Grapevine Phenolic Compounds in Xylem Sap and Tissues Are Significantly Altered During Infection by *Xylella fastidiosa*

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ABSTRACT

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Pierce's disease of grapevine (PD), caused by the bacterial pathogen *Xylella fastidiosa*, remains a serious problem for grape production in California and elsewhere. This research examined induction of phenolic compounds in grapevines ('Thompson Seedless') infected with *X. fastidiosa* over a 6-month period. Two months postinoculation with *X. fastidiosa*, catechin, digalloylquinic acid, and astringin were found at greater levels in xylem sap; multiple catechins, procyanidins, and stilbenoids were found at greater levels in xylem tissues; and precursors

to lignin and condensed tannins were found at greater levels in xylem cell walls. However, such large-scale inductions of phenolic compounds were not observed 4 months after inoculation. Six months after inoculation, infected plants had significantly reduced phenolic levels in xylem sap and tissues when compared with control plants, including lowered levels of lignin and condensed tannins. At 6 months, PD symptoms were severe in infected plants and most photosynthetic tissue was abscised. These results suggest that, even though grapevine hosts may initially respond to *X. fastidiosa* infections with increased production of phenolic compounds, ultimately, PD causes grapevines to enter a state of decline whereby diseased hosts no longer have the resources to support secondary metabolite production, including defense-associated phenolic compounds.

Pierce's disease (PD), caused by the xylem-limited bacterium *Xylella fastidiosa* Wells et al., remains a serious threat to the \$3 billion Californian grape (*Vitis vinifera* L.) industry (8). One recent epidemic caused a loss of 30% of vineyards in Temecula Valley, CA for an estimated cost of \$46 million (8,40).

Despite recent research on PD in California, including efforts to breed resistance into commercial grapevines (11,15,27,41), little is known about grapevine responses to *X. fastidiosa* infection. Furthermore, it is unclear what, if any, induced defense responses occur in the xylem of *X. fastidiosa*-infected grapevines and whether or not such defense responses can limit *X. fastidiosa* infections or the progression of PD.

Recent efforts have examined key components of grapevine resistance to *X. fastidiosa* infection; in particular, the upregulation of certain defense-associated proteins or RNAs following infection (5,26,45). Furthermore, the role of grapevine xylem anatomy in effecting *X. fastidiosa* intra-plant movement also has been confirmed (4,9,38).

This current study complements previous grapevine resistance research by exploring the role secondary metabolites could play in pathogen-induced host defense responses that could occur during *X. fastidiosa* infections. Phenolic compounds produced by grapevines range from cell-wall-thickening compounds such as lignin (and its coumaric acid derivative precursors) and tannins

(both condensed and hydrolysable) to compounds associated with antibiotic activity (e.g., stilbenoids and flavonoids) (2). Many of these phenolic compounds were shown to be induced when grapevines were infected by fungal pathogens such as *Botrytis cinerea* (12,21,24,34,39), viruses (17), and phytoplasmas (33). Presumably, grapevines produce greater amounts of phenolic compounds following infections because these compounds act to inhibit, impair, or otherwise kill pathogens. Some grapevine phenolic compounds were positively associated with resistance to infection by pathogens such as *B. cinerea*, *Plasmopara viticola*, and *Erysiphe* spp. (13,14,16,35,36). Furthermore, phenolic compounds were observed to limit the in vitro growth of certain pathogens (1,30,31), including *X. fastidiosa* (28). One of the most important grape phenolics in host defense against pathogens is resveratrol, a phytoalexin stilbenoid called the "key to resistance in grape" (18).

Because of the well-documented roles that phenolics have in providing resistance to other pathogens (2), the objective of this study was to observe changes in concentrations of grapevine xylem phenolics that occur following inoculation with *X. fastidiosa*. Phenolics were monitored within xylem sap, tissue, and cell walls 2, 4, and 6 months after initial inoculation to observe any changes and determine whether they were consistent. We hypothesized that infected grapevines possess greater phenolic concentrations in xylem sap and tissues (both methanol-soluble and cell-wall-embedded phenolics). Few studies have focused on woody plant biochemical responses to xylem-dwelling bacteria. Therefore, this study is important because it not only expands our understanding about the host-pathogen interactions that occur in grapevines infected with *X. fastidiosa* but also expands our knowledge about plant responses that occur to xylem-fastidious bacteria in general.

MATERIALS AND METHODS

Plant materials and experimental design. In total, 30 *V. vinifera* 'Thompson Seedless' vines (on 'Freedom' rootstock)

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were arranged in two completely randomized blocks within a climate-controlled greenhouse running a 14-h day-length cycle. Thompson Seedless grapevines were chosen because this cultivar was previously observed to be moderately tolerant of *X. fastidiosa* infection (3,23,32). In November 2010, half of the plants in each block were mechanically inoculated with the Stag's leap strain of *X. fastidiosa* by using a modification of the pin-prick method of Hopkins (20), using water to resuspend bacterial cultures prior to infection. Three inoculations were made of the scion just above the rootstock and, to increase success, grapevines were not watered 24 h prior to inoculation. The other half of the plants were mock inoculated similarly with water. Two weeks after the initial inoculations, the inoculation treatments were repeated to further increase inoculation efficiency. One month after the initial inoculation and 2 weeks after the second inoculation, *X. fastidiosa* infection success was determined using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) according to the methods described below.

At 2, 4, and 6 months after the initial inoculation, 10-cm-long shoot samples, weighing ≈ 10 g each, were collected from all plants ≈ 30 cm apically from the inoculation point. Different shoots were sampled at each time. Collected samples were placed into 15-ml centrifuge tubes, immediately flash frozen in liquid nitrogen, and stored at -20°C until chemical analyses.

At the same time, an additional 5 g of plant material was harvested directly underneath the chemistry samples and immediately processed for both ELISA and PCR in order to detect *X. fastidiosa*. ELISA detection was made following the manufacturer's instructions using ELISA kits from Promega Corp. (Fitchberg, WI). PCR was conducted using the methods and primers as described by Chen et al. (10). Plants inoculated with *X. fastidiosa* that failed to test positive for *X. fastidiosa* were excluded from chemical analyses.

At the completion of the experiment (6 months), plants were evaluated for PD symptoms by placing them into one of the following categories: 0 (no disease), 1 ($<25\%$ symptomatic leaf tissue), 2 (25 to 50% symptomatic leaf tissue), 3 (51 to 75% symptomatic leaf tissue and some stunting of new shoots), 4 (76 to 100% symptomatic leaf tissue and severe stunting of shoots), or 5 (dead). Dead plants were excluded from chemical analyses. Some noninfected controls received non-zero ratings because symptom ratings were independent of tests for *X. fastidiosa*.

