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# CHARACTERIZATION OF *XYLELLA FASTIDIOSA* PLANT CELL WALL DEGRADATION AND INHIBITION OF THE TYPE II SECRETION MACHINERY


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## ABSTRACT

*Xylella fastidiosa* (*Xf*) is the causal agent of Pierce's Disease (PD) of grapevine. This xylem-limited bacterial pathogen systemically colonizes the xylem by using cell wall-degrading enzymes (CWDEs) to dismantle the pit membrane barriers that separate xylem vessels. Tylose formation is the predominant vascular occlusion associated with *Xf* infection, and excessive tylose development has been linked to the extreme susceptibility of *Vitis vinifera* wine grapes to PD. Thus, we sought out to better understand this host defense response in the context of *Xf*-mediated cell wall degradation. By using visual evidence (SEM and microCT), coupled with transcriptome analyses of inoculated grapevines, we determined that endoglucanase-deficient *Xf* mutants differentially induce tylose production relative to the wild-type *Xf* strain. These findings indicate that *Xf* endoglucanases play a role in facilitating host tylose production. Given these findings and that *Xf* CWDEs are important for the degradation of pit membranes (thus allowing systemic colonization), it is imperative that these virulence factors are targeted for inhibition. However, inhibiting each CWDE individually as a commercial strategy for controlling *Xf* is both impractical and costly. As these CWDEs are predicted to be secreted by the Type II secretion system (T2SS), we are currently searching for natural products that block the T2SS, thus preventing the secretion of CWDEs, and subsequently minimizing both *Xf* systemic colonization and excessive host tylose production.


## LAYPERSON SUMMARY

*Xylella fastidiosa* (*Xf*) relies on degradation of the plant cell wall to move within the grapevine, which occurs through cooperation between at least two classes of enzymes that target different carbohydrate components of the complex scaffold of the plant cell wall. A major goal of this project is to determine the mechanisms that lead to disassembly of the plant cell wall that eventually leads to systemic colonization of *Xf* in grapevines. Here we have performed experiments designed to better understand what facilitates movement of the bacterium and the subsequent clogging of the water-conducting cells that worsens Pierce's Disease severity. In addition, we are designing experiments to inhibit the secretion machinery responsible for delivering the *Xf* enzymes that are involved in *Xf* movement throughout the plant, thus, providing a comprehensive approach to restriction of *Xf* and disease development rather than targeting individual enzymes.

## INTRODUCTION

*Xylella fastidiosa* (*Xf*) is the causal agent of Pierce's Disease (PD) of grapevine, a serious and often lethal disease (Hopkins and Purcell, 2002, Chatterjee et al., 2008, Purcell and Hopkins, 1996). This xylem-limited bacterial pathogen colonizes the xylem and in doing so must be able to move efficiently from one xylem vessel element to adjacent vessels (Roper et al. 2007). Xylem conduits are separated by pit membranes (PMs) that are composed of cellulose microfibrils embedded in a meshwork of pectin and hemicellulose, and prevent the movement of air embolisms and pathogens within the xylem (Buchanan, 2000). The pore sizes within that meshwork range from 5 to 20 nM, which will not allow passive passage of *Xf* cells whose size is 250-500 x 1,000-4,000 nM (Perez-Donoso et al., 2010, Mollenhauer & Hopkins, 1974). Based on functional genomics and *in planta* experimental

evidence, *Xf* utilizes cell wall-degrading enzymes (CWDEs) to actively digest the polymers within the PMs, thereby facilitating its movement throughout the xylem network (Simpson et al. 2000, Roper et al., 2007, Perez-Donoso et al., 2010). It is known that polygalacturonase (PG) is a major pathogenicity factor for *Xf* (Roper et al., 2007) and that it acts in concert with at least one EGase to breach the PM barrier (Perez-Donoso et al. 2010). EGases are implicated in virulence and colonization of the xylem in other bacterial phytopathogens, such as *Pantoea stewartii* subsp. *stewartii*, *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *campestris* (Gough, 1988, Roberts et al., 1988, Saile et al., 1997, Mohammadi et al., 2012). In our previous study (project # 14-0144-SA), we tested the role of the *Xf* EGases *in planta* by constructing deletion mutants in two of the EGases (Δ*engXCA1* and Δ*engXCA2*) and mechanically inoculating the modified *Xf* lines into *Vitis vinifera* cv. Cabernet Sauvignon grapevines. Interestingly, both Δ*engXCA1* and Δ*engXCA2* achieved the same titers (*data not shown*) in the Cabernet Sauvignon vines as wild-type *Xf,* yet they were less virulent and elicited fewer PD symptoms (Fig. 1).

