# MANAGEMENT OF PIERCE'S DISEASE OF GRAPE BY INTERFERING WITH CELL-CELL COMMUNICATION IN *XYLELLA FASTIDIOSA*

**Project Leader:**
Steven E. Lindow
Dept. of Plant and Microbial Biology
University of California
Berkeley, CA 94720

**Cooperators:**
Subhadeep Chatterjee
Dept. of Plant and Microbial Biology
University of California
Berkeley, CA 94720

Alexander Purcell
Dept of Env. Sci. Policy & Mgmt.
University of California
Berkeley, CA 94720

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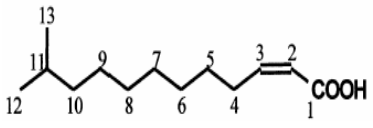
## ABSTRACT

*Xylella fastidiosa* (*Xf*) produces an unsaturated fatty acid signal molecule called DSF that changes its gene expression in cells as they reach high numbers in plants. We have investigated DSF-mediated cell-cell signaling in *Xf* with the aim of developing cell-cell signaling disruption as a means of controlling Pierce's disease (PD). We have extensively investigated both the role of DFS-production by *Xf* on its behavior within plants, the manner in which other bacterial strains affect such cell signaling, the extent to which other endophytes could modulate density-dependent behaviors and virulence in *Xf* by interfering with cell-cell signaling, performed genetic transformation of grape to express DSF, and explored other means to alter DSF abundance in plants to achieve PD control. *Xf* strains that overproduce DSF cause disease symptoms in grape, but only at the site of inoculation and the cells do not move within the plant as do wild-type strains. Thus elevating DSF levels in plants should reduce movement of *Xf* in the plant and also reduce the likelihood of transmission by sharpshooters. We identified bacterial strains that can interfere with *Xf* signaling both by producing large amounts of DSF or by degrading DSF. We have identified the genes needed to degrade DSF and when they were transferred to and over-expressed in other strains they conferred the ability of these strains to degrade DSF. When co-inoculated into grape with *Xf*, both DSF-producing strains and DFS-degrading strains greatly reduced the incidence and severity of disease in grape. Non-endophytic bacterial species were also established in high numbers inside grape leaves and petioles following spray application to plants with a high concentration of a silicon-based surfactant with a low surface tension. PD was reduced in plants after topical application of a DSF-producing strain of *Erwinia herbicola*. To verify that disease control is due to DSF interference, we have constructed mutants of these strains blocked in their ability to produce or degrade DSF and showed that the mutants are deficient in disease control. Given that DSF overabundance appears to mediate an attenuation of virulence in *Xf* we have transformed grape with the *rpfF* gene of *Xf* to enable DSF production in plants. Initial results indicate that plants produce at least some DSF and are much less susceptible to disease.

## INTRODUCTION

Endophytic bacteria such as *Xf* colonize the internal tissues of the host, forming a biofilm inside the plant. A key determinant of success for an endophyte is the ability to move within the plant. We expect activities required for movement to be most successful when carried out by a community of cells since individual cells may be incapable of completing the feat on their own. Cells assess the size of their local population via cell-cell communication and coordinately regulate the expression of genes required for such processes. Our study aims to determine the role of cell-cell communication in *Xf* in colonization and pathogenicity in grapevines and transmission by the insect vector. *Xf* shares sequence similarity with the plant pathogen *Xanthomonas campestris* pathovar *campestris (Xcc).* In *Xcc,* expression of pathogenicity genes is controlled by the Rpf system of cell-cell communication, enabling a population of cells to launch a pathogenic attack in a coordinated manner. Two of the Rpf proteins, RpfB and RpfF, work to produce a diffusible signal factor (DSF) which has recently been described as an alpha,beta unsaturated fatty acid.

As the population grows, the local concentration of DSF increases. Other Rpf proteins are thought to sense the increase in DSF concentration and transduce a signal, resulting in expression of pathogenicity factors. We now have shown that *Xf* makes a molecule that is recognized by *Xcc* but probably slightly different than the DSF of *Xcc*. Based on our knowledge of density-dependent gene regulation in other species, we predict the targets of Rpf regulation would be genes encoding extracellular polysaccharides, cellulases, proteases and pectinases necessary for colonizing the xylem and spreading from vessel to vessel and perhaps adhesins that modulate movement.