Chemical analyses. The frozen shoots were debarked by peeling away the outermost (phloem and bark) layers with a straight razor, and the denuded wood segments were then divided into two equal portions. One wood segment was cut into portions to fit inside 1.5-ml centrifuge tubes with 5 to 10 glass beads on the bottom. These tubes were centrifuged at $12,000 \times g$ for 5 min at 4°C to cause the xylem sap to leave the xylem tissue and collect in the bottom of the tubes. The xylem sap was moved to clean tubes and diluted 1:1 (vol/vol) in water for high-performance liquid chromatography (HPLC) analyses as described below.

The remaining (uncentrifuged) tissue was ground to a powder in liquid nitrogen with a mortar and pestle grinder (Retsch, Newtown, PA). The ground tissue was weighed out into two 100-mg aliquots in separate microcentrifuge tubes. One of these tubes received 500 μl of methanol (Thermo-Fisher Inc., Pittsburgh, PA) and was stored overnight at 4°C , had the methanol removed to a clean tube, and then was re-extracted with an additional 500 μl of methanol overnight at 4°C (for a total of 1 ml of methanol in a combined extract). The remaining pellet was extracted for cell-wall-bound phenolics (6) by washing once in 1 ml of water, then in 1 ml of methanol, and finally in 1 ml of *tert*-butyl methyl ether (Sigma-Aldrich, St. Louis). After drying overnight in a chemical hood, the pellet was resuspended in 400 μl of 1 N NaOH (Sigma-Aldrich) with 100 mM ascorbic acid (Sigma-Aldrich) and 0.2% (vol/vol) of NaBH_4 (Sigma-Aldrich). This sample incubated for 24 h on a shaker at 40°C , after which

the solution was acidified with 400 μl of 1.5 M formic acid (Sigma-Aldrich) and then 200 μl of methanol was added. After centrifugation, the cell-wall-bound phenolic solution was transferred to a clean tube before analysis by HPLC, as described below.

The remaining pellet from the previous extraction was washed twice in water, treated with 800 μl of 2 N HCl (Sigma-Aldrich) and 300 μl of thioglycolic (mercaptoacetic) acid (Sigma-Aldrich), then heated at 86°C for 4 h to extract lignin (6,42). The suspension was centrifuged and the supernatant removed. The pellet was washed twice in 1 ml of water, resuspended in 1 ml of 0.5 M NaOH, and shaken for 18 h. The NaOH supernatant was then removed and transferred to clean 2-ml centrifuge tubes, and the pellet was again suspended in 0.5 ml of 0.5 M NaOH and shaken for 18 h. Both extracts were combined for a total of 1.5 ml of supernatant. To this supernatant, 300 μl of 12 N HCl was added and the mixture was incubated at 25°C for 4 h. The tubes were centrifuged ($12,000 \times g$ for 5 min), the supernatant was removed, and the pellet was resuspended in 1 ml of 0.5 M NaOH. Then 100 μl of this solution was pipetted into a 2-ml cuvette with 1.9 ml of 0.5 M NaOH and absorbance was read at 280 nm using a Libra S22 spectrophotometer (Biochrom, Miramar, FL). Absorbance values were recorded and compared with a standard curve of lignin (Sigma-Aldrich) suspended in 0.5 M NaOH at concentrations of 20, 40, 80, 160, or 320 $\mu\text{g}/\text{ml}$ in order to calculate the total weight of lignin in each sample.

The second aliquot of 100 mg of finely ground xylem tissue was extracted for condensed tannins using the methods of Wallis et al. (44). In brief, the samples were twice extracted in 0.5 ml of 70% acetone (Sigma-Aldrich) overnight at 4°C . The combined 1 ml of 70% acetone extract was then washed once with 0.5 ml of petroleum ether (Sigma-Aldrich), followed by a wash with 0.5 ml of ethyl acetate (Sigma-Aldrich), centrifuging at $10,000 \times g$ after each wash to cause phase separation. The top organic solvent phase was discarded each time. The remaining acetone solution was evaporated overnight in a fume hood. To the remaining solution, 0.5 ml of water was added. Of this solution, 0.2 ml was added to 1.2 ml of acidified n-butanol (95:5 [vol/vol] n-butanol:12 N HCl [Sigma-Aldrich]), and 20 μl of iron reagent (2% [wt/vol] ferric ammonium sulfate dodecahydrate salt [Sigma-Aldrich] diluted in 2 M HCl). This mixture was vortexed until the solution turned clear, and it was then heated at 95°C for 1 h. Each sample solution then had 100 μl pipetted into a microplate and absorbance was read at 550 nm using a Biotek Epoch reader (Winooski, VT). Condensed tannin standards were prepared using the ytterbium acetate-based gravimetric method (44), and used to establish condensed tannin standard curves (tannin amounts were adjusted 1:1 in order to account for the weight of bound ytterbium acetate).

The xylem sap, methanol extracts, and cell-wall-bound extracts were analyzed by HPLC as described by Wallis et al. (43), with a few modifications. In total, 50 μl of each sample was injected into a Shimadzu (Columbia, MD) LC-20AD dual-pump system equipped with a SIL-20A HT auto-sampler cooled to 4°C , a Shimadzu Shim-Pack XR-ODS 4.6-by-100-mm column in a CTO-20 column oven set at 50°C , and an SPD-20A photodiode array detector. A binary gradient with a flow rate of 1.0 ml/min was used, with an initial hold for 2 min of 95% solvent A (water with 2% [vol/vol] acetic acid) (Sigma-Aldrich), changing to 52.5% solvent B (methanol with 2% [vol/vol] acetic acid) over the next 20 min, then changing to 100% solvent B over the next 10 min, holding at 100% solvent B for 3 min, and finally changing back to 95% solvent A over 5 min to prepare for the next run (a total run time of ≈ 40 min). Compounds were quantified with the SPD-20A scanning at 280 or 320 nm depending on the compound. Additional wavelengths such as 370 and 525 nm also were monitored but no peaks were consistently observed at high-enough levels to be quantifiable at these wavelengths. The threshold for quantification by peak areas was $\approx 10,000$ mAU/min;

therefore, compounds whose peak areas were below this value were not quantified.

