster leafhopper, *Macrosteles fascifrons* Stal (Homoptera, Cicadellidae). *J. Morphol.* 172:361–79
12. Backus EA, McLean DL. 1983. The sensory systems and feeding behavior of leafhoppers. II. A comparison of the sensillar morphologies of several species (Homoptera:Cicadellidae). *J. Morphol.* 176:3–14
13. Barber CE, Tang JL, Feng JX, Pan MQ, Wilson TJG, et al. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol. Microbiol.* 24:555–66
14. Barnard EL, Ash EC, Hopkins DL, McGovern RJ. 1998. Distribution of *Xylella fastidiosa* in oaks in Florida and its association with growth decline in *Quercus laevis*. *Plant Dis.* 82:569–72
15. Bhattacharyya A, Stilwagen S, Ivanova N, D'Souza M, Bernal A, et al. 2002. Whole-genome comparative analysis of three phytopathogenic *Xylella fastidiosa* strains. *Proc. Natl. Acad. Sci. USA* 99:12403–88
16. Bhattacharyya A, Stilwagen S, Reznik G, Feil H, Feil WS, et al. 2002. Draft sequencing and comparative genomics of *Xylella fastidiosa* strains reveal novel biological insights. *Genome Res.* 12:1556–634
17. Blua MJ, Perring TM, Madore MA. 1994. Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. *J. Chem. Ecol.* 20:691–708
18. Brlansky RH, Timmer LW, French WJ, McCoy RE. 1983. Colonization of the sharpshooter vectors, *Oncometopia nigricans* and *Homalodisca coagulata*, by xylem-limited bacteria. *Phytopathology* 73:530–35
19. Burr TJ, Hoch HC, Cursino L, Li Y. 2007. The role that different pili classes in *Xylella fastidiosa* play in colonization of grapevines and pierces disease pathogenesis: Chemosensory cluster controlling twitching motility. In *Pierce's Disease Research Symp. Proc.*, pp. 123–26. San Diego: Calif. Dep. Food Agric.
20. Chang CJ, Donaldson RC. 2000. Nutritional requirements of *Xylella fastidiosa*, which causes Pierce's disease in grapes. *Can. J. Microbiol.* 46:291–93
21. Chatelet DS, Matthews MA, Rost TL. 2006. Xylem structure and connectivity in grapevine (*Vitis vinifera*) shoots provides a passive mechanism for the spread of bacteria in grape plants. *Ann. Bot.* 98:483–94
22. Chatterjee S, Sonti RV. 2002. *rpfF* mutants of *Xanthomonas oryzae* pv. *oryzae* are deficient for virulence and growth under low iron conditions. *Mol. Plant Microbe Interact.* 15:463–71
23. Chatterjee S, Wistrom C, Lindow SE. 2008. A cell-cell signaling sensor is required for virulence and insect transmission of *Xylella fastidiosa*. *Proc. Natl. Acad. Sci. USA*. In press
24. Chisholm ST, Coaker G, Day B, Staskawicz BJ. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124:803–14
25. Chou FL, Chou HC, Lin YS, Yang BY, Lin NT, et al. 1997. The *Xanthomonas campestris* gumD gene required for synthesis of xanthan gum is involved in normal pigmentation and virulence in causing black rot. *Biochem. Biophys. Res. Commun.* 233:265–79
26. Costa HS, Guzman A, Hernandez-Martinez R, Gispert C, Cooksey DA. 2006. Detection and differentiation of *Xylella fastidiosa* strains acquired and retained by glassy-winged sharpshooters (Hemiptera: Cicadellidae) using a mixture of strain-specific primer sets. *J. Econ. Entomol.* 99:1058–64
27. Costa HS, Raetz E, Pinckard TR, Gispert C, Hernandez-Martinez R, et al. 2004. Plant hosts of *Xylella fastidiosa* in and near Southern California vineyards. *Plant Dis.* 88:1255–61

28. Da Silva AC, Ferro JA, Reinach FC, Farah CS, Furlan LR, et al. 2002. Comparison of the genome of two *Xanthomonas* pathogens with different host specificities. *Nature* 417:459–63