Given that the DSF signal molecule greatly influences the behavior of *Xf* including both its virulence to grape and ability to be vectored by sharpshooters we have investigated various ways by which this pathogen can be "confused" by altering the local concentration of the signal molecule in plants to disrupt disease and/or transmission. Our preliminary work revealed that several other bacterial species can both positively and negatively interact with the DSF-mediated cell-cell signaling in *Xf*, but until this study we did not know of the manner in which the interaction occurred nor whether such strains had the potential to affect the virulence of *Xf* in grape. In this period we have extensively investigated both the role of DFS-production by *Xf* on its behavior within plants, the manner in which other bacterial strains affect such cell signaling, the extent to which other endophytes could modulate density-dependent behaviors and virulence in *Xf* by interfering with cell-

cell signaling, obtained genetic transformation of grape to express DSF, and explored other means to alter DSF abundance in plants to achieve PD control.

**OBJECTIVES**

1. Identify bacteria that interfere with DSF-mediated cell-cell signaling in *Xf*, and conduct pathogenicity tests on grapevines colonized by DSF-interfering bacteria to determine potential for PD control
2. Isolation of mutant strains of DSF-degrading and DSF-producing bacteria that can no longer interfere in cell-cell signaling to verify that disease control is linked to cell-cell signal interference
3. Molecular identification of genes conferring DSF-degrading activity
4. Engineer the grapevine endophytes to express genes conferring DSF-degradation and DSF-synthesis activities and test whether the resulting transgenic endophytes are capable of disease control
5. Creation of grapevines expressing genes conferring DSF-degradation and DSF-synthesis activity to test for PD resistance
6. Evaluate topical application of DSF-degrading and DSF-producing bacteria with penetrating surfactants for PD control

**RESULTS**

**Objective 1. Disease control with DSF-interfering strains**

We have previously isolated a variety of bacteria and tested them for their ability to interfere with cell-cell signaling in *Xf*. We found several strains that negatively affected signaling in *Xcc* while several strains were found to produce DSF. When co-inoculated into grapevines with *Xf,* strains that either inhibit or activate cell-cell signaling in greenhouse studies both reduced PD; DSF-producing strains tended to be most effective in blocking disease. To better understand the contribution of DSF production by *Xf* on virulence to grape we varied DSF in *Xf* in several ways. As we observed earlier, RpfF- mutants of *Xf* are hypervirulent to grape, producing more than twice the level of disease symptoms and producing symptoms earlier than a wild-type strain (Figure 1). In contrast, when DSF is over-produced in Xf by expressing *rpfF* under the control of the highly active and constitutive *kan* promoter, such strain are greatly reduced in virulence (Figure 1). While DS-overproducing strains caused infection at the site of inoculation, the pathogen and diseased leaves were not seen away from that site. A similar reduced virulence phenotype was observed in an RpfC- mutant of *Xf*. Such a mutant over-expresses DSF compared to a wild-type strain, suggesting that RpfC negatively regulates DSF production in *Xf*. The RpfC- mutant caused infection only at the site of inoculation, and hence was much less virulent than the wild-type strain (Figure 2). The population size of the RpfC- mutant in the plant was always much lower than that of the wild-type or RpfF- mutant and was undetectable further than about 30 cm from the site of inoculation, unlike the wild-type strain (Figure 2). In contrast, the population size of the RpfF- mutant was higher at all points away from the site of inoculation than the wild-type strain (Figure 2). These results all support our model that DFS regulates genes required for movement of *Xf* from colonized vessels. We hypothesize that *rpfF*-deficient mutants may be causing increased vessel blockage in the grapevine, leading to increased symptom expression.

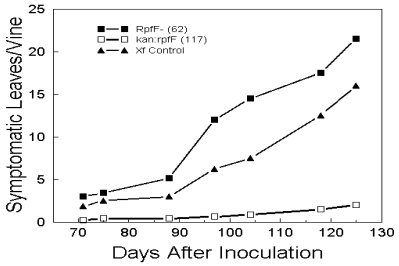



**Figure 1.** Severity of PD in grape inoculated with a DSF-over-producing strain of *Xf* or with an RpfF- mutant compared to plants inoculated with wild-type Temecula.

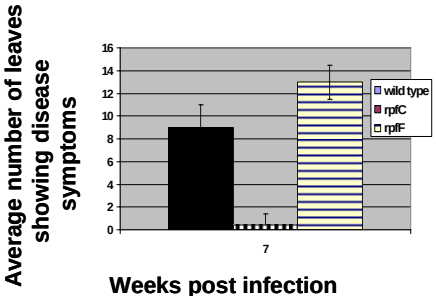



**Figure 2A.** Severity of PD on grape inoculated with the *rpfC* mutant of *Xf* which overproduce DSF or the RpfF- mutant, which is unable to produce DSF and the wild type Temecula.