PD symptom development is tightly correlated with the ability of *Xf* to degrade specific polysaccharides, namely fucosylated xyloglucans (part of the hemicellulosic component) and weakly esterified homoglacturonans (part of the pectin portion), that make up the intervessel PMs (Sun et al., 2011). In general, pectin is one of the first targets of cell wall digestion for invading pathogens and the resulting oligogalacturonides (OGs), which are smaller pieces of the pectin polymer, that are released are likely used as a carbon source for the invading pathogen. In addition, specific OGs with a degree of polymerization in the size range of 10-15 residues can also serve as signals that trigger host defense responses (Benedetti et al., 2015). These responses include accumulation of reactive oxygen species (ROS), expression of pathogenesis-related proteins, deposition of callose, activation of mitogen-activated protein kinases (MAPKs), among other defense related processes (Boller & Felix, 2009, Benedetti et al., 2015).

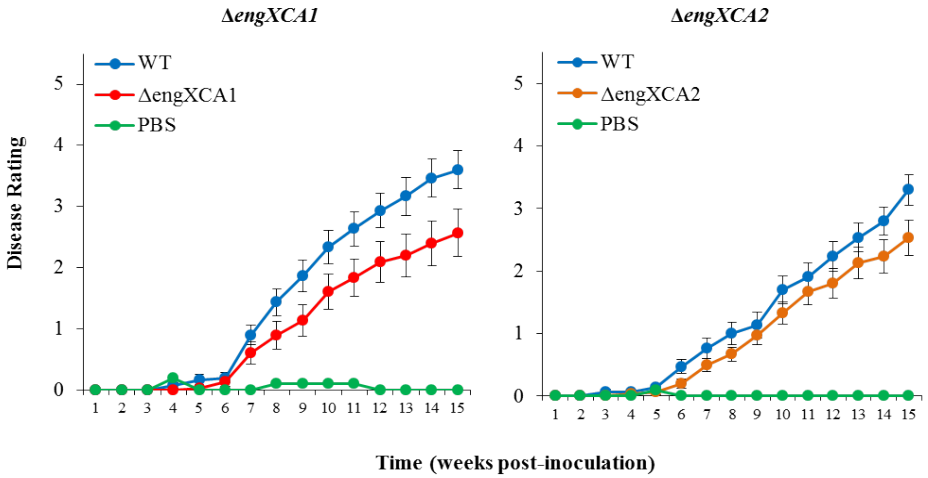


**Figure 1:** Pierce's disease development over 15 weeks in Cabernet Sauvignon grapevines after inoculation with wild-type Temecula 1 (blue), and the Δ*engXCA1* (red) or Δ*engXCA2* (orange) mutant strains. 1X PBS (green) served as the negative control. All vines were rated on a disease scale of 0-5, where 0 = healthy, 1-4 = increasing degrees of scorching, and 5 = vine death. Data are the means of three independent assays with ten replicates each. Bars represent the standard error of the mean.

Tyloses are outgrowths of parenchyma cells that emerge through vessel-parenchyma pits into vessel lumen, and are common in a wide range of species (Bonsen and Kučera 1990; Esau 1977; Tyree and Zimmermann 2002). Tyloses impede fluid penetration (Parameswaran et al. 1985) and induce a permanent state of reduced hydraulic conductivity, and are triggered by abiotic and biotic stresses, such as pathogen infection (Aleemullah and Walsh 1996; Collins et al. 2009; Dimond 1955; Parke et al. 2007). Tylose formation is the predominant vascular occlusion associated with *Xf* infection (Fig. 2A, B), and excessive tylose development has been linked to the extreme susceptibility of *Vitis vinifera* wine grapes to PD (Fritschi et al. 2008; Sun et al. 2013).

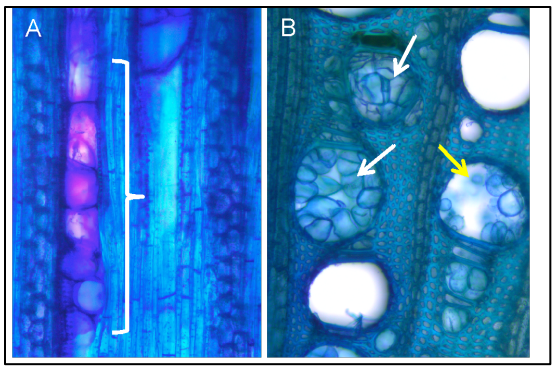


**Figure 2:** Xylem vessels of *V. vinifera* grapevines inoculated with *Xf*. **A.** Longitudinal section **B.** cross-section. Grapevine petiole sections were stained with toluidine blue O (0.05%). White arrows and bracket indicate vessels that are completely occluded with tyloses, and yellow arrow indicates a partially occluded vessel. Images taken by J. Rapicavoli (Roper Lab).

