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TECHNICAL ADVANCE

Evaluation of *Arabidopsis thaliana* as a Model Host for *Xylella fastidiosa*

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The bacterium *Xylella fastidiosa* causes a number of plant diseases of significant economic impact. To date, progress determining mechanisms of host-plant susceptibility, tolerance, or resistance has been slow, due in large part to the long generation time and limited available genetic resources for grape, almond, and other known hosts of *X. fastidiosa*. To overcome many of these limitations, *Arabidopsis thaliana* has been evaluated as a host for *X. fastidiosa*. A pin-prick inoculation method has been developed to infect *Arabidopsis* with *X. fastidiosa*. Following infection, *X. fastidiosa* multiplies and can be detected by microscopy, polymerase chain reaction, and isolation. The ecotypes Van-0, LL-0, and Tsu-1 all allow more growth of strain *X. fastidiosa* Temecula than the reference ecotype Col-0. Affymetrix ATH1 microarray analysis of inoculated vs. noninoculated Tsu-1 reveals gene expression changes that differ greatly from changes seen after infection with apoplast-colonizing bacteria such as *Psuedomonas syringae* pvs. *tomato* or *syringae*. Many genes responsive to oxidative stress are differentially regulated, while classic pathogenesis-related genes are not induced by *X. fastidiosa* infection.

Xylella fastidiosa is a xylem-limited, gram-negative bacteria and an economically important plant pathogen causing scorching and dieback symptoms of perennial crops and ornamental landscape plants (Purcell and Hopkins 1996). Pierce's disease is a chronic threat to California's grape industry, while citrus variegated chlorosis (CVC) has devastated Brazilian citrus crops (Simpson et al. 2000); both diseases are caused by *X. fastidiosa*. Almond leaf scorch disease (ALSD) is also caused by *X. fastidiosa*. Xylellae diseases are transmitted to new host plants by xylem-feeding sharpshooter vectors. Bacteria then multiply in the xylem, forming biofilm-like structures that can grow large enough to occlude xylem vessels and block water transport (Alves et al. 2004; Fry and Milholland 1990a; Newman et al. 2003). Disease symptoms, including leaf scorching and fruit shriveling, are consistent with xylem blockage and resulting water stress.

European bunch grape (*Vitis vinifera*) is cultivated worldwide as a high-quality , wine, and raisin grape; unfortunately, it is highly susceptible to Pierce's disease. In contrast, muscadine grape species native to the southeastern United States support much lower titers of *X. fastidiosa* and do not develop disease. Bacterial population size in resistant and susceptible grapevines is highly correlated with symptom expression (Alves et al. 2004; Fry and Milholland 1990a and b; Krivanek and Walker 2005). Although grape resistance loci have been mapped (Krivanek et al. 2005b, 2006), the molecular basis for resistance is unknown. Likewise, peach trees are not susceptible to *X. fastidiosa* strains capable of causing ALSD, even though peach and almond are closely related and can be hybridized. While one peach-almond hybrid has been shown to be resistant to an ALSD (Ledbetter and Rogers 2009), neither the genetic nor molecular basis of resistance has been elucidated.

A model host plant for *X. fastidiosa* would be valuable for the rapid testing of *X. fastidiosa* isolates and mutants. Screens for novel pathogenesis mutants could be more readily conducted in a model host; such screens are labor and time intensive in grape. Also, genetic resources in the host would facilitate dissection of the host response and identification of host defenses that are effective against *X. fastidiosa*. The model plant *Arabidopsis thaliana* is an ideal system for rapid progress in genetic and molecular studies (Meinke et al. 1998). *Arabidopsis* has proven useful in elucidating the pathogenicity of bacterial, viral, fungal, and oomycete pathogens (Carr and Whitham 2007; Oliva et al. 2010; Thatcher et al. 2005). The objective of this work was to determine if *Arabidopsis* was susceptible to *X. fastidiosa* and could be developed into a useful model host. We demonstrate that, after mechanical inoculation, *X. fastidiosa* persists and multiplies in *Arabidopsis* xylem elements. The relative susceptibility of different ecotypes to *X. fastidiosa* was evaluated and genes that were differentially expressed following infection were identified.

RESULTS

Inoculation method development.

The most effective way to inoculate *X. fastidiosa* into *Arabidopsis* was to place 1 µl of bacterial suspension or water onto the midrib at the leaf-petiole junction and pierce the midrib four or five times with a minuten pin. It was necessary to use a very small pin (0.15 mm diameter) so as not to break the

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midrib or excessively damage the leaf. Plants were visualized under a dissecting microscope during inoculation to ensure precise placement of the pin pricks. The 1-µl drop of bacterial suspension provided an adequate reservoir of bacteria that were able to enter the xylem. Simply dipping the pin in the bacterial suspension prior to poking did not result in lasting infection, probably because too few bacteria were introduced. During mechanical inoculation of stems of larger plants (e.g., tobacco, grape, and almond), the applied bacterial suspension enters the transpiration stream and rapidly disappears. A single *Arabidopsis* leaf has a much smaller transpiration volume and the 1-µl drop did not noticeably decrease in size. *X. fastidiosa* was detectable by polymerase chain reaction (PCR) in the middle of the leaf in samples harvested within 5 min of inoculation (data not shown), demonstrating bacterial entry into and movement within the vascular system. *X. fastidiosa* was not detected by either PCR or microscopy in the distal third of infected leaves until approximately 10 days postinoculation (dpi) (data not shown).