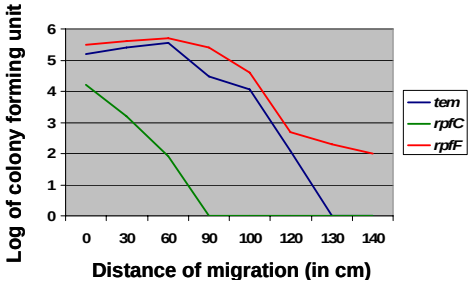



**Figure 2B.** Migration assay of wild type, RpfC and RpfF- mutants in the grapevines. Bacteria were isolated from the petioles at different distances from the point of inoculation 14 weeks after inoculation.

To test the model that DSF controls the process of colonization of grape we evaluated the process of colonization of grape by wild-type and RpfF- mutants of *Xf*. This was done using a gfp-marked strain of *Xf* that could be visualized within sections of inoculated grape by confocal laser microscopy. The RpfF- mutant colonized many more vessels than did the wild-type strain (Figure 3). Furthermore, this mutant blocked many more vessels by growing to larger population sizes in the xylem vessels (Figure 4). Thus RpfF, by synthesizing DSF appears to down-regulate virulence in *Xf*. These results explain why an RpfF- mutant is more virulent; by spreading much more extensively, and especially by blocking vessels it will cause more disease symptoms. These results all suggest that altering DSF levels in the xylem will alter the behavior of *Xf* and reduce disease.

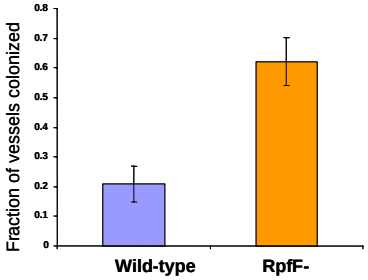



**Figure 3**. Fraction of grape xylem vessels colonized by wild type *Xf* or an RpfF- mutant 10 weeks after inoculation. The more extensive movement of the RpfF- mutant in grape suggests that RpfF suppresses extracellular enzymes presumably involved in movement of *Xf* between xylem cells.

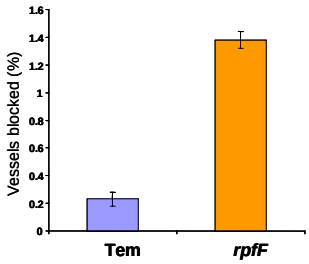



**Figure 4**. Percent of grape xylem vessels blocked by wild type *Xf* or an RpfF- mutant 10 weeks after inoculation as determined by confocal microscopy. The more extensive growth of the RpfF- mutant in the xylem suggests that virulence traits such as extracellular enzymes may contribute to growth of cells in the plant.

**Objective 2. Linking biocontrol of PD to DSF interference**

To establish a rigorous connection between DSF production and disease control, we have constructed mutant strains of those DSF-producing bacteria that perform best in the disease control assays that no longer could produce DSF. These mutants were then compared to their parent strains in disease control assays. We also made mutants of DSF-degrading strains that no longer could degrade DSF. We expected that if DSF interference can provide disease control, then strains no longer able to interfere with DSF signaling will also no longer be able to control disease. All mutants unable to produce DFS (such mutant V134-1 of strain V in Figure 5) were diminished in ability to reduce PD when co-inoculated with *Xf* compared to their DSF-producing wild-type strain. Likewise, mutant strain G741, a mutant of DSF-degrading parental strain G that no longer could degrade DSF also was greatly reduced in ability to control PD when co-inoculated with *Xf* compared to its parental strain (Figure 6). These results suggest strongly that it is the production of, or degradation of DSF in plants by these antagonistic bacteria that makes a large contribution to their ability to reduce PD. The results thus strongly suggest that any method that either increases or decreased DSF abundance in *Xf*-infected plants will have a large effect on the incidence and/or severity of PD.

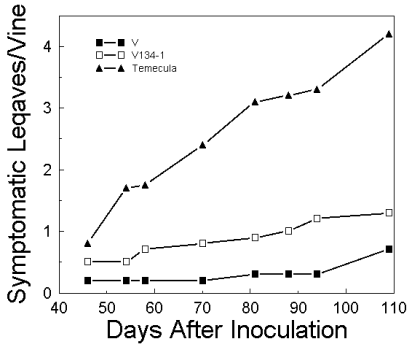



**Figure 5.** Severity of PD on grape co-inoculated with DSF-producing strain V or mutant V134-1 that is unable to produce DSF and *Xf* strain Temecula. The mutant is less effective in reducing disease compared to the parental strain.