Peaks were putatively identified to compounds by matching UV/Vis spectra, relative retention times, and masses as determined by running voucher xylem sap, tissue, and cell wall samples on a Shimadzu LCMS-2020 HPLC-mass spectrometer, running the same gradient program as that of the HPLC analyses. The liquid chromatography–mass spectrometry analysis used an ESI probe, and masses for each peak were matched to compounds previously identified as occurring in grapevine (e.g., those described by Ali et al. [2]). Unequivocal identifications of peaks to compounds was made by matching retention times, UV/Vis spectra, and masses with those of known standards for the following compounds: caffeic acid, catechin, caftaric acid (albeit not to enantiomers), chicoric acid, coumaric acid, epicatechin, epicatechin gallate, ferulic acid, gallic acid, isoquercetin, piceid, procyanidin B1, procyanidin B2, procyanidin C1, quercetin, quinic acid, and resveratrol (trans-resveratrol) (all from Sigma-Aldrich). All other identified compounds (e.g., astilbin, coutaric acid, fetaric acid, and other procyanidins) were unable to be obtained commercially and, therefore, remain putatively identified. Some peaks were unable to be matched to compounds that are known to occur in grapevines and, therefore, were labeled as unknowns.

Compounds were quantified using equations generated from analyses of standard curves at the appropriate wavelength (either 280 or 320 nm). Catechin, epicatechin, and other flava-3-ols were converted to gram amounts by using a standard curve of catechin; procyanidins (both dimer and trimer forms) were converted based on a standard curve of procyanidin B2; other flavonoids were converted using standard curves from quercetin or quercetin-3- β -D-glucoside; caftaric, coutaric, and fetaric acids were converted to gram amounts using a standard curve of caftaric acid; other coumaric acid derivatives were converted to gram amounts using a standard curve of ferulic acid; hydrolysable tannins compounds were converted using a standard curve of ellagic acid; stilbenoids were converted using a standard curve of resveratrol; and chicoric acid was converted using a standard curve of itself. Unknown compounds were not converted to gram amounts, and were analyzed by raw peak areas (mAU/min) at 280 or 320 nm.

Concentrations of compounds in the same phenolic class were summed together for statistical analyses, depending on the sample, as follows: procyanidins and catechins (flava-3-ols), other flavonoids, hydroxycinnamoyltartaric acids (e.g., caftaric, coutaric, or fetaric acids), coumaric acid derivatives, hydrolysable tannins or precursors (including ellagitannins), or stilbenoids. In cases where certain compounds were unable to be resolved from a particular plant, that plant was excluded from analyses of grouped compounds to avoid an inaccurate sum due to missing data.

Statistical analyses. For all statistical analyses, SPSS (ver. 19.0; IBM, Armonk, NY) was used with $\alpha = 0.05$. All data were screened prior to analyses using the EXPLORE feature for normality confirmation and outlier removal, with outliers defined as data points occurring greater than two standard errors away from the mean. Because each plant was sampled multiple times, repeated-measures analyses of variance (ANOVAs) were performed to examine differences resulting from inoculation treatment and multiple sampling of the same plant. However, because the same shoots were not measured at each time, univariate ANOVAs (model: sampling time, inoculation treatment, spatial block, and all interactions) also were utilized to assess total effects of sampling time and infection status for each compound and compound grouping. When spatial block terms (both block effect and interactions) were not significant, they were excised from ANOVAs. Due to strong effects of time and time–treatment interactions, each sampling time had infection status assessed by t tests to observe significant differences for a single sampling time (2, 4, or 6 months). Final PD ratings were associated with phenolic levels at 6 months postinoculation using Spearman's rho correlations.

RESULTS

Inoculation success. Of the 15 grapevines inoculated, 12 were positive for *X. fastidiosa* infection at all the sampling times. Three plants were initially positive at the beginning of the study (1 month after the first inoculation) but ceased to be ELISA and PCR positive when samples were collected at 2 months and, therefore, were excluded from subsequent analyses. One infected plant was excluded at 6 months because it was dead but, because it was still alive at 2 and 4 months, its chemistry was assessed at those times. At 6 months, 3 of the 11 remaining infected grapevines received the disease severity rating of 1, 4 received a rating of 2, 3 received a rating of 3, and 1 received a rating of 4.

Xylem sap phenolics. In total, 25 phenolic compound peaks were quantified for the xylem sap samples (Table 1). Repeated-measures ANOVAs determined that there were no significant effects ($P < 0.05$) of *X. fastidiosa* inoculation on chemistry, with the exception of the compound catechin ($F = 5.837$, $P = 0.027$, $N = 20$).

Nonrepeated-measures univariate ANOVAs determined that time was a major source of variation for sap concentrations of total phenolics ($F = 4.477$, $P = 0.016$, $N = 63$), catechins and procyanidins ($F = 3.441$, $P = 0.038$, $N = 69$), hydroxycinnamoyltartaric acids ($F = 27.326$, $P < 0.001$, $N = 72$), flavonoids ($F = 15.896$, $P < 0.001$, $N = 69$), and hydrolysable tannins ($F = 9.702$, $P < 0.001$, $N = 73$) (Fig. 1). All individual compound concentrations also were significantly ($P < 0.05$) affected by time, with the exception of procyanidin B1, a procyanidin C isomer, epicatechin, caftaric isomer 1, a coutaric acid dimer, engeletin, and unknown 2. *X. fastidiosa* infections significantly increased levels of procyanidin B1 ($F = 4.097$, $P = 0.047$, $N = 70$), procyanidin C1 ($F = 5.950$, $P = 0.017$, $N = 72$), and catechin ($F = 4.720$, $P = 0.033$, $N = 73$). Analyses of catechin concentrations revealed a significant ($P < 0.05$) interaction between time and infection treatment, whereas analyses of procyanidin B1 and procyanidin C isomer did not reveal any significant interactions. Spatial block effects and block–treatment interactions were not significant ($P < 0.05$) and, therefore, were removed from ANOVAs.

The t tests of the separate sampling times revealed that concentrations of total phenolics, catechins and procyanidins, and hydroxycinnamoyltartaric acids were not affected by *Xylella* infection 2 or 4 months postinoculation; however, significantly fewer levels of these compound groups were observed in infected plants compared with noninfected controls 6 months after inoculation treatment (Fig. 1). Concentrations of catechin, digalloylquinic acid, and astringin were greater in sap from infected plants 2 months after inoculation treatments, and catechin concentrations were greater in infected plants 4 months after inoculation (Table 2). One unknown (unknown 1) had greater sap levels in infected plants than noninfected plants 2 months postinoculation treatment ($t = 2.666$, $P = 0.014$, $N = 24$). In contrast, sap concentrations of procyanidin B1, a procyanidin C isomer, procyanidin C1, two caftaric acid isomers, and digalloylquinic acid were significantly less in infected plants than noninfected plants 6 months postinoculation (Table 2).