Microscopy of *X. fastidiosa* in planta.

Green fluorescent protein (GFP) fluorescence associated with the *X. fastidiosa* Temecula strain (Newman et al. 2003) was clearly visible starting approximately 7 dpi. Figure 1 shows typical pictures of two infected xylem elements running down the midrib (Fig. 1A), single infected xylem elements in both the midrib and a lateral vein (Fig. 1B), and in branching lateral veins (Fig. 1C). Figure 2A and B show scanning electron micrographs of xylem vessels from a mock-inoculated and a *X. fastidiosa*–inoculated leaf, respectively. Secondary wall thickenings characteristic of xylem elements are clearly visible in both micrographs, while rod-shaped bacteria and thread-like bacterial exopolysaccharides (EPS) are only present in the *X. fastidiosa*–inoculated leaf. Transmission electron micrographs show a xylem vessel (again, note the secondary wall thickenings characteristic of xylem in Figure 2C) largely occluded by bacterial cells and other osmophillic substances. These substances could be plant-derived tyloses, gums, bacterial EPS, or other material. The double membrane structure characteristic of a gram-negative bacterium like *X. fastidiosa* is clearly visible surrounding bacterial cells in Figure 2E and F.

Symptom development was not observed in inoculated *Arabidopsis* leaves. An individual *Arabidopsis* leaf has a lifespan on the plant of approximately 4 weeks; the whole *Arabidopsis* plant has a lifespan of 4 to 5 months when grown under 10-h light. Leaf senescence begins with yellowing of the leaf tip, and then, the leaf droops and decay starts. The onset and progression of senescence did not differ among *X. fastidiosa*–inoculated, mock-inoculated, and uninoculated leaves of similar age. Additionally, spread of *X. fastidiosa* out of the inoculated leaf was not observed. Again, this could be due to the relatively short lifespan of *Arabidopsis*.

Ecotype screening.

Initial development of the inoculation method was done using the reference ecotype Col-0. To determine if there are ecotype sensitivity differences, all 96 ecotypes in the Nordborg 96 collection were tested (Nordborg et al. 2005). Each accession was planted and inoculated at least twice; Col-0 was planted with each experiment to serve as an internal control. Bacterial growth was assayed by fluorescence microscopy and by PCR at 14 dpi. Any accession showing at least a fivefold higher *X. fastidiosa* titer than the Col-0 control in either replicate was screened at least twice more. There were three ecotypes, i.e., LL-0, Tsu-1, and Van-0, that reproducibly allowed more growth (Fig. 3A). A time course comparing Col-0 and Tsu-1 confirmed the growth differential with both PCR (Fig. 3B) and with bacterial isolation and dilution plating (Fig. 3C). PCR detects bacterial DNA from either living or dead bacterial cells, while isolation only enumerates live cells. The two detection methods give comparable results, indicating that the fraction of live bacterial cells in each ecotype are also similar. *X. fastidiosa* growth is also detectable by enzyme-linked immunosorbent assay (ELISA) (data not shown).

To investigate the genetic basis for the observed difference in bacterial growth, reciprocal crosses were performed between Col-0 and Tsu-1. F1 plants from both crosses demonstrated intermediate growth of *X. fastidiosa* as measured by PCR (data not shown), indicating a probable quantitative, multilocus inheritance. A tenfold difference in bacterial growth is not large enough to use for mapping multiple loci; therefore, F2 plants were not examined.