**Objective 3. Characterizing DFS degradation pathways**

To better exploit DSF degradation as a means of disease control we need to understand how DSF degradation occurs and what genes are involved. We thus molecularly identified the genes conferring the DSF-interference phenotype in *Pseudomonas* strain G, one of the most active DSF degrading bacteria. We have inactivated the genes for interference in *Pseudomonas* strain G individually by random Tn5 mutagenesis and cloned the disrupted loci. We obtained many individual transposon mutants that were completely blocked in DSF degradation. Interestingly, all of the mutations were in the *carAB* genes, encoding carbamoyl-phosphate synthetase activity degradation suggesting that this locus was particularly (solely?) responsible for DSF degradation. These genes are involved in purine and pyrimidine biosythessis. We speculate that the cells require UDP-galactose for the breakdown of DSF, which is an alpha, beta fatty acid. The *carAB* genes have been cloned and shown to restore DSF-degradation in strain G mutants, verifying that these genes were indeed responsible for DSF degradation (Figure 7). Importantly, over-expression of *carAB* in a mutant of strain G that was previously unable to degrade DSF conferred upon it the ability to degrade DSF that is greater than the original strain G (Figure 7). Also, over-expression of *carAB* in *E. coli*, a bacterium with little ability to degrade DS confers upon it the ability to rapidly degrade DSF (Figure 7). This exciting result suggests that not only can we impart DSF degradation activity upon other endophytic bacteria that are good colonizers of grape, but that we can produce bacteria with superior abilities to degrade DSF by over-expressing *carAB*. In very exciting results, we find that the ability of *Pseudomonas* strain G to reduce PD of grape when co-inoculated with *Xf* is greatly increased when the cloned *carAB* is over-expressed in this strain (Figure 8). We thus will be exploring the possibilities of enhancing control of PD by introducing cloned *carAB* genes into better endophytes of grape.

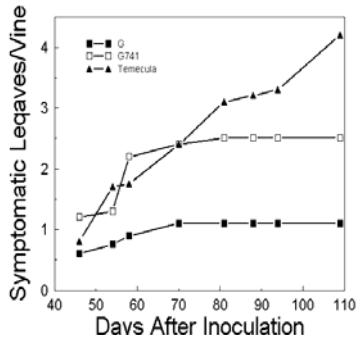



**Figure 6**. Severity of PD on grape co-inoculated with DSF-degrading *Pseudomonas* strain G or mutant G741 that is unable to degrade DSF and *Xf* strain Temecula. The mutant is less effective in reducing disease compared to the parental strain.

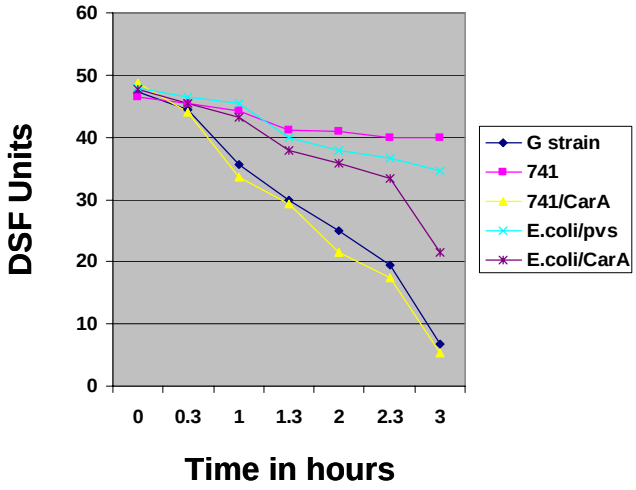



**Figure 7**. DSF degradation kinetics of bacteria harboring carAB. DSF was added to an overnight grown culture of *Pseudomonas* strain G, mutant G741 (G Mutant), G741/Pcar(G- mutant with the complementing clone), *E.coli* with the vector alone and *E.coli* with pCAB (cloned *carAB* gene). DSF was extracted at different time points and was assayed with an indicator strain.