29. da Silva FR, Vettore AL, Kemper EL, Leite A, Arruda P. 2001. Fastidian gum: the *Xylella fastidiosa* exopolysaccharide possibly involved in bacterial pathogenicity. *FEMS Microbiol. Lett.* 203:165–71
30. da Silva JF, Koide T, Gomes SL, Marques MV. 2007. The single "Extracytoplasmic-Function" sigma factor of *Xylella fastidiosa* is involved in the heat shock response and presents an unusual regulatory mechanism. *J. Bacteriol.* 189:551–60
31. da Silva FG, Shen Y, Dardick C, Burdman S, Yadav RC, de Leon AL, Ronald PC. 2004. Bacterial genes involved in Type I secretion and sulfation are required to elicit the rice *Xa21*-mediated innate immune response. *Mol. Plant Microbe Interact.* 17:593–01
32. Damsteegt VD, Brlansky RH, Phillips PA, Roy A. 2006. Transmission of *Xylella fastidiosa*, causal agent of citrus variegated chlorosis, by the glassy-winged sharpshooter, *Homalodisca coagulata*. *Plant Dis.* 90:567–70
33. D'Argenio DA, Miller SI. 2004. Cyclic di-GMP as a bacterial second messenger. *Microbiology* 150:2497–02
34. De La Fuente L, Burr TJ, Hoch HC. 2007. Mutations in Type I and Type IV pilus biosynthetic genes affect twitching motility rates in *Xylella fastidiosa*. *J. Bacteriol.* 189:7507–10
35. de Souza AA, Takita MA, Coletta-Filho HD, Caldana C, Goldman GH, et al. 2004. Gene expression profile of the plant pathogen *Xylella fastidiosa* during biofilm formation in vitro. *FEMS Microbiol. Lett.* 237:341–53
36. de Souza AA, Takita MA, Pereira EO, Coletta HD, Machado MA. 2005. Expression of pathogenicity-related genes of *Xylella fastidiosa* in vitro and in planta. *Curr. Microbiol.* 50:223–28
37. Denny TP. 1995. Involvement of bacterial polysaccharides in plant pathogenesis. *Annu. Rev. Phytopathol.* 33:173–97
38. Dharmapuri S, Sonti RV. 1999. A transposon insertion in the *gumG* homologue of *Xanthomonas oryzae* pv. *oryzae* causes loss of extracellular polysaccharide production and virulence. *FEMS Microbiol. Lett.* 179:53–59
39. Doddapaneni H, Yao JQ, Lin H, Walker MA, Civerolo EL. 2006. Analysis of the genome-wide variations among multiple strains of the plant pathogenic bacterium *Xylella fastidiosa*. *BMC Genomics* 7:225
40. Dow JM, Crossman L, Findlay K, He YQ, Feng JX, Tang JL. 2003. Biofilm dispersal in *Xanthomonas campestris* is controlled by cell-cell signaling and is required for full virulence to plants. *Proc. Natl. Acad. Sci. USA* 100:10995–1000
41. Dow JM, Daniels MJ. 2000. *Xylella* genomics and bacterial pathogenicity to plants. *Yeast* 17:263–71
42. Dow JM, Fouhy Y, Lucey JF, Ryan RP. 2006. The HD-GYP domain, cyclic Di-GMP signaling, and bacterial virulence to plants. *Mol. Plant Microbe Interact.* 19:1378–84
43. Dow JM, Scofield G, Trafford K, Turner PC, Daniels MJ. 1987. A gene cluster in *Xanthomonas campestris* pv. *campestris* required for pathogenicity controls the excretion of polygalacturonate lyase and other enzymes. *Physiol. Mol. Plant Pathol.* 31:261–71
44. Ebbert MA, Nault LR. 2001. Survival in *Dalbulus* leafhopper vectors improves after exposure to maize stunting pathogens. *Entomol. Exp. Appl.* 100:311–24
45. Fedatto LM, Silva-Stenico ME, Etchegaray A, Pacheco FTH, Rodrigues JLM, Tsai SM. 2006. Detection and characterization of protease secreted by the plant pathogen *Xylella fastidiosa*. *Microbiol. Res.* 161:263–72
46. Feil H, Purcell AH. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. *Plant Dis.* 85:1230–34

47. Feil H, Feil WS, Lindow SE. 2007. Contribution of fimbrial and afimbrial adhesins of *Xylella fastidiosa* to attachment to surfaces and virulence to grape. *Phytopathology* 97:318–24