Xylem tissue phenolics. In total, 22 phenolic compound peaks were quantified for finely ground xylem tissue samples (Table 1). Repeated-measures ANOVAs determined that *X. fastidiosa* infection significantly increased concentrations of total non-catechin flavonoids ($F = 5.682$, $P = 0.030$, $N = 18$), total stilbenoids ($F = 6.167$, $P = 0.024$, $N = 18$), gallic acid hexoside ($F = 5.100$, $P = 0.045$, $N = 13$), taxifolin pentoside ($F = 16.393$, $P = 0.002$, $N = 14$), resveratrol ($F = 5.674$, $P = 0.035$, $N = 14$), and an astringin dimer ($F = 7.444$, $P = 0.018$, $N = 14$) in xylem tissue.

Nonrepeated-measures univariate ANOVAs observed time to be a major source of variation for concentrations of total phenolics ($F = 20.890$, $P < 0.001$, $N = 58$), total catechins and procyanidins ($F = 17.243$, $P < 0.001$, $N = 64$), coumaric acid derivatives ($F =$

7.163, $P = 0.002$, $N = 63$), other flavonoids ($F = 20.690$, $P < 0.001$, $N = 67$), and stilbenoids ($F = 11.876$, $P < 0.001$, $N = 64$) (Fig. 1). All individual compound concentrations also were significantly ($P < 0.05$) influenced by sampling time, with the exception of catechin and astringin. *X. fastidiosa* infection significantly increased levels of coumaric acid derivatives ($F = 4.765$, $P = 0.033$, $N = 63$), other (non-catechin) flavonoids ($F = 4.155$, $P = 0.046$, $N = 63$), stilbenoids ($F = 8.163$, $P = 0.006$, $N = 64$), procyanidin B2 ($F = 10.149$, $P = 0.002$, $N = 66$), epicatechin gallate ($F = 7.614$, $P = 0.008$, $N = 66$), gallic acid hexoside ($F =$

4.411, $P = 0.040$, $N = 66$), taxifolin pentoside ($F = 14.020$, $P < 0.001$, $N = 67$), resveratrol ($F = 8.030$, $P = 0.006$, $N = 67$), an astringin dimer ($F = 11.356$, $P = 0.001$, $N = 67$), unknown 1 ($F = 4.088$, $P = 0.048$, $N = 66$), and unknown 2 ($F = 4.564$, $P = 0.037$, $N = 67$). For analyses of most compounds, significant interactions were observed between time and treatment ($P < 0.05$), with the exception of total coumaric acid derivatives, gallic acid hexoside, and a ferulic acid derivative. Spatial blocks were not significant and did not significantly interact with treatment; therefore, blocks were removed from ANOVAs.

TABLE 1. Compounds quantified in xylem sap, pulverized tissue, or cell walls using high-performance liquid chromatography^a

Tissue, class	Compound	Retention Time	UV/Vis max	Mass
Sap				
Catechins and procyanidin	Catechin	8.685	278	290
	Epicatechin	11.11	273	290
	Procyanidin B isomer	4.96	279, 310	594
	Procyanidin B1	7.69	277, 320	578
	Procyanidin C1	8.1	276, 326	866
	Procyanidin C isomer	5.65	279, 312	866
Other flavonoids	Engeletin	16.65	261, 360	434
	Isoquercetin	14.16	360	464
	Quercetin	17.315	300, 365	302
	Miquelianin	18.3	360	478
Hydroxycinnamoyltartaric acids	Caftaric acid isomer 1	6.63	278	312
	Caftaric acid isomer 2	7.07	278	312
	Coutaric acid isomer 1	10.43	271	296
	Coutaric acid isomer 2	10.8	271	296
	Fetaric acid isomer 1	11.625	280	326
	Fetaric acid isomer 2	12	280	326
Hydrolyzable tannins	Digalloyl quinic acid	4.545	279	496
	Ellagitannin derivative 1	2.657	273	482
	Ellagitannin derivative 2	3.4	263	566
	Quinic acid	2.355	263	192
Stilbenoid	Astringin	18.18	283, 345	406
Unidentified	Unknown 1	1.803	274	268
	Unknown 2	9.245	274	600
	Unknown 3	12.86	276, 340	292
	Unknown 4	12.94	271	446
	Unknown 5	21.055	308	430
Xylem				
Catechins and procyanidin	Catechin	8.687	279	290
	Epicatechin	11.497	273	290
	Epicatechin gallate	14.524	272	442
	Procyanidin B1	7.727	279	578
	Procyanidin B2	9.757	277	578
	Procyanidin C1	8.073	279	866
	Procyanidin gallate	10.817	276	730
Other flavonoids	Astilbin	16.38	274, 317	450
	Engeletin	16.983	276	434
	Taxifolin pentoside	24.587	274	436
Stilbenoids	Astringin	18.313	273	406
	Astringin dimer	22.88	273	810
	Resveratrol	17.417	279, 315	228
	ϵ -Viniferin	20.9	275, 320	454
Coumaric acid derivative	Ferulic acid derivative	14.507	275	476
Hydrolyzable tannin	Gallic acid glucoside	6.227	279	332
Lignan	Chicoric acid	28.113	279, 314	474
Unidentified	Unknown 1	21.01	274	386
	Unknown 2	26.423	279, 314	506
	Unknown 3	27.78	285, 318	700
	Unknown 4	28.95	288, 319	682
Cell wall				
Coumaric acid derivatives	Caffeic acid	10.423	277, 322	180
	Coumaric acid	13.143	309	164
	Ferulic acid	14.45	322, 292	194
Catechins	Catechin	8.824	278	290
	Epicatechin	11.226	275	290
Hydrolyzable tannin	Gallic acid	3.09	269	170
Stilbenoid	Piceid	15.61	280, 318	390
Unidentified	Unknown 1	21.26	274	386
	Unknown 2	23.328	274	328
	Unknown 3	23.82	281, 324	330
	Unknown 4	24.467	274	342

^a Putative identifications were made based on matching of UV/Vis spectra (λ_{max}) or masses from liquid chromatography–mass spectrometry analyses.

Concentrations of total phenolics, catechins and procyanidins, and stilbenoids were greater in *X. fastidiosa* infected plants than noninfected controls 2 and 4 months following inoculation treatments (Fig. 2). Non-catechin flavonoid concentrations were

greater 2 months after inoculation treatments (Fig. 2). Levels of catechin, epicatechin, epicatechin gallate, procyanidin B2, procyanidin gallate, engeletin, taxifolin pentoside, astringin, astringin dimer, resveratrol, and a ferulic acid derivative were greater in

Fig. 1. Means ($\pm$ standard error) sap concentrations of phenolics, separated by compound class, present within both noninfected and *Xylella fastidiosa*-infected grapevines. Statistics (*t* test) for each sampling time are provided. Different capital letters next to sample times signify that mean concentrations were different between sampling periods regardless of infection status. In all cases, *N* = 63 to 73.

infected plants than controls 2 months postinoculation (Table 3). Epicatechin, epicatechin gallate, procyanidin B2, and taxifolin pentoside also were greater in infected plants than controls 4 months postinoculation (Table 3). Two unknowns were at greater levels in xylem tissue from infected plants 2 months postinoculation: unknown 2 ($t = 2.239$, $P = 0.036$, $N = 23$) and unknown 3 ($t = 2.416$, $P = 0.024$, $N = 23$). At 6 months postinoculation treatment, only taxifolin pentoside concentrations remained greater in infected plants than controls, whereas engeletin was present in greater amounts in noninfected plants than *X. fastidiosa*-infected plants (Table 3).