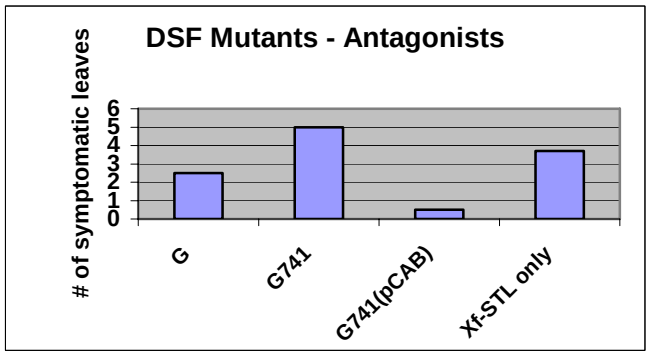



**Figure 8**. Severity of PD on grape co-inoculated with *Pseudomonas* strain G that can degrade DSF, mutant G741 that can not degrade DSF, or with mutant G741 over-expressing the cloned *carAB* genes on plasmid pCAB compared with plants inoculated with *Xf* strain STL alone. Note that mutant G741 did not reduce disease but over-expression of *carAB* resulted in great disease control.

**Objective 4. Expressing DSF degradation and production in grape endophytes**

Disease control by DSF-interfering strains will be optimized if they are good colonists of grapevine. To maximize disease control we are expressing the various genes conferring DSF interference in effective non-pathogenic endophytic colonists of grapevine such as *Erwinia herbicola*. We expect that this strategy will deliver the disease control agent directly to the site of the pathogen and result in highly effective control. Since the *rpfF* gene of *Xf* is sufficient to confer expression of DSF in other bacteria we are introducing it into these two species. We have also examined ways of over-expressing *rpfF* in various bacteria to enhance their production of DSF. We have constructed DSF over-producing strains of *E.coli* and 299R (*Erwinia herbicola*) expressing the DSF biosynthetic gene *rpfF* from *Xcc* and X*f*. Dramatic increases in production of extracellular DSF is observed specially with the *Xf* DSF relative to the parental strain (Table 1). We are further studying the ability of the purified DSF from these overproducing strains as well as the strains itself in controlling PD by spraying them on infected grape vines.

**Objective 5. Production of DSF in transgenic plants for disease control**

We have expressed the *rpfF* gene in grape and tomato transformed at the Ralph M. Parsons Foundation Plant Transformation Facility at the University of California, Davis. Initially, we submitted a tested but un-optimized *rpfF* construct to the facility. We transformed both tomato and grape since we could more quickly obtain evidence of DSF production in tomato since it is much quicker and easier to transform than grape, and since it is susceptible to *Xanthomonas campestris pv.vesicatoria (Xcv)*, a pathogen that produces a DSF similar to that of *Xf* we could more quickly evaluate the effect of *in planta* production of DSF on disease control. The first transformed plants are now mature and have been tested for DFS production. Initial assays reveal that DSF is rapidly degraded by damaged plant tissue during extraction procedures, making it hard to estimate the abundance of DSF within the plants. Therefore different assays are being developed to avoid this complication in assessing DSF abundance. Mature grape plants have now been rooted to produce large numbers of clonal plants that were inoculated with *Xf*. Transformed tomato plants have also been grown to maturity and selfed to produce sufficient seed for inoculation experiments. Our initial results from inoculation of tomato transformed with the *rpfF* gene of *Xcc* reveals that they are much more susceptible to infection from topical applications of *Xcv* (Figure 9). Since RpfF- mutants of *Xcv* are less virulent to tomato unlike RpfF- mutants of *Xf* that are more virulent, DSF increases the virulence of *Xcv* (the opposite of its affect in *Xf*) and hence our finding that the *rpfF*-transformed tomato are more susceptible to *Xcv* suggests that DSF was produced by these plants. Large numbers of clonal rpfF-expressing grapes have

**Table 1**. Production of DSF by *E. coli* and *Erwinia herbicola* haboring cloned *rpfF* genes from *Xf* and *Xcc*.

| Strains | Relative DSF production (Units) |
|---|---|
| *Xcc* | 100 |
| *X. fastidiosa* Temecula | 4-5 |
| *E. coli* DH10B (ptrpXccrpfF) | 3000 |
| *E. coli* DH10B (ptrpXfrpfF) | 100 |
| *E. herbicola* (ptrpXccrpfF) | 6000 |
| *E. herbicola* (ptrpXfrpfF) | 200 |