48. Fereres A, Kampmeier GE, Irwin ME. 1999. Aphid attraction and preference for soybean and pepper plants infected with Potyviridae. *Ann. Entomol. Soc. Am.* 92:542–48
49. Fouhy Y, Scanlon K, Schouest K, Spillane C, Crossman L, et al. 2007. Diffusible signal factor-dependent cell-cell signaling and virulence in the nosocomial pathogen *Stenotrophomonas maltophilia*. *J. Bacteriol.* 189:4964–68
50. Freitag AH. 1951. Host range of Pierce's disease virus of grapes as determined by insect transmission. *Phytopathology* 41:920–34
51. Fritschi FB, Lin H, Walker MA. 2007. *Xylella fastidiosa* population dynamics in grapevine genotypes differing in susceptibility to Pierce's disease. *Am. J. Enol. Viticult.* 58:326–32
52. Guilhabert MR, Kirkpatrick BC. 2005. Identification of *Xylella fastidiosa* antivirulence genes: hemagglutinin adhesins contribute to *X. fastidiosa* biofilm maturation and colonization and attenuate virulence. *Mol. Plant Microbe Interact.* 18:856–68
53. He YW, Wang C, Zhou L, Song H, Dow JM, et al. 2006. Dual signaling functions of the hybrid sensor kinase RpfC of *Xanthomonas campestris* involved either phosphorelay or receiver domain-protein interaction. *J. Biol. Chem.* 281:33414–21
54. He YW, Xu M, Lin K, Ng YJA, Wen CM, et al. 2006. Genome scale analysis of diffusible signal factor regulon in *Xanthomonas campestris* pv. *campestris*: identification of novel cell-cell communication-dependent genes and functions. *Mol. Microbiol.* 59:610–22
55. He YWM, Ng AYJ, Xu M, Lin K, Wang LH, et al. 2007. *Xanthomonas campestris* cell-cell communication involves a putative nucleotide receptor protein Clp and a hierarchical signaling network. *Mol. Microbiol.* 64:281–92
56. Hendson M, Purcell AH, Chen DQ, Smart C, Guilhabert M, Kirkpatrick B. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. *Appl. Environ. Microbiol.* 67:895–903
57. Henneberger TSM, Stevenson KL, Britton KO, Chang CJ. 2004. Distribution of *Xylella fastidiosa* in sycamore associated with low temperature and host resistance. *Plant Dis.* 88:951–58
58. Hernandez-Martinez R, Costa HS, Dumenyo CK, Cooksey DA. 2006. Differentiation of strains of *Xylella fastidiosa* infecting grape, almonds, and oleander using a multiprimer PCR assay. *Plant Dis.* 90:1382–88
59. Hernandez-Martinez R, de la Cerda KA, Costa HS, Cooksey DA, Wong FP. 2007. Phylogenetic relationships of *Xylella fastidiosa* strains isolated from landscape ornamentals in Southern California. *Phytopathology* 97:857–64
60. Hernandez-Martinez R, Pinckard TR, Costa HS, Cooksey DA, Wong FP. 2006. Discovery and characterization of *Xylella fastidiosa* strains in southern California causing mulberry leaf scorch. *Plant Dis.* 90:1143–49
61. Hill BL, Purcell AH. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. *Phytopathology* 85:209–12
62. Hill BL, Purcell AH. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. *Phytopathology* 85:1368–72
63. Hill BL, Purcell AH. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. *Phytopathology* 87:1197–201
64. Hopkins DL. 1989. *Xylella fastidiosa*—xylem-limited bacterial pathogen of plants. *Annu. Rev. Phytopathol.* 27:271–90
65. Hopkins DL, Purcell AH. 2002. *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. *Plant Dis.* 86:1056–66

66. Hu NT, Hung MN, Chiou SJ, Tang F, Chiang DC, et al. 1992. Cloning and characterization of a gene required for the secretion of extracellular enzymes across the outer membrane by *Xanthomonas campestris* pv. *campestris*. *J. Bacteriol.* 174:2679–87