Xylem cell wall phenolics. In total, 11 phenolic compound peaks were quantified by HPLC from the cell-wall-bound phenolic extractions (Table 1). Lignin and condensed tannin content also were quantified by spectrophotometric methods. Repeated-measures ANOVAs determined that infection significantly increased levels of cell-wall-bound total phenolics ($F = 8.345$, $P = 0.009$, $N = 21$) and total catechins ($F = 8.953$, $P = 0.007$, $N = 22$) but significantly decreased levels of condensed tannins ($F = 8.473$, $P = 0.008$, $N = 24$). Individually, catechin ($F = 9.018$, $P = 0.007$, $N = 22$), epicatechin ($F = 5.227$, $P = 0.033$, $N = 22$), and coumaric acid ($F = 5.103$, $P = 0.035$, $N = 22$) were at greater concentrations in *X. fastidiosa*-infected plants than noninfected plants throughout the entire experiment.

Nonrepeated-measures univariate ANOVAs confirmed that time was a major source of variation for levels of cell-wall-bound total phenolics ($F = 24.899$, $P < 0.001$, $N = 74$), catechins ($F = 30.296$, $P < 0.001$, $N = 76$), and coumaric acid derivatives ($F = 13.014$, $P < 0.001$, $N = 75$), as well as lignin ($F = 47.238$, $P < 0.001$, $N = 71$) and condensed tannin ($F = 45.676$, $P < 0.001$, $N = 77$) (Fig. 1). All individual compound levels except caffeic acid also were significantly ($P < 0.05$) influenced by sampling time. *X. fastidiosa* infection significantly increased concentrations of cell-wall-bound total phenolics ($F = 10.313$, $P = 0.002$, $N = 74$), total catechins ($F = 10.409$, $P = 0.002$, $N = 76$), coumaric acid derivatives ($F = 5.854$, $P = 0.018$, $N = 75$), catechin ($F = 8.249$, $P =$

0.005, $N = 76$), epicatechin ($F = 8.364$, $P = 0.005$, $N = 76$), and coumaric acid ($F = 6.846$, $P = 0.011$, $N = 76$). Levels of condensed tannin were significantly lower in infected plants compared with noninfected plants ($F = 4.304$, $P = 0.042$, $N = 77$). Significant ($P < 0.05$) interactions of time and treatment were observed for all analyses of phenolic levels, with the exception of lignin and coumaric acid. Spatial blocks and block-treatment interactions were not significant. Therefore, blocks were removed from ANOVAs.

Total phenolics, catechins, and coumaric acid derivative concentrations were greater in cell walls of infected plants than in controls 2 months after inoculation treatments (Fig. 3). Total phenolics and catechins remained at greater concentrations in xylem cells walls 4 months postinoculation treatments (Fig. 3). Lignin and condensed tannin levels were greater in cell walls of noninfected plants than in infected plants 6 months after inoculation treatments (Fig. 3). Coumaric acid, ferulic acid, catechin, epicatechin, and gallic acid concentrations were greater in the cell walls of infected plants than controls 2 months postinoculation (Table 4). Concentrations of caffeic acid, epicatechin, and gallic acid were greater in the cell walls of infected plants than controls 4 months postinoculation (Table 4). Unknown 2 also was at greater levels in infected plants 2 months after inoculation ($t = 2.286$, $P = 0.032$, $N = 24$).

PD symptoms and correlations with grapevine chemistry. Symptoms of PD in this experiment were not visibly observed until 4 months after inoculation. Symptom ratings 6 months postinoculation were significantly greater in infected (2.33 ± 0.31) than noninfected controls (1.07 ± 0.12) ($t = 4.140$, $P < 0.001$, $N = 26$). Control plants exhibited minor leaf necrosis due to causes other than *X. fastidiosa* infection, because none of those plants tested positive for *X. fastidiosa* (whereas all *X. fastidiosa*-inoculated plants were positive for *X. fastidiosa* by both ELISA and PCR). Lignin, a major constituent of cell walls, had concentrations negatively associated with symptom ratings ($\rho = -0.512$, $P = 0.010$, $N = 24$) when both control and infected plants were

TABLE 2. *Xylella fastidiosa* infection effects on individual phenolic compound mean levels present within grapevine xylem sap at 2, 4, or 6 months after inoculation