now been produced and inoculated with *Xf* to test for susceptibility to PD. The rpfF-expressing grape are much less susceptible to PD (Figure 10). The severity of disease was reduced over 10-fold compared to non-transformed plants. While *Xf* spread throughout non-transformed plants causing disease on petioles located great distances from the point of inoculation, disease was observed only very close to the point of inoculation in *rpfF*-expressing plants. We thus expect to find that *Xf* is limited in its movement in plants having high indigenous levels of DSF due to the expression of *rpfF*, in a manner similar to what we have observed in DFS-overproducing strains of *Xf*. We are thus very excited about the prospects of enhancing DFS levels in plants as a means of reducing disease. This might best be done in transgenic plants or perhaps in topical applications of DSF or analogs or also by expression in plants by other endophytic bacteria. We are transforming additional plants with both *rpfF* and *rpfB* genes that have been modified to direct the protein product to the chloroplast where fatty acid synthesis (and DSF synthesis) should be much enhanced compared to its production in the cytosol, the presumed location of RpfF in the current transformed plants.

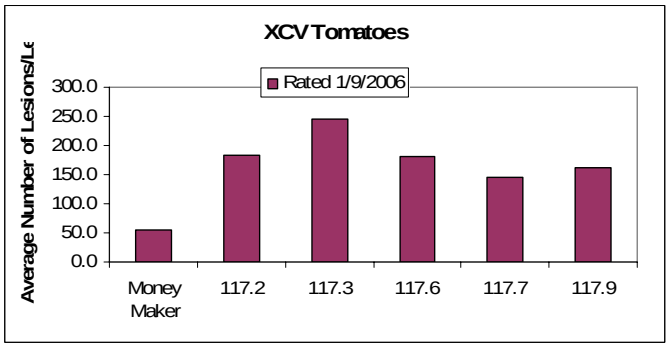



**Figure 9**. Average number of lesion spots per tomato leaflet.caused by *Xanthomonas campestris* pv. *vesicatoria* on wild type tomato (Money maker) and five transgenic lines expressing the *Xcc rpfF* gene.

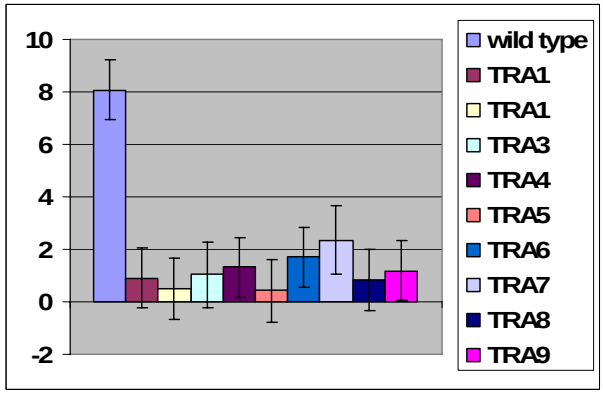



**Figure 10**. Disease severity (# symptomatic leaves/plant) on Freedom grape transformed with the r*pfF* gene encoding DSF production and inoculated with *Xf*.

**Objective 6. Topical application of DFS-interfering bacteria for disease control**

We have found that it is possible to establish large populations of bacteria within grape leaves, stems and petioles by simple topical applications of bacterial suspensions to plants in solutions of organosilicon surfactants having very low surface tensions. A variety of bacteria were found to colonize grape for at very high population sizes (> $10^6$ cells/petiole) for extended periods of time following topical application (Figure 11). While these bacteria apparently do not spread throughout the plant after inoculation as does *Xf*, by introducing them into the intercellular spaces and perhaps even the xylem of the plant by use of the surfactants that stimulate spontaneous infiltration of the plant, we can inoculate the bacteria into all sites within the plant. Initial studies have shown that topical applications of an *Erwinia herbicola* strain harboring the *Xf rfpF* gene can provide some control of PD (Figures 12 and 13). As noted above, we now can produce much more DSF in surrogate hosts such as *E. herbicola* and will be testing these new stains for disease control. In addition, we have isolated large amounts of DFS from such over-producing strains and have applied it topically with surfactants to determine if it will be taken into the plant and alter pathogen behavior. These inoculated plants will be rated for disease within a few more weeks.