67. Huang TP, Wong ACL. 2007. A cyclic AMP receptor protein-regulated cell-cell communication system mediates expression of a FecA homologue in *Stenotrophomonas maltophilia*. *Appl. Environ. Microbiol.* 73:5034–40
68. Jha G, Rajeshwari R, Sonti RV. 2005. Bacterial type two secretion system secreted proteins: double-edged swords for plant pathogens. *Mol. Plant-Microbe Interact.* 18:891–98
69. Killiny N, Almeida RPP. 2007. In vitro attachment of *Xylella fastidiosa* to polysaccharides. *Phytopathology* 97:S57
70. Koide T, Vencio RZN, Gomes SL. 2006. Global gene expression analysis of the heat shock response in the phytopathogen *Xylella fastidiosa*. *J. Bacteriol.* 188:5821–30
71. Krivanek AF, Walker MA. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. *Phytopathology* 95:44–52
72. Lee BM, Park YJ, Park DS, Kang HW, Kim JG, et al. 2005. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. *Nucleic Acids Res.* 33:577–86
73. Leopold RA, Freeman TP, Buckner JS, Nelson DR. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera:Cicadellidae). *Arthropod Struct. Dev.* 32:189–99
74. Lin H, Doddapaneni H, Takahashi Y, Walker MA. 2007. Comparative analysis of ESTs involved in grape responses to *Xylella fastidiosa* infection. *BMC Plant Biol.* 7:8
75. Lubelsky J, Konings WN, Driessen AJM. 2007. Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. *Microbiol. Mol. Biol. Rev.* 71:463–76
76. Marucci RC, Lopes JRS, Vendramim JD, Corrente JE. 2005. Influence of *Xylella fastidiosa* infection of citrus on host selection by leafhopper vectors. *Entomol. Exp. Appl.* 117:95–103
77. McElrone AJ, Sherald JL, Forseth IN. 2001. Effects of water stress on symptomatology and growth of *Parthenocissus quinquefolia* infected by *Xylella fastidiosa*. *Plant Dis.* 85:1160–64
78. McGaha LA, Jackson B, Bextine B, McCullough D, Morano L. 2007. Potential plant reservoirs for *Xylella fastidiosa* in South Texas. *Am. J. Enol. Viticult.* 58:398–401
79. Meidanis J, Braga MDV, Almeida SV. 2002. Whole genome analysis of transporters in the plant pathogen *Xylella fastidiosa*. *Microbiol. Mol. Biol. Rev.* 66:272–99
80. Meng Y, Li Y, Galvani CD, Hao G, Turner JN, et al. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. *J. Bacteriol.* 187:5560–67
81. Minsavage GV, Thompson CM, Hopkins DL, Leite R, Stall RE. 1994. Development of a polymerase chain-reaction protocol for detection of *Xylella fastidiosa* in plant tissue. *Phytopathology* 84:456–61
82. Mittler TE. 1967. Water tensions in plants—an entomological approach. *Ann. Entomol. Soc. Am.* 60:1074–76
83. Montero-Astua M, Saborio-R G, Chacon-Diaz C, Garita L, Villalobos W, et al. 2008. First report of *Xylella fastidiosa* in avocado in Costa Rica. *Plant Dis.* 92:175
84. Muniz JRC, Alves CA, de Pieri C, Beltramini LM, Selistre-De-Araujo HS, et al. 2004. Overexpression, purification, biochemical characterization, and molecular modeling of recombinant GDP-mannosyltransferase (GumH) from *Xylella fastidiosa*. *Biochem. Biophys. Res. Commun.* 315:485–92
85. Naito A. 1977. Feeding habits of leafhoppers. *Jpn. Agric. Res. Q.* 11:115–19
86. Nault LR. 1997. Arthropod transmission of plant viruses: a new synthesis. *Ann. Entomol. Soc. Am.* 90:521–41

87. Newman KL, Almeida RPP, Purcell AH, Lindow SE. 2003. Use of a green fluorescent strain for analysis of *Xyella fastidiosa* colonization of *Vitis vinifera*. *Appl. Environ. Microbiol.* 69:7319–27