Importantly, rates of tylose development in *V. arizonica*, a resistant species, are much lower than those in *V. vinifera*, which may reflect differing innate immune responses to the presence of *Xf* in the xylem. To our knowledge, no one has looked at the molecular mechanisms underlying the differences in response to *Xf* among different *V. vinifera* cultivars. Thus, we sought out to better understand this difference in cultivar response to *Xf* in the context of host cell wall degradation and the elicitation of specific defense responses that lead to tylose formation in grapevines. Interestingly, a preliminary analysis of tylose formation in Cabernet Sauvignon vines inoculated with the Δ*engXCA1* mutant using a high resolution microCT technique (a kind of CAT scan) by the McElrone laboratory determined that these vines exhibited fewer tyloses than those inoculated with wild-type *Xf* (*data not shown*). Therefore, our hypothesis is that enzymatic degradation of the plant cell wall by *Xf* CWDEs is generating cell wall fragments that elicit DAMP signaling defense pathways, which leads to downstream tylose production and PD symptom development in certain grape cultivars.

Given that *Xf* CWDEs are important for the degradation of pit membranes (thus allowing systemic colonization), it is imperative that these virulence factors are targeted for inhibition. However, inhibiting each CWDE individually as a commercial strategy for controlling *Xf* is both impractical and costly. Interestingly, these CWDEs are predicted (using SignalP software) to be secreted via the Type II secretion system (T2SS). The T2SS is a molecular nanomachine that transports pre-folded proteins from the periplasm across a dedicated channel in the outer membrane (Cianciotto, 2005, Korotkov et al., 2012). The T2SS systems of many plant and animal pathogens are either known or predicted to secrete proteins, namely polymer degrading enzymes, which are involved in nutrient acquisition (Jha et al., 2005). Proteins destined for secretion by the T2SS are first delivered to the periplasm via the Sec or Tat-dependent secretion pathway where they are folded (Slonczewski, 2014). *Xf* appears to only possess the Sec-dependent secretion pathway. Because of our interest in *Xf* CWDEs and their mechanism of secretion, we created a mutation in the *xpsE* gene, which encodes the putative ATPase that powers the T2SS**.** Grapevines inoculated with the *xpsE* mutant never developed PD symptoms and remained healthy, a phenotype similar to the grapevine response to the *Xf* Δ*pglA* mutant (Fig. 3).

We hypothesize that this is due to the pathogen's inability to secrete the CWDEs necessary for xylem colonization. In addition, we have indirect experimental evidence that *Xf* utilizes the T2SS to secrete PG. We observed that the Δ*xpsE* mutant produces visibly less EPS on XFM minimal medium containing pectin as the sole carbon source, resulting in a much less mucoid phenotype (*data not shown)*. However, when wild-type *Xf* and Δ*xpsE* are grown on XFM+galacturonic acid (i.e., the monomeric sugar that makes up the pectin polymer) or on XFM+glucose, both strains produce similar amounts of EPS. We infer from this that the breakdown of the pectin substrate is necessary to produce EPS and when the T2SS is disrupted this prevents secretion of PG and the subsequent breakdown of pectin.

Thus, we have compelling *in planta* and *in vitro* preliminary data indicating that *Xf* has a functional T2SS system and the proteins secreted by T2SS are critical for the infection process. From this we reason that the T2SS represents an excellent target for disease control because disrupting this system would provide comprehensive inhibition of secretion of PG (the major pathogenicity factor for *Xf*) and the other auxiliary CWDEs (Roper et al. 2007 and recent results discussed above). Therefore, identifying molecules that can inhibit T2SS function is an excellent avenue of research to pursue to develop strategies that mitigate PD by preventing pathogen ingress.