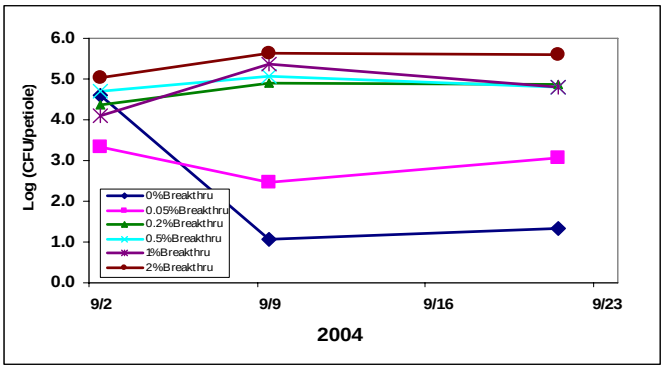
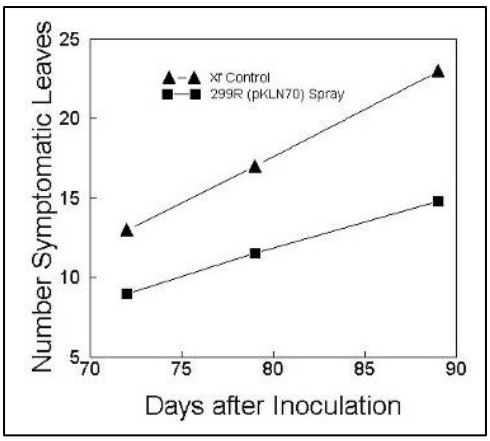
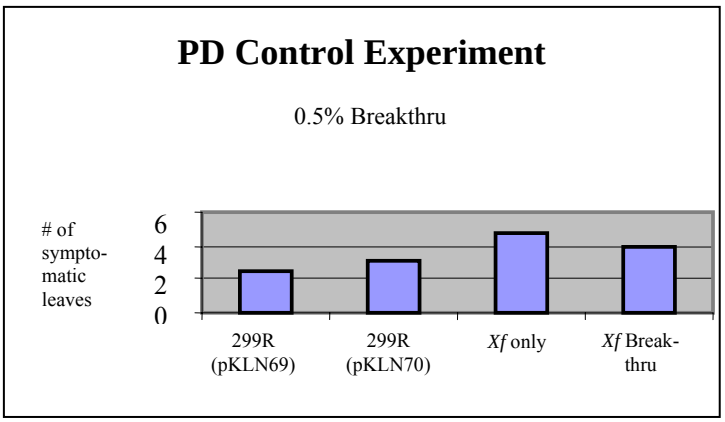



**Figure 11**. Population size of *E. herbicola* strain 299R in petioles at different times after spray inoculation with different concentrations of Breakthru.




**Figures 12 and 13**. Severity of PD of grape sprayed with *E. herbicola* harboring plasmids pKLN69 or pKLN70 encoding RpfF and thus DSF production compared to plants inoculated with *Xf* Temecula alone.

**CONCLUSIONS**

Substantial data now show that cell-cell signaling plays a major role in the epidemiology and virulence of *Xf* and that disruption of cell signaling is a promising means of controlling PD. Cell-cell signaling strongly controls movement and hence disease since *Xf* mutants unable to signal are hypervirulent while strains that overproduce DSF have low virulence and do not move within grape. This suggests that, it will be more efficient to elucidate and target *Xf*'s regulation of colonization strategies rather than individual traits predicted to contribute to virulence based. We have identified bacterial strains that can interfere with *Xf* signaling. These strains proved very effective as protective agents for grapevines when co-inoculated with *Xf*. Both positive and negative interference with DSF signaling reduced disease in grape suggesting that signaling is normally finely balanced in the disease process; such a finely balanced process might be readily disrupted. Since in bacteria *rpfF* is sufficient to encode a synthase capable of DSF production, expression of DFS directly in plants is an attractive approach for disease control. Preliminary results are very encouraging that DSF can be made in plants and will dramatically reduce PD. Alternatively, the use of various bacteria to express DSF in plants may prove equally effective in altering *Xf* behavior and hence disease control. Our observation that large numbers of bacteria could be introduced into grape plants by simple topical applications of cell suspensions in a penetrating surfactant has enabled us to pursue a new strategy of disease control that will enable us to efficiently test those strains that are found to be effective in PD control by a method that should prove practical for commercial use. Thus our investigation of the fundamental issues associated with interactions of *Xf* with grape has led to several very practical possible control measures of PD that can be evaluated over the short term.