Class, compound	Mean ($\pm$ standard error) levels ($\mu\text{g/ml}$) ^a								
	2 months			4 months			6 months		
	Control	Inoculated	<i>t</i>	Control	Inoculated	<i>t</i>	Control	Inoculated	<i>t</i>
Catechins and procyanidins									
Catechin	47.0 $\pm$ 7	132.0 $\pm$ 33	2.609*	35.5 $\pm$ 3.7	51.5 $\pm$ 6.7	2.207*	70.0 $\pm$ 10.0	51.5 $\pm$ 13.5	1.121
Epicatechin	24.1 $\pm$ 4.1	18.2 $\pm$ 2.2	1.208	15.6 $\pm$ 1.6	17.5 $\pm$ 2.9	0.611	22.0 $\pm$ 2.7	15.8 $\pm$ 2.3	1.610
Procyanidin B1	21.0 $\pm$ 6.0	122.0 $\pm$ 32.0	1.258	82.7 $\pm$ 18.2	82.8 $\pm$ 30.0	0.005	146.0 $\pm$ 33	46.0 $\pm$ 14	2.245*
Procyanidin B2	49.0 $\pm$ 10.4	54.6 $\pm$ 8.0	0.393	16.8 $\pm$ 2.5	15.8 $\pm$ 3.0	0.263	83.4 $\pm$ 20.5	49.1 $\pm$ 14.4	1.219
Procyanidin C isomer	25.4 $\pm$ 5.3	32.6 $\pm$ 5.5	0.948	23.6 $\pm$ 5.4	26.5 $\pm$ 4.2	0.398	47.4 $\pm$ 7.7	27.2 $\pm$ 3.1	2.037*
Procyanidin C1	54.8 $\pm$ 12.3	35.8 $\pm$ 6.6	1.332	54.2 $\pm$ 16.5	50.9 $\pm$ 13.6	0.147	151.0 $\pm$ 28	64.0 $\pm$ 16.0	2.225*
Other flavonoids									
Engeletin	6.21 $\pm$ 0.74	6.85 $\pm$ 1.08	0.486	7.39 $\pm$ 1.09	5.88 $\pm$ 0.97	1.006	6.92 $\pm$ 1.08	8.96 $\pm$ 2.18	0.928
Isoquercetin	9.08 $\pm$ 1.07	8.78 $\pm$ 1.04	0.204	6.66 $\pm$ 0.60	8.18 $\pm$ 1.34	1.119	20.90 $\pm$ 3.70	19.40 $\pm$ 1.3	0.295
Quercetin	9.70 $\pm$ 1.30	12.40 $\pm$ 1.60	1.344	2.77 $\pm$ 0.51	3.57 $\pm$ 0.91	0.806	8.21 $\pm$ 1.25	7.62 $\pm$ 0.75	0.354
Miquelianin	5.39 $\pm$ 0.55	5.83 $\pm$ 0.68	0.499	4.69 $\pm$ 0.66	4.82 $\pm$ 0.59	0.140	8.22 $\pm$ 1.21	7.89 $\pm$ 1.35	0.176
Hydroxycinnamoyltartaric acids									
Caftaric acid isomer 1	33.3 $\pm$ 3.3	32.6 $\pm$ 5.0	0.119	6.13 $\pm$ 1.6	4.47 $\pm$ 0.67	0.807	11.20 $\pm$ 2.5	4.80 $\pm$ 0.6	2.071*
Caftaric acid isomer 2	9.58 $\pm$ 2.70	8.69 $\pm$ 1.96	0.262	5.74 $\pm$ 1.22	4.96 $\pm$ 0.96	0.482	9.00 $\pm$ 0.93	5.52 $\pm$ 1.03	2.448*
Coutaric acid isomer 1	13.60 $\pm$ 1.6	14.00 $\pm$ 2.3	0.118	6.26 $\pm$ 0.75	7.97 $\pm$ 1.79	0.959	8.71 $\pm$ 1.85	5.30 $\pm$ 0.51	1.444
Coutaric acid isomer 2	5.61 $\pm$ 0.70	5.36 $\pm$ 0.62	0.274	5.10 $\pm$ 0.50	4.35 $\pm$ 0.59	0.988	7.24 $\pm$ 1.09	5.45 $\pm$ 0.74	1.196
Fetarinic acid isomer 1	5.51 $\pm$ 1.22	6.20 $\pm$ 1.03	0.431	3.74 $\pm$ 0.52	3.38 $\pm$ 0.60	0.451	4.73 $\pm$ 0.76	3.37 $\pm$ 0.45	1.341
Fetarinic acid isomer 2	4.37 $\pm$ 0.74	6.20 $\pm$ 0.96	1.520	3.13 $\pm$ 0.28	3.72 $\pm$ 0.55	1.017	4.25 $\pm$ 0.42	4.11 $\pm$ 0.54	0.211
Hydrolyzable tannins									
Digalloylquinic acid	10.4 $\pm$ 1.3	21.7 $\pm$ 4.4	2.540*	6.29 $\pm$ 0.43	7.00 $\pm$ 1.02	0.696	9.55 $\pm$ 1.77	4.84 $\pm$ 1.22	1.935
Ellagitannin derivative 1	11.6 $\pm$ 1.1	11.1 $\pm$ 1.3	0.319	7.98 $\pm$ 0.51	7.63 $\pm$ 0.71	0.399	5.82 $\pm$ 1.10	3.89 $\pm$ 0.63	1.323
Ellagitannin derivative 2	10.7 $\pm$ 1.0	12.0 $\pm$ 1.6	0.714	6.68 $\pm$ 0.51	7.20 $\pm$ 0.87	0.487	6.61 $\pm$ 1.22	4.76 $\pm$ 0.98	1.081
Quinic acid	14.2 $\pm$ 3.9	13.5 $\pm$ 1.7	0.228	10.70 $\pm$ 1.7	11.00 $\pm$ 1.3	0.115	17.30 $\pm$ 3.0	14.10 $\pm$ 3.0	0.710
Stilbenoid									
Astringin	0.31 $\pm$ 0.04	0.58 $\pm$ 0.12	2.300*	0.58 $\pm$ 0.10	0.50 $\pm$ 0.09	0.558	0.84 $\pm$ 0.10	0.89 $\pm$ 0.13	0.317

^a Inoculated = inoculated by *X. fastidiosa*; * indicates $P < 0.050$.

considered. Taxifolin pentoside concentrations from ground xylem tissue were positively associated with symptom ratings ($\rho = 0.527$, $P = 0.014$, $N = 21$) when both control and infected plants were considered. When correlations were made using infected plants only, the sap levels of miquelianin ($\rho = 0.788$, $P = 0.012$,

$N = 9$); xylem tissue levels of total stilbenoids ($\rho = 0.926$, $P = 0.008$, $N = 6$) as well as resveratrol ($\rho = 0.797$, $P = 0.032$, $N = 7$) and astringin dimer ($\rho = 0.896$, $P = 0.006$, $N = 7$); and total cell-wall-bound phenolics ($\rho = 0.749$, $P = 0.013$, $N = 10$) were significantly positively associated with symptom severity ratings. No

Fig. 2. Means ($\pm$ standard error) xylem concentrations of phenolics, separated by compound class, present within both noninfected and *Xylella fastidiosa*-infected grapevines. Statistics (t test) for each sampling time are provided. Different capital letters next to sample times signify that mean concentrations were different between sampling periods regardless of infection status. In all cases, $N = 58$ to 67.

other significant correlations were observed between PD symptom ratings and compound concentrations.

DISCUSSION

This research observed that grapevines might respond to *X. fastidiosa* infection initially (i.e., 2 months postinoculation) by producing increased amounts of phenolic compounds, including both phytoalexins (stilbenoids) and phytoanticipins (coumaric acid derivatives and flavonoids). As such, these results possible reveal that grapevines respond to *X. fastidiosa* with inductions of defense-associated compounds in the xylem. Previous studies have demonstrated the inducibility of phenolic compounds in grapevine xylem via abscisic acid application (29).

In general, compound levels in sap did not vary as much in response to *X. fastidiosa* infection as those from methanol extracts of pulverized xylem or from cell wall extractions. The relatively low water solubility of many phenolic compounds may have kept levels below detectable limits in xylem sap. Likewise, it

is possible that environmental influences on xylem sap flow added extra variability to sap chemical analysis. Future studies examining host biochemical changes due to *X. fastidiosa* infections may prefer to focus on analyzing pulverized xylem tissue levels in addition to sap analyses because of these reasons. Ultimately, accumulation of phenolics likely occurs in the xylem tissues surrounding the xylem itself before importation into the sap and, therefore, the biochemical makeup of the surrounding xylem tissue should be of interest in comparable studies.