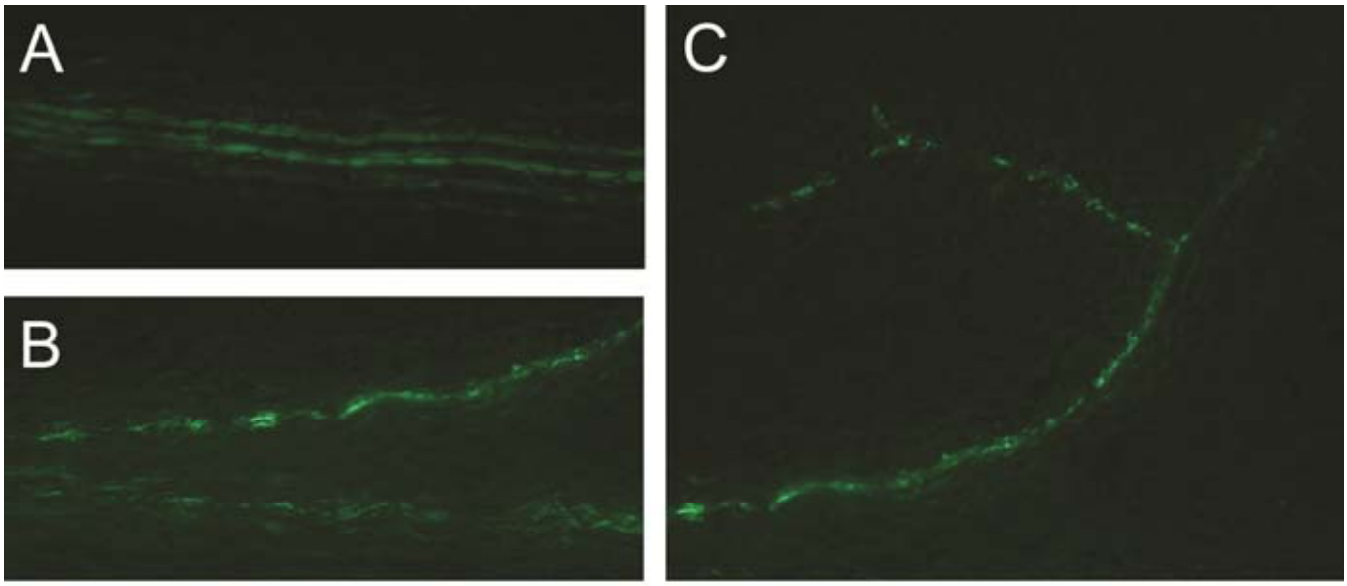

Fig. 1. Green fluorescent protein (GFP)-labeled *Xylella fastidiosa* is visible in *Arabidopsis* Van-0 xylem vessels at 10 days postinoculation. **A,** Two infected xylem vessels in the midrib. **B,** Infected element in the midrib (bottom) and infected branching secondary vein (top). **C,** Several branching secondary veins with infected xylem vessels.

Infection with *X. fastidiosa* virulence mutants.

To further examine the utility of *Arabidopsis* as a model host for *X. fastidiosa*, ecotype Tsu-1 was infected with three known bacterial virulence mutants. The *tolC* mutant did not grow and live cells were not recoverable from *Arabidopsis* (data not shown), a result similar to infection of grapevine (Reddy et al. 2007). The *rpfF* mutant grew to higher titer than the corresponding wild type (Fig. 3D), again similar to infec-