**FUNDING AGENCIES**

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# EFFECTS OF CHEMICAL MILIEU ON ATTACHMENT, AGGREGATION, BIOFILM FORMATION, AND VECTOR TRANSMISSION OF *XYLELLA FASTIDIOSA* STRAINS

**Project Leader:**
Alexander H. Purcell
ESPM-Division of Insect Biology
University of California
Berkeley CA 94720
purcell@nature.berkeley.edu

**Researcher:**
Clytia Montllor Curley
ESPM-Division of Insect Biology
University of California
Berkeley CA 94720
montllor@nature.berkeley.edu

**Cooperators:**
Steven E. Lindow
Dept. of Plant and Microbial Biology
University of California
Berkeley CA 94720
icelab@socrates.berkeley.edu

Harvey Hoch
Dept. of Plant Pathology, NYSAES
Barton Laboratory, Cornell University
Geneva, NY 14456
hch1@nysaes.cornell.edu

Thomas Burr
Dept. of Plant Pathology, NYSAES
Barton Laboratory, Cornell University
Geneva, NY 14456

**Reporting Period**: The results reported here are from work conducted September 2005 to August 2006.

## ABSTRACT
An *rpfF* DSF-deficient mutant that does not adhere to the inside of insect vector mouthparts formed a reduced biofilm *in vitro*, but contributed to biofilm formation when combined with a wild-type *Xylella fastidiosa* (*Xf*)strain. Growth of the DSF-deficient mutant in media PW was comparable to that of the wild type *in vitro* and neither strain appeared to outcompete the other when grown in PW liquid media over two weeks. However the mutant strain showed reduced growth alone, and in co-culture with the wild-type, in a second medium (BHF) that promoted biofilm formation in the wild-type strain. A fluorescent (*gfp*) mutant of Temecula was also used on occasion, but was dropped when it became clear that the mutation reduced growth ability in media and plants. Competitive behavior of the various strains *in planta* is also being investigated in our laboratory. Studies were also undertaken to try to enhance uptake and delivery of *Xf* from artificial feeding sachets to plants. A wild-type and a DSF-overproducing *rpfC* mutant strain of *Xf* were added to sachets and fed to leafhopper vectors. Only one out of 85 insects tested positive for *Xf* after sachet feeding, and no test grape plants have been infected by *Xf* from insects fed this way. Wild-type *Xf* (strain STL), grown on solid media, was also presented for sachet feeding to sharpshooter vectors; one of 24 insects that acquired *Xf* this way was able to transmit to a healthy seedling.

## INTRODUCTION
Studies from this lab (R.P.P. Almeida and A.H. Purcell, unpublished) showed that sharpshooters could acquire cultured *Xylella fastidiosa* (*Xf*) cells added to expressed xylem sap in an artificial feeding apparatus (sachet), but did not transmit these bacteria to grapevines, as measured by subsequent symptom development. The assumption was that these cells did not attach to the foregut of the feeding insects. This sort of circumstantial evidence points up the complexity of the bacterial / insect / plant factors necessary for transmission (uptake and delivery) to occur. The importance of attachment and subsequent biofilm formation in this process is also indicated by the behavior of mutants that do not attach to the vector mouthparts and are not insect transmissible (Newman et al. 2004). Studies of *Xf* biofilm formation *in vitro* indicate that chemical make-up of media, substrate, and bacterial genotype/phenotype all play roles in aggregation behavior of *Xf* (e.g., Marques et al. 2002, Leite et al. 2004, Feil et al. 2003, Hoch et al. 2004). Our ultimate objective is to understand the factors that affect the process of attachment and biofilm formation of *Xf* under different environmental conditions. Investigating the conditions, be they environmental or genetic, that promote attachment and subsequent detachment from insect mouthparts is crucial to understanding transmission from insects to plants.

## OBJECTIVES
1. Determine whether vector retention (and subsequent delivery) of *Xf* is related to the chemical and physical environment from which the bacteria are grown or acquired.
2. Investigate how *Xf* cells attach (and detach) to specific foregut regions of sharpshooter vectors. *NB: this objective being carried out in collaboration with the Hoch/Burr labs.*

## RESULTS
In six experiments in which the *rpfF* mutant KLN61 was grown alone, it made much reduced or no discernible biofilms in glass flasks compared to the wild type Temecula, or the *gfp*-Temecula strain. In an experiment in which biofilms were quantified by sonication of the film after rinsing, and subsequent plating, KLN61 was estimated to make only 6% of the biofilm made by *gfp*-Temecula under identical circumstances. However, both qualitative and quantitative measurement of biofilms created by KLN61 grown together with Temecula (or *gfp*-Temecula) indicated that when the two strains were grown together they were consistently as thick or thicker than those created by Temecula alone.