88. Newman KL, Almeida RPP, Purcell AH, Lindow SE. 2004. Cell-cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. *Proc. Natl. Acad. Sci. USA* 101:1737–42
89. Oliveira MA, Guimaraes BG, Cussiol JRR, Medrano FJ, Gozzo FC, Netto LES. 2006. Structural insights into enzyme-substrate interaction and characterization of enzymatic intermediates of organic hydroperoxide resistance protein from *Xylella fastidiosa*. *J. Mol. Biol.* 359:433–45
90. Prado SS, Lopes JRS, Demetrio CGB, Borgatto AF, Almeida RPP. 2008. Host colonization differences between citrus and coffee isolates of *Xylella fastidiosa* in reciprocal inoculation. *Sci. Agric.* 65: In press
91. Purcell AH, Finlay AH. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. *Phytopathology* 69:393–95
92. Purcell AH, Finlay AH, McLean DL. 1979. Pierce's disease bacterium: mechanism of transmission by leafhopper vectors. *Science* 206:839–41
93. Purcell AH, Hopkins DL. 1996. Fastidious xylem-limited bacterial plant pathogens. *Annu. Rev. Phytopathol.* 34:131–51
94. Purcell AH, Saunders SR, Hendson M, Grebus ME, Henry MJ. 1999. Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. *Phytopathology* 89:53–58
95. Purcell AH. 1980. Almond leaf scorch: leafhopper and spittlebug vectors. *J. Econ. Entomol.* 73:834–38
96. Rajeshwari R, Jha G, Sonti RV. 2005. Role of an in planta expressed xylanase of *Xanthomonas oryzae* pv. *oryzae* in promoting virulence on rice. *Mol. Plant Microbe Interact.* 18:830–37
97. Ray SK, Rajeshwari R, Sharma Y, Sonti RV. 2002. A high molecular-weight outer membrane protein of *Xanthomonas oryzae* pv. *oryzae* exhibits homology to non fimbrial adhesins of animal pathogenic bacteria and is required for optimum virulence. *Mol. Microbiol.* 46:637–47
98. Ray SK, Rajeshwari R, Sonti RV. 2000. Mutants of *Xanthomonas oryzae* pv. *oryzae* deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. *Mol. Plant Microbe Interact.* 13:394–401
99. Redak RA, Purcell AH, Lopes JRS, Blua MJ, Mizell RF, Andersen PC. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annu. Rev. Entomol.* 49:243–70
100. Reddy JD, Reddy SL, Hopkins DL, Gabriel DW. 2007. TolC is required for pathogenicity of *Xylella fastidiosa* in *Vitis vinifera* grapevines. *Mol. Plant Microbe Interact.* 20:403–10
101. Roper MC, Greve LC, Labavitch JM, Kirkpatrick BC. 2007. Detection and visualization of an exopolysaccaride produced by *Xylella fastidiosa* in vitro and in planta. *Appl. Environ. Microbiol.* 73:7252–58
102. Roper MC, Greve LC, Warren JG, Labavitch JM, Kirkpatrick BC. 2007. *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. *Mol. Plant Microbe Interact.* 20:411–19
103. Rosselli LK, Oliveira CLP, Azzoni AR, Tada SFS, Catani CF, et al. 2006. A new member of the aldo-keto reductase family from the plant pathogen *Xylella fastidiosa*. *Arch. Biochem. Biophys.* 453:143–50
104. Ryan RP, Fouhy Y, Lucey JF, Crossman LC, Spiro S, et al. 2006. Cell-cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in cyclic di-GMP turnover. *Proc. Natl. Acad. Sci. USA* 103:6712–17

105. Severin HHP. 1949. Transmission of the virus of Pierce's disease of grapevines by leafhoppers. *Hilgardia* 19:190–206
106. Severin HHP. 1950. Spittle-insect vectors of Pierce's disease virus II. Life history and virus transmission. *Hilgardia* 19:357–81
107. Shen Y, Chern MS, Siva FG, Ronald P. 2001. Isolation of a *Xanthomonas oryzae* pv. *oryzae* flagellar operon region and molecular characterization of *flhF*. *Mol. Plant Microbe Interact.* 14:204–13