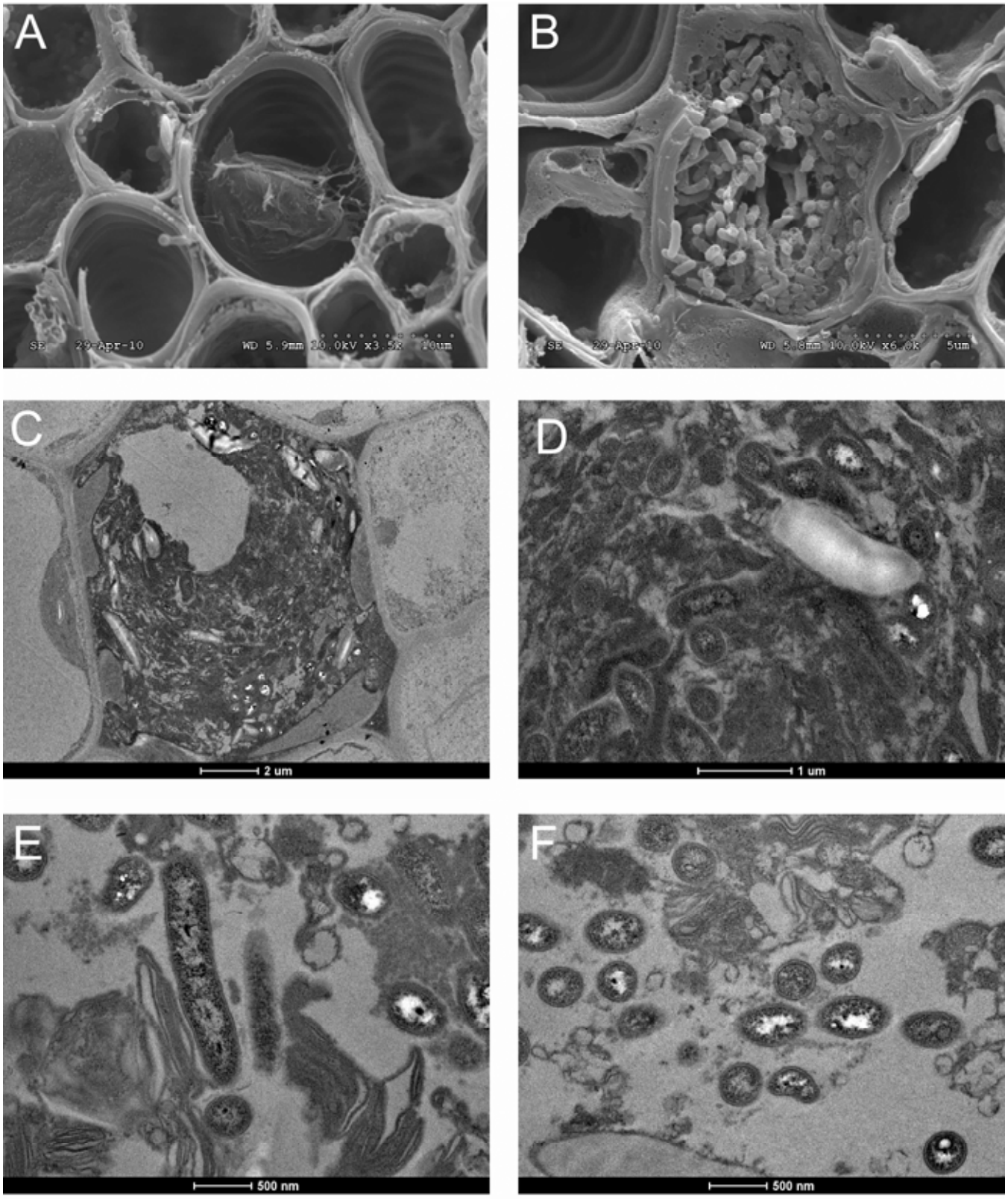

Fig. 2. Electron microscopy reveals *Xylella. Fastidiosa*–infected xylem vessels at 15 days postinoculation. **A,** Scanning electron micrographs (SEM) of a mock-inoculated leaf with empty xylem vessels. **B,** SEM of an infected leaf showing bacteria largely occluding an infected xylem vessel. **C,** Transmission electron micrographs (TEM) of an infected leaf, again showing a largely occluded xylem vessel; **D, E,** and **F,** close-up TEM of bacteria and other osmophillic substances in infected xylem vessels.

tion of grapevine (Chatterjee et al. 2008a). However, the *pglA* mutant grew to similar levels as did the wild-type Fetzer strain; *pglA* mutant is nonpathogenic in grape. Figure 3D also demonstrates that the Fetzer strain grows to similar levels as Temecula. The Stag's Leap strain displays growth indistinguishable from that of Temecula and Fetzer (data not shown).

Gene expression analysis.

To identify genes differentially regulated in response to *X. fastidiosa*, three replicate pairs of RNA samples from mock- and *X. fastidiosa*–inoculated Tsu-1 were hybridized to Affymetrix ATH-1 *Arabidopsis* whole-genome microarray chips. Probe set intensities were averaged across replicates prior to comparison of mock- and *X. fastidiosa*–inoculated results. Genes with expression changes of at least twofold and significant *t*-test values (<0.05) were considered differentially expressed genes (DEG) and are listed in Supplementary Table 1. These 86 genes fell into many different functional categories, as predicted by Gene Ontology annotation (Fig. 4A), with more than half of all DEG involved in either basic metabolism or of unknown function. Only three DEG were in the biotic stress category. To create a visual representation of the data, the 86 DEG were clustered in a heat map according to similar expression patterns (Fig. 4B). The dendogram for the probe sets groups genes upregulated by *X. fastidiosa* infection on the top of the heat map and downregulated genes on the bottom. Each group shows complex structure, implying diverse regulatory mechanisms leading to the up- or downregulation of various genes. The heat map also visually demonstrates good reproducibility among the three replicates. Differential expression as detected by microarray was confirmed using quantitative real-time reverse transcription (qRT)-PCR. Figure 4C demonstrates that four of the five genes tested actually showed greater regulation by qRT-PCR than by microarray, as is often observed (Brady et al. 2006).

DISCUSSION

To date, experimental hosts for *X. fastidiosa* have been limited to the woody agricultural species that are affected by xylellae diseases and to *Nicotiana tabacum*. The time period between inoculation and symptom development or bacterial titer determination can range between about one month for tobacco (Alves et al. 2003; Francis et al. 2008; Lopes et al. 2000) to several months for grape or almond (Almeida and Purcell 2003; Krivanek and Walker 2005; Krivanek et al. 2005a).

Here, we demonstrate that the use of *Arabidopsis thaliana* Tsu-1 as a model experimental host can reduce the time between inoculation and assay to 10 to 14 days. *Arabidopsis* also has numerous publicly available genetic resources, making it