Regardless, increased stilbenoid production 2 months after *X. fastidiosa* infection was observed and likely significant because of previous associations found between stilbenoids and antibiotic effects (2,18), even though levels were not increased 4 and 6 months after inoculation. A study by Maddox et al. (28) previously demonstrated that *X. fastidiosa* growth in vitro was significantly inhibited by considerable low (200 μ M) levels of resveratrol, which was the most abundant stilbenoid found in xylem tissue. Even though resveratrol was not observed at measureable levels in xylem sap, it was possible that grapevine

TABLE 3. *Xylella fastidiosa* infection effects on individual phenolic compound mean levels present within grapevine xylem tissue at 2, 4, or 6 months after inoculation

Class, compound	Mean ($\pm$ standard error) levels (μ g/g FW) ^a								
	2 months			4 months			6 months		
	Control	Inoculated	<i>t</i>	Control	Inoculated	<i>t</i>	Control	Inoculated	<i>t</i>
Catechins and procyanidins									
Catechin	250 $\pm$ 31	423 $\pm$ 84	2.176*	293 $\pm$ 25	347 $\pm$ 17	1.654	537 $\pm$ 61	329 $\pm$ 93	1.909
Epicatechin	188 $\pm$ 38	322 $\pm$ 48	2.214*	56.7 $\pm$ 9.0	90.4 $\pm$ 14.3	2.060*	114 $\pm$ 21	78 $\pm$ 23	1.062
Epicatechin gallate	230 $\pm$ 23	359 $\pm$ 56	2.368*	90 $\pm$ 8	157 $\pm$ 20	3.439**	95.3 $\pm$ 14.0	85.3 $\pm$ 20.4	0.408
Procyanidin B1	203 $\pm$ 20	290 $\pm$ 48	1.860	80 $\pm$ 8	120 $\pm$ 20	1.948	130 $\pm$ 15	102 $\pm$ 22	1.075
Procyanidin B2	141 $\pm$ 20	293 $\pm$ 53	3.019**	32.9 $\pm$ 6.2	65.0 $\pm$ 10.1	2.845*	80.7 $\pm$ 11.5	91.0 $\pm$ 23.6	0.446
Procyanidin C isomer	238 $\pm$ 18	271 $\pm$ 35	0.910	75 $\pm$ 8	117 $\pm$ 17	2.305*	134 $\pm$ 15	106 $\pm$ 22	1.081
Procyanidin gallate	135 $\pm$ 18	221 $\pm$ 39	2.193*	45.9 $\pm$ 6.4	70.8 $\pm$ 11.7	1.954	72.3 $\pm$ 10.4	54.4 $\pm$ 16.6	0.954
Other flavonoids									
Astilbin	58.1 $\pm$ 10.8	89.2 $\pm$ 16.8	1.638	12.9 $\pm$ 3.0	15.6 $\pm$ 3.7	0.579	27.3 $\pm$ 4.6	23.4 $\pm$ 11.1	0.391
Engeletin	69.6 $\pm$ 12.2	165 $\pm$ 44	2.390*	21.9 $\pm$ 2.8	36.2 $\pm$ 7.7	1.868	35.1 $\pm$ 4.8	13.1 $\pm$ 2.8	3.102**
Taxifolin pentoside	3.0 $\pm$ 0.7	21.0 $\pm$ 6.7	3.167**	1.49 $\pm$ 0.17	4.81 $\pm$ 1.32	2.737*	2.14 $\pm$ 0.32	4.67 $\pm$ 0.71	3.799**
Stilbenoids									
Astringin	0.271 $\pm$ 0.045	0.527 $\pm$ 0.104	2.495*	0.589 $\pm$ 0.064	0.617 $\pm$ 0.149	0.182	0.498 $\pm$ 0.101	0.522 $\pm$ 0.153	0.135
Astringin dimer	0.78 $\pm$ 0.11	2.85 $\pm$ 0.76	3.197**	0.60 $\pm$ 0.10	1.16 $\pm$ 0.29	1.975	0.857 $\pm$ 0.155	0.906 $\pm$ 0.147	0.198
Resveratrol	3.6 $\pm$ 1.1	19.0 $\pm$ 6.0	2.955**	1.87 $\pm$ 0.23	3.39 $\pm$ 0.82	1.943	2.27 $\pm$ 0.30	1.70 $\pm$ 0.32	1.171
ϵ -Viniferin	1.26 $\pm$ 0.58	2.13 $\pm$ 0.47	1.088	0.154 $\pm$ 0.067	0.304 $\pm$ 0.212	0.732	0.540 $\pm$ 0.228	0.290 $\pm$ 0.286	0.657
Coumaric acid derivative									
Feulic acid derivative	14.8 $\pm$ 3.2	42.6 $\pm$ 14.0	2.253*	15.1 $\pm$ 2.6	17.3 $\pm$ 4.0	0.471	39.5 $\pm$ 8.6	48.7 $\pm$ 10.1	0.660
Hydrolyzable tannin									
Gallic acid hexoside	14.4 $\pm$ 3.9	11.1 $\pm$ 3.3	0.600	9.44 $\pm$ 2.60	8.02 $\pm$ 2.08	0.407	33.9 $\pm$ 6.5	14.7 $\pm$ 3.9	1.970
Lignan									
Chicoric acid	7.73 $\pm$ 0.48	8.26 $\pm$ 0.64	0.678	6.00 $\pm$ 0.51	6.83 $\pm$ 0.41	1.233	6.31 $\pm$ 0.54	6.20 $\pm$ 0.79	0.118

^a Inoculated = inoculated by *X. fastidiosa*; * and ** indicate $P < 0.050$ and 0.010 , respectively.