108. Shi XY, Dumenyo CK, Hernandez-Martinez R, Azad H, Cooksey DA. 2007. Characterization of regulatory pathways in *Xylella fastidiosa*: genes and phenotypes controlled by *algU*. *Appl. Environ. Microbiol.* 73:6748–56
109. Siciliano F, Torres P, Sendin L, Bermejo C, Filippone P, et al. 2007. Analysis of the molecular basis of *Xanthomonas axonopodis* pv. *citri* pathogenesis in *Citrus limon*. *Electron. J. Biotechnol.* 9:200–4
110. Simionato AVC, da Silva DS, Lambais MR, Carrilho E. 2007. Characterization of a putative *Xylella fastidiosa* diffusible signal factor by HRGC-EI-MS. *J. Mass Spectrom.* 42:490–96
111. Simpson AJG, Reinach FC, Abreu FA, Acencio M, Alvarenga R, et al. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature* 406:151–59
112. Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM. 2000. A two-component system involving an HD-GYP domain protein links cell-cell signaling to pathogenicity gene expression in *Xanthononas campestris*. *Mol. Microbiol.* 38:986–1003
113. Souza LCA, Wulff NA, Gaurivaud P, Mariano AG, Virgilio ACD, et al. 2006. Disruption of *Xylella fastidiosa* CVC *gumB* and *gumF* genes affect biofilm formation without a detectable difference in exopolysaccharide production. *FEMS Microbiol. Lett.* 257:236–42
114. Stevenson JF, Matthews MA, Greve LC, Labavitch JM, Rost TL. 2004. Grapevine susceptibility to Pierce's disease II: progression of anatomical symptoms. *Am. J. Enol. Viticult.* 55:238–45
115. Tang JL, Feng JX, Li QQ, Wen HX, Zhou DL, et al. 1996. Cloning and characterization of the *rpfC* gene of *Xanthomonas oryzae* pv. *oryzae*: involvement in exopolysaccharide production and virulence to rice. *Mol. Plant Microbe Interact.* 9:664–66
116. Thorne ET, Stevenson JF, Rost TL, Labavitch JM, Matthews MA. 2006. Pierce's disease symptoms: comparison with symptoms of water deficit and the impact of water deficits. *Am. J. Enol. Viticult.* 57:1–11
117. Torres PS, Malamud F, Rigano LA, Russo DM, Marano MR, et al. 2007. Controlled synthesis of the DSF cell-cell signal is required for biofilm formation and virulence in *Xanthomonas campestris*. *Environ. Microbiol.* 9:2101–9
118. Tyree MT, Zimmermann MH. 2002. *Xylem Structure and the Ascent of Sap*. New York: Springer-Verlag
119. Tyson GE, Stojanovic BJ, Kuklinski RF, Divittorio TJ, Sullivan ML. 1985. Scanning electron microscopy of Pierce's disease bacterium in petiolar xylem of grape leaves. *Phytopathology* 75:264–69
120. Van der Linden MG, Rego TG, Araujo DAM, Farias ST. 2006. Prediction of potential thermostable proteins in *Xylella fastidiosa*. *J. Theor. Biol.* 242:421–25
121. Van Sluys MA, de Oliveira MC, Monteiro-Vitorello CB, Miyaki CY, Furlan LR, et al. 2003. Comparative analysis of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strain of *Xylella fastidiosa*. *J. Bacteriol.* 185:1018–26
122. Wang LH, He Y, Gao Y, Wu JE, Dong YH, et al. 2004. A bacterial cell-cell communication signal with cross-kingdom structural analogues. *Mol. Microbiol.* 51:903–12

123. Wang N, Feil W, Lindow S. 2007. Identification of traits of *Xylella fastidiosa* conferring virulence to grape and insect transmission by analysis of global gene expression using DNA microarrays. *Phytopathology* 97:S120
124. de Weert S, Vermeiren H, Mulders IHM, Kuiper I, Hendrickx N, et al. 2002. Flagella-driven chemotaxis towards exudates components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. *Mol. Plant Microbe Interact.* 15:1173–80
125. Wistrom C, Purcell AH. 2005. The fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts in California. *Plant Dis.* 89:994–99