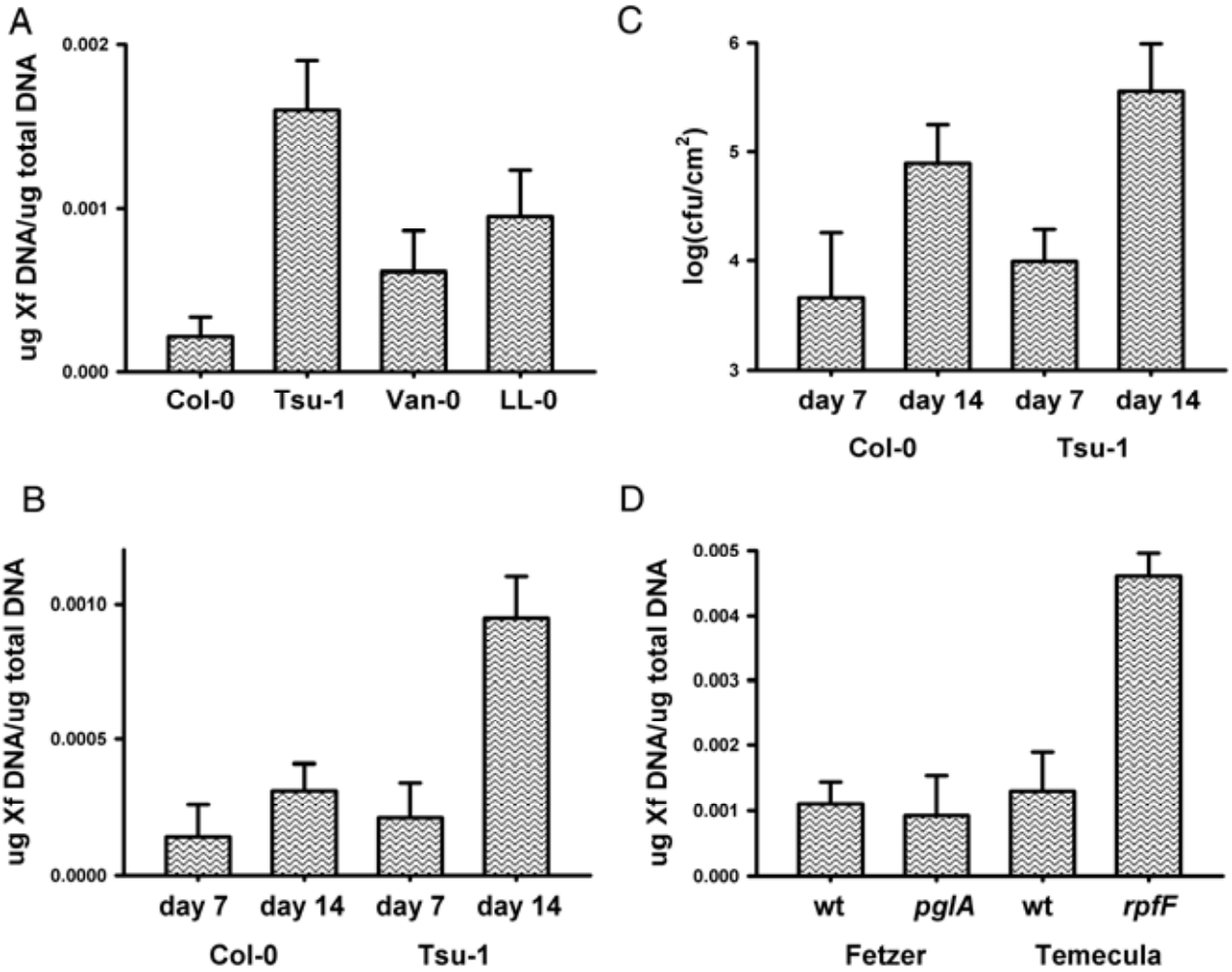

Fig. 3. *Arabidopsis* ecotypes display differential susceptibility to *Xylella fastidiosa*. **A,** Quantitative real-time polymerase chain reaction (PCR) measurement of bacterial titers at 14 days postinoculation (dpi). **B,** Time course of bacterial titers in Col-0 and Tsu-1 measured by PCR and **C,** isolation. **D,** Quantitative real-time PCR measurement of *rpfF* and *pglA* mutants and corresponding wild-type strains at 14 dpi. Each point is the average of at least eight biological replicate samples; error bars show standard deviation. Each experiment was repeated at least three times; representative data sets are shown.

an ideal model plant for the dissection of host-pathogen responses. *Arabidopsis* supports significant growth of *X. fastidiosa*, although symptom development was not observed. There are inter-ecotype differences in susceptibility, with Tsu-1 being the most susceptible ecotype tested. However, mapping the loci responsible for differences between Tsu-1 and the reference ecotype Col-0 is not practical, given the relatively small difference in bacterial growth and the probable multilocus nature of the susceptibility.

In grape, *X. fastidiosa* rapidly spreads the length of an infected xylem vessel. Spread is halted by pit membranes until bacterial density is high enough to trigger expression of the *pglA* polygalacturonase and other enzymes that degrade pit membranes and are thought to allow bacterial cells to pass into adjacent xylem vessels. A mutant in *pglA* lost its pathogenicity and ability to systemically colonize grapevine (Roper et al. 2007). Systemic spread of any *X. fastidiosa* strain was not observed in *Arabidopsis*. This could be explained if the *pglA* gene and other cell wall–degrading enzymes of *X. fastidiosa* are not expressed during infection of *Arabidopsis* or if such enzymes are not effective at degrading *Arabidopsis* pit membranes. Either of these possibilities would also explain the similar growth of wild type and the *pglA* mutant in *Arabidopsis*.

Various measurements of xylem vessel length in grape have shown that most vessels average about 10 cm in length with occasional connections between vessels that extend up to 1 meter (Chatelet et al. 2006; Chatterjee et al. 2008c). To our knowledge, similar studies in *Arabidopsis* have not been published. The observation that *X. fastidiosa* does not enter the distal third of an infected leaf until at least 10 dpi does imply that some plant structure, possibly pit membranes, is able to halt bacterial spread. With time and the accumulation of bacterial cells, *X. fastidiosa* is able to move throughout the inoculated leaf.