TABLE 4. *Xylella fastidiosa* infection effects on individual phenolic compound mean levels present within grapevine xylem cell walls at 2, 4, or 6 months after inoculation

Class, compound ^b	Mean ($\pm$ standard error) levels (μ g/g FW) ^a								
	2 months			4 months			6 months		
	Control	Inoculated	<i>t</i>	Control	Inoculated	<i>t</i>	Control	Inoculated	<i>t</i>
Coumaric acid deriv.									
Caffeic acid	5.80 $\pm$ 0.92	7.37 $\pm$ 1.06	1.102	3.63 $\pm$ 0.53	7.06 $\pm$ 1.07	3.005**	4.82 $\pm$ 0.89	4.17 $\pm$ 0.84	0.504
Coumaric acid	73.6 $\pm$ 4.2	95.1 $\pm$ 6.9	2.853**	46.5 $\pm$ 3.9	51.4 $\pm$ 4.3	0.851	64.2 $\pm$ 3.7	68.1 $\pm$ 6.2	0.582
Ferulic acid	74 $\pm$ 16	158 $\pm$ 35	2.443*	63.8 $\pm$ 5.8	60.8 $\pm$ 6.1	0.361	84.8 $\pm$ 6.6	74.3 $\pm$ 5.5	1.143
Catechins									
Catechin	483 $\pm$ 54	740 $\pm$ 83	2.704*	283 $\pm$ 27	370 $\pm$ 49	1.615	315 $\pm$ 18	299 $\pm$ 34	0.465
Epicatechin	267 $\pm$ 20	371 $\pm$ 28	3.055**	199 $\pm$ 17	264 $\pm$ 21	2.470*	216 $\pm$ 13	185 $\pm$ 16	1.507
Hydrolyzable tannin									
Gallic acid	1,010 $\pm$ 60	1,420 $\pm$ 160	2.705*	633 $\pm$ 43	857 $\pm$ 63	2.998**	1,740 $\pm$ 190	1,620 $\pm$ 110	0.470
Stilbenoid									
Piceid	47.3 $\pm$ 3.1	41.3 $\pm$ 3.7	1.215	31.8 $\pm$ 3.5	32.7 $\pm$ 4.2	0.170	40.6 $\pm$ 2.6	36.0 $\pm$ 2.3	1.252

^a Inoculated = inoculated by *X. fastidiosa*; * and ** indicate $P < 0.050$ and 0.010 , respectively.

^b Abbreviation: deriv. = derivatives.

hosts accumulated this and other stilbenoids in order to delay the progression of *X. fastidiosa* infections. Positive correlations were observed between stilbenoid concentrations of infected plants and symptom ratings, which suggests that severely diseased plants responded more strongly to *X. fastidiosa* with increased stilbenoid production than plants with more mild infections. However, mean

stilbenoid concentrations of all infected grapevines did not differ than those in noninfected grapevines. Regardless, because *X. fastidiosa* infections can cause death in Pierce's diseased grapevines, *X. fastidiosa* likely possess mechanisms to ultimately overcome the negative effects that stilbenoids have on bacterial growth in vivo.

Fig. 3. Means ($\pm$ standard error) cell wall concentrations of phenolics, separated by compound class, present within both noninfected and *Xylella fastidiosa*-infected grapevines. Statistics (t test) for each sampling time are provided. Different capital letters next to sample times signify that mean concentrations were different between sampling periods regardless of infection status. In all cases, $N = 71$ to 77 .

Increased levels of catechins and procyanidins also were observed 2 months after *X. fastidiosa* inoculation, although increases were not observed 4 and 6 months after infection for all of these compounds. Catechin concentrations 2 months postinoculation (131.5 µg/ml) exceeded the concentration determined to inhibit *X. fastidiosa* growth (200 µM or 116 µg/ml) by Maddox et al. (28). Considering that concentrations of catechin in non-infected grapevines (46.5 µg/ml) were well below inhibitory levels, these data suggest that, 2 months postinoculation, infected grapevines were successful at increasing sap catechin concentrations to levels detrimental to *X. fastidiosa* growth.

Increased concentrations of cell-wall-bound phenolics also were observed 2 months after inoculation (albeit not 4 or 6 months postinoculation), including phenolics that are precursors to lignin (coumaric acid and ferulic acid) and catechins. Increased levels of these compounds from cell wall extractions could suggest that cell walls of infected plants become more specialized in order to limit *X. fastidiosa* infection. Increased lignin levels could result in thicker cell walls that were more resistant to degradation by *X. fastidiosa* and, therefore, limit the ability of the pathogen to move into adjacent vessel elements and spread throughout the plant. Partial evidence for this may be negative correlations between lignin levels and symptoms. However, a lack of increased lignin or condensed tannin content suggests that chemical changes to cell walls might not be enough of a significant response to help limit *X. fastidiosa* infection.

Although phenolic compounds were increased in infected grapevines 2 months after *X. fastidiosa* inoculation, by 6 months, phenolic production was significantly diminished. All *X. fastidiosa*-infected plants at 6 months exhibited severe signs of stress such as abscission of ≈50% of leaves. Remaining leaves were highly necrotic, and stunting of new shoots was apparent. Under this state of severe stress, it was likely that negative trade-offs occurred between primary and secondary metabolism, with host resources shifted toward primary metabolism (19,22,37). Hence, infected grapevines may have shifted away the resources needed to maintain a proper host defense response against *X. fastidiosa* in order to address a severe reduction in host primary metabolite production due to lost photosynthetic tissues (22,37). An exception to this may have been stilbenoid production and cell wall phenolic production, because disease severity at 6 months was positively associated with these compounds. Infected grapevines might have encountered a “point of no return” between 4 and 6 months whereby the disease was too far progressed to allow normal physiology to occur, the grapevines became symptomatic, and the plants entered a “survival mode” where most photosynthate was allocated for basic growth and maintenance (7).

Regardless, early induction of phenolic compounds may have benefited Thompson Seedless grapevines by delaying the progress of *X. fastidiosa* infections because of antibiotic activities. PD symptoms may have developed much quicker in the absence of induction of phenolic compounds in response to *X. fastidiosa*. This could explain, for instance, why certain grapevine cultivars, such as ‘Chardonnay’ or ‘Crimson Seedless’, exhibited greater *X. fastidiosa* titers and quicker symptom development than Thompson Seedless, whereas other cultivars, such as ‘Rubired’, may have slower symptom development and lower titers than Thompson Seedless (3). In other words, the capacity of particular cultivars to induce phenolics to higher concentrations may, in part, explain why some cultivars do not develop PD symptoms as quickly as others. If it is true that phenolics can delay or limit *X. fastidiosa* infections, then cultivars with naturally greater levels of phenolic induction following *X. fastidiosa* infection should be preferred over those with decreased phenolic production. Among other advantages, such cultivars could have increased instances of “winter curing”, a phenomenon which has been documented to allow infected grapevines to recover from PD (25).

In conclusion, this study observed changes in phenolic compound production that occurred when grapevines were infected with *X. fastidiosa*. Grapevine hosts responded to *X. fastidiosa* infection initially by producing greater levels of phenolics in xylem tissue, likely for release into the xylem sap as an attempt to limit *X. fastidiosa* infection. However, efforts to limit *X. fastidiosa* infection ultimately failed in Thompson Seedless grapevines. Future studies should examine phenolic production in various cultivars of grapevine, both susceptible and resistant, to observe whether constitutive or inducible phenolic concentrations are associated with delaying, preventing, or abating PD symptoms.

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