X. fastidiosa, like many bacteria, has a cell-to-cell signaling system that uses a set of regulation of pathogenicity factor (*rpf*) genes and a low molecular weight diffusible signaling factor (DSF) to control the expression of genes important for virulence and transmission (Chatterjee et al. 2008c). Mutation of the DSF synthetase gene (*rpfF*) results in increased pathogenicity and higher bacterial titer in infected grapevine (Newman et al. 2004). A similar effect, although not as pronounced, was observed in *Arabidopsis* (Fig. 3D). It has been hypothesized that enhanced *pglA* expression is a contributing factor to the increased growth and pathogenicity exhibited by *rpfF* mutants (Chatterjee et al. 2008a). Because growth of the *pglA* mutant was not impaired in *Arabidopsis*, it is unlikely that enhanced *pglA* expression is contributing to the elevated bacterial titer observed with the *rpfF* mutant. It is likely that other differences between the *rpfF* mutant and wild type, such as reduced biofilm formation, account for the increased growth of the mutant.

The TolC protein is the periplasmic and outer membrane component of all five classes of characterized multidrug resistance (MDR) efflux pumps in gram-negative bacteria. TolC is also essential for type I–dependent protein secretion. While the genomes of many gram-negative bacteria contain multiple TolC homologues per strain, the first two published genome sequences of *X. fastidiosa* strains revealed a single TolC homolog per genome (Simpson et al. 2000; Van Sluys et al. 2003). It was, therefore, not surprising that a *tolC* mutant completely abolished pathogenicity in grapevine and rendered *X. fastidiosa* unable to survive in planta (Reddy et al. 2007). Results presented here demonstrate a similar phenotype of the *tolC* mutant in *Arabidopsis*, indicating that either MDR efflux pumps, a functional type I secretion system, or both are necessary for survival and growth in *Arabidopsis*. The fact that no viable *tolC* mutant cells were recoverable from either grape or *Arabidopsis*, even at time points less than 1 h after inoculation, also implies that the *tolC* mutant is hypersensitive to many plant compounds or, at least, to compounds present in both disparately related plant species.

Gene expression profiling revealed a number of interesting changes in gene expression in *Arabidopsis* Tsu-1 after inoculation with *X. fastidiosa*. In general, responses to pathogen infection result from a complex interplay of at least three different signaling pathways, namely, salicylic acid (SA), ethylene and jasmonic acid (Et/JA), and abscissic acid (Berrocal-Lobo and Molina 2008; Glazebrook 2005); significant antagonism has been observed between the SA and the Et/JA pathways. Many biotrophic bacterial pathogens primarily activate the SA pathway, which acts through NPR1 and EDS1 leading to the upregulation of PR-1 and other classical pathogenesis-related (PR) genes and repress the Et/JA pathway. *X. fastidiosa* infection of Tsu-1 affected the expression of only three genes known to be upregulated by either SA, the biotrophic bacterial pathogen *Pseudomonas syringae*, or both, downregulating EPS1/At5g67160 and a receptor-like kinase, At2g37710 (Blanco et al. 2005; Zheng et al. 2009), while upregulating HSPRO2/At2g4000 (Murray et al. 2007). Two ethylene-responsive transcription factors, At1g21910 and At1g77640, were also upregulated by *X. fastidiosa* infection. Taken together, these changes in gene expression indicate that *X. fastidiosa* infection tends to activate the Et/JA pathway while largely repressing the SA pathway. However, a much larger number of genes are differentially regulated by *P. syringae* than by *X. fastidiosa*, possibly because an apoplastic pathogen like *P. syringae* makes direct contact with a much larger number of living cells than does a xylem-limited pathogen like *X. fastidiosa*.

A significant number of genes responsive to oxidative stress are upregulated by *X. fastidiosa* infection. Most notable are two thioredoxin superfamily proteins, At4g15700 and At4g15660; both genes are predicted to be involved in limiting oxidative stress and are also upregulated by heat stress (Larkindale and Vierling 2008). Overexpression of HSPRO2/At2g4000 has been shown to confer enhanced tolerance to oxidative stress (Luhua et al. 2008); as mentioned above, HSPRO2 is upregulated in response to *P. syringae* and by SA as well, as showing significant homology to a nematode-resistance protein from sugar beet (Murray et al. 2007). Many more genes predicted to be involved in limiting oxidative damage are upregulated by *X. fastidiosa* infection than genes responsive to pathogens or biotic stresses. This is consistent with *Arabidopsis* responding more to the water deficit induced by xylem blockage than directly to the infecting bacteria.

A published study of differential gene expression in grapevine in response to *X. fastidiosa* infection (Lin et al. 2007) shows a number of similarities to the results presented here. Seven of the 1,942 expressed sequence tags (EST) sequenced by Lin and associates (2007) are highly similar to differentially regulated *Arabidopsis* genes identified here. For example, the protein sequence of AtXTR3 (At5g57550), a putative xyloglucan endotransglycosylase, is 74% identical and 89% similar to an EST identified by Lin and associates (2007). These proteins are likely to be involved in cell-wall biogenesis and in strengthening cell walls as a defense response against certain pathogens. However, a number of classical PR genes (PR-2/β 1-3 glucanase, chitinase, and proline-rich proteins) were differentially expressed in grape but not in *Arabidopsis* (Lin et al. 2007). It may be necessary to use laser capture microdissection or a similar technique to examine changes in gene regulation specifically induced by *X. fastidiosa* in cells directly contacting the pathogen early in the infection process.

In conclusion, this work describes the evaluation of *Arabidopsis thaliana* as a model host for *X. fastidiosa*. Although *X.*

fastidiosa does multiply and spread through the infected *Arabidopsis* leaf, it does not reach as high titers in *Arabidopsis* as it does in susceptible grapevines. Nor does it cause symptoms or move systemically. The latter is probably the biggest drawback of *Arabidopsis* as a model host. Neither pathogen factors that are necessary for systemic movement, like the *pglA* gene, nor host responses that limit movement can be studied in *Arabidopsis*. Lower bacterial titers are also an important difference between grape and *Arabidopsis*; it is possible that *Arabidopsis* may be a better model for the asymptomatic plant hosts that serve as agronomically important disease reservoirs. The lack of symptom development is a smaller drawback. While it is certainly reassuring to observe symptoms during the course of infection, it is not necessary. The vast majority of experiments infecting grape or other agronomic hosts of *X. fastidiosa* quantitate bacterial growth by PCR, ELISA, or isolation, as must be done for *Arabidopsis*. Symptoms are rarely used as the only measure of bacterial growth. It is possible that the lack of symptom development in *Arabidopsis* is due mainly to the short lifespan of an individual *Arabidopsis* leaf. However, the lack of symptoms could also reflect significant biological differences in the host-pathogen interaction. A marked advantage of *Arabidopsis* is the availability of insertional mutations in almost all genes. Experiments examining the susceptibility of mutants in differentially expressed genes are underway. If such experiments identify defense responses important for limiting the growth of *X. fastidiosa*, *Arabidopsis* will certainly be a useful model host. Currently, *Arabidopsis* can be used to test the pathogenicity of certain kinds of bacterial mutants (*tolC* for example).

MATERIALS AND METHODS

Bacterial strains and culture conditions.

Xylella fastidiosa Temecula marked with GFP (Newman et al. 2003) was used unless stated otherwise. The *pglA* mutant and corresponding Fetzer wild type were obtained from B. Kirkpatrick (University of California, Davis, CA, U.S.A.) (Roper et al. 2007). The *tolC* mutant (in the Temecula strain) was obtained from D. Gabriel (University of Florida, Gainsville, FL, U.S.A.) (Reddy et al. 2007) and *rpfF* mutant (also in Temecula) from S. Lindow (University of California, Berkeley, CA, U.S.A.) (Chatterjee et al. 2008b). The strain Stag's Leap was obtained from J. C. Chen (United States Department of Agriculture Agricultural Research Service, Parker, CA, U.S.A.) (Krivanek et al. 2005a). PWG plates (Davis et al. 1981) were streaked with *X. fastidiosa* and were allowed to grow at 27°C for 10 to 14 days. Bacteria were harvested by scraping the plates and were washed three times in water and were resuspended at an optical density at 600 nm = 0.2 for inoculation.

Arabidopsis growth and inoculations.

Arabidopsis thaliana seeds were obtained from the Arabidopsis Biological Resource Center and were grown in Metro-Mix200 (SunGro, Bellevue, WA, U.S.A.) at 22°C under a 10-h light and 14-h dark cycle. Plants were inoculated at 4 to 4.5 weeks of age by dropping 1 µl of *X. fastidiosa* suspension or water (for mock inoculations) on the midrib at the petiole-leaf junction and piercing under the drop four to five times with a 0.15-mm diameter minuten pin (Sphinx/BioQuip, Rancho Dominguez, CA, U.S.A.). Three to four leaves at similar developmental stages were inoculated per plant. The Nordborg

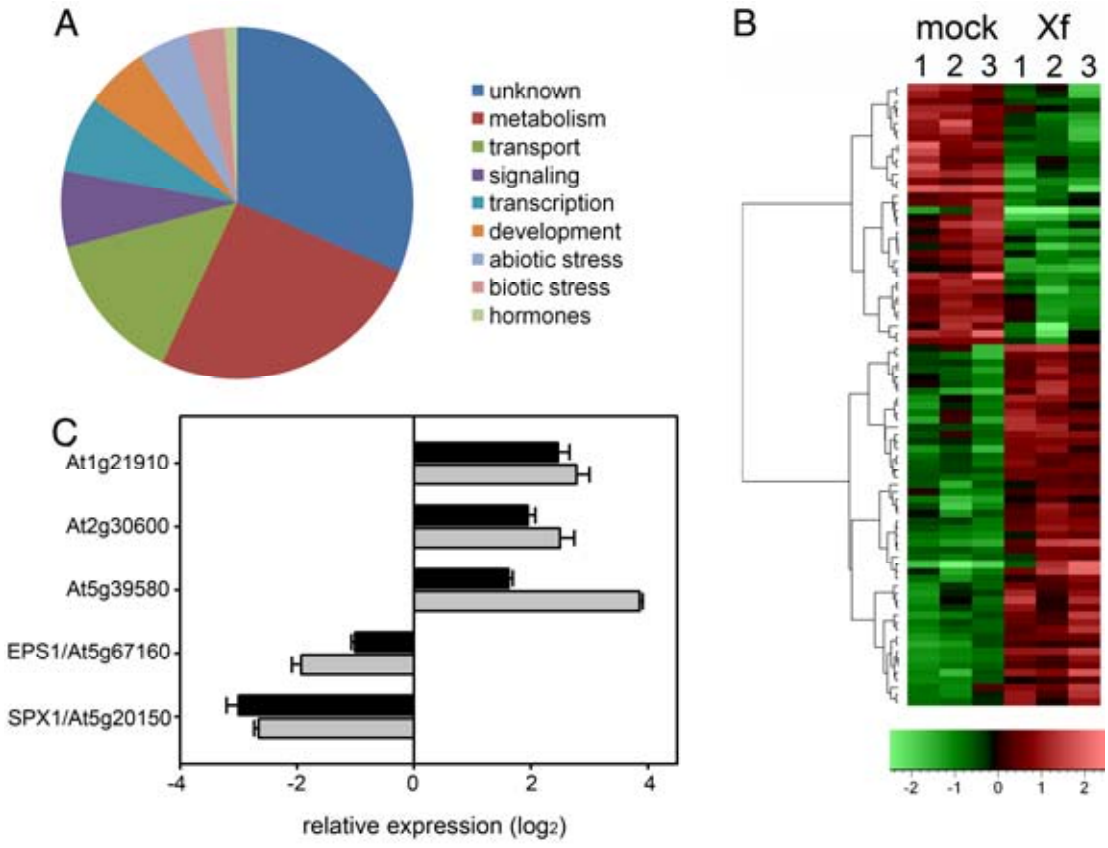

Fig. 4. A, Genes differentially regulated by *Xylella fastidiosa* infection fall into different gene ontology categories. **B,** Heat map clusters differentially regulated genes based on similar expression patterns. Each row depicts the expression of one gene across all six arrays (columns). **C,** Quantitative reverse-transcriptase polymerase chain reaction (PCR) confirms differential regulation of all five genes tested. PCR was run on three paired sets of RNA. Gray bars represent average expression, while error bars indicate standard deviations. Microarray results (black bars) are included for comparison.

96, a collection of 96 genetically and geographically disparate ecotypes (Nordborg et al. 2005), was obtained from the Arabidopsis Biological Resource Center.

Microscopy of plant tissues.

Light micrographs of fresh-mounted leaves were taken with a Leica DM6000B fluorescence microscope using an L5 filter at 10 dpi. Electron microscopy was performed as previously described (Francis et al. 2008).

Bacterial detection in plant tissues.

For qRT-PCR, total DNA was extracted from two infected leaves, using a modification of a previously published protocol (Dellaporta et al. 1983). Typically, leaves were harvested at 7 and 14 dpi. PCR was performed using Xf145 primers (Ledbetter and Rogers 2009) and Applied Biosystem's Sybr Green Master Mix in a Step One Plus thermocycler (Applied Biosystems, Foster City, CA, U.S.A.) following manufacturer's protocols. Prior to isolation of *X. fastidiosa* from *Arabidopsis*, detached leaves were surface-sterilized in 10% bleach for 2 min, followed by two washes in sterile water. Two punches per leaf were cut with a #3 cork borer, were placed in a sterile tube containing a $^1/_4$-inch glass bead, and were macerated using a FastPrep-24 tissue homogenization system (MP Biomedicals, Solon, OH, U.S.A.). The macerate was diluted in sterile water and was plated on PWG plates. Colonies were verified to be *X. fastidiosa* by GFP fluorescence and were counted 12 to 14 days after plating. Eight to 12 replicate samples were assayed per time point and genotype. ELISA was performed using a commercially available kit according to the manufacturer's directions (Agdia Biofords, Elkhart, IN, U.S.A.).

Gene expression analysis.

X. fastidiosa– and mock-inoculated *Arabidopsis* leaves were harvested at 9 dpi and midribs were dissected out. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.), and integrity was assessed using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, U.S.A.). Three biological replicate pairs of *X. fastidiosa*– and mock-inoculated RNA was hybridized to Affymetrix ATH-1 *Arabidopsis* whole-genome arrays according to the manufacturer's instructions. Probe sets with detection P values <0.05 in any of the three replicates were removed. DEG are those that show an absolute fold change >2.0, independent t-test P values <0.05, and paired t-test P values <0.05. All post–RNA isolation microarray protocols were performed by Genome Explorations, Inc. (Memphis, TN, U.S.A.). DEG heat maps for each comparison were generated after $\log_2$ transformation and row mean centering of MAS5 signal values. Raw microarray data has been deposited at NASCarrays. Real-time qRT-PCR confirmation of DEG was performed using a Step One Plus thermocycler (Applied Biosystems) and Applied Biosystem's High Capacity RNA-to-cDNA kit and SYBR Green Master Mix following manufacturer's protocols. Primers were designed using QuantPrime.

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