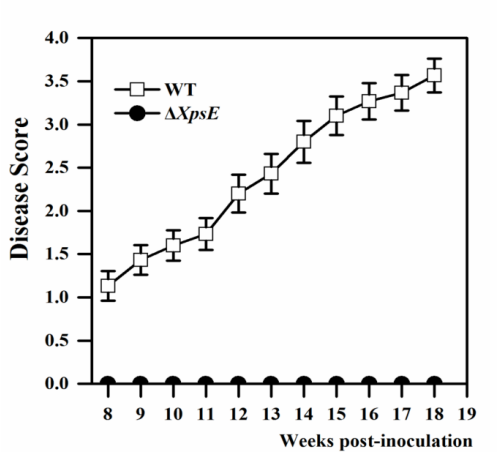


**Figure 3:** The *Xf* T2SS is necessary for PD development in grapevine. The Δ*xpsE* mutant does not induce PD symptoms in *V. vinifera* grapevines. Disease severity was based on a visual disease scale from 0 (no disease) to 5 (dead). Vines inoculated with 1X PBS (negative control) did not develop PD symptoms.

## OBJECTIVES

1) Qualitative analysis of the effect of cell wall degradation on the grapevine response to *Xf*.
2) Quantitative analysis of plant defense pathways induced by *Xf* cell wall degrading enzyme activity: biochemical and transcriptional studies.
3) Inhibition of the Type II secretion system using natural products produced by grapevine microbial endophytes.

## RESULTS AND DISCUSSION

**Qualitative analysis of the effect of cell wall degradation on the grapevine response to *Xf*.**

In the context of plant cell wall degradation, we examined the effects that different *Xf* endoglucanase mutants (Δ*engXCA1*, Δ*engXCA2*, and Δ*engXCA1*/Δ*engXCA2*) have on the integrity and carbohydrate composition of grapevine pit membranes using both microscopic and immunological techniques coupled with fluorescence (Sun et al., 2011) and/or electron (Sun et al., unpublished) microscopy. We coupled these microscopic observations with macroscopic studies of the spatial distribution of tyloses and other vascular occlusions, such as plant-derived gels and bacterial aggregates using high resolution micro-computed tomography (microCT). This non-destructive method technique uses x-rays to create cross-sections of an object that can be used to re-create a virtual model (3D model). These experiments will allow us to match degradation of specific host cell wall carbohydrates with spatiotemporal patterns of production of tyloses in 3 dimensions.

Wild-type *Xf* (Temecula 1) and *Xf* endoglucanase mutant strains have been used to inoculate Cabernet Sauvignon grapevines in the greenhouse. PBS-inoculated vines were used as negative controls. Each *Xf* strain was inoculated into 27 plants (3 biological replicates with 9 technical replicates each) and PD symptoms were rated each week using the 0 – 5 PD rating index (Guilhabert and Kirkpatrick, 2005). Vine samples (stem and petiole) were collected at three time-points covering early-, mid-, and late-infection based on the PD rating index (Early infection = 1 – 2, Mid-infection = 2 – 3, Late-infection = 3 – 4). Each sampling consisted of three biological replications (each with three technical replications) per treatment. All stem samples were analyzed using RNAseq, microCT, and EM to determine host response when challenged with either wild-type *Xf* or the *Xf* mutant strains.

**Modifications of different *Xf* strains on xylem structures of Cabernet Sauvignon vines –** Late time-point stem samples of Cabernet Sauvignon vines that were inoculated with PBS, wild-type *Xf*, or the *Xf* endoglucanase mutant strains were analyzed using SEM. We found that at the late time-point of PD symptom development, certain *Xf* strains display differences in vascular occlusion, intervessel PM integrity and *Xf* cell presence.

In the vines inoculated with PBS, vascular occlusion and *Xf* cells were not observed, and intervessel PMs remained mostly intact at the late time-point (Fig. 4). Vines inoculated with wild-type *Xf* also displayed significant xylem structural modifications at the late time-point. Over 50% of the vessels in the transverse section of a stem were occluded by tyloses, *Xf* cells occurred as large clusters in addition to individual occurrence or

small clusters, and intervessel PMs were completely degraded (Fig. 5). In late time-point samples from Δ*engXCA2*-inoculated vines, 30% of the total vessels were occluded with tyloses (Fig. 6A, B). Several broken intervessel PMs were present, and clusters of Δ*engXCA2* cells were seen near these broken PMs (Fig. 6C, D). However, late time-point samples from Δ*engXCA1*-inoculated vines showed relatively few tyloses despite several instances of significant intervessel PM degradation (Fig. 7). Interestingly, in the late time-point samples inoculated with the Δ*engXCA1*/Δ*engXCA2* double mutant, tyloses occurred in very few vessels (Fig. 8A, B), intervessel PMs were mostly intact, and Δ*engXCA1*/Δ*engXCA2* cells were not observed (Fig. 8C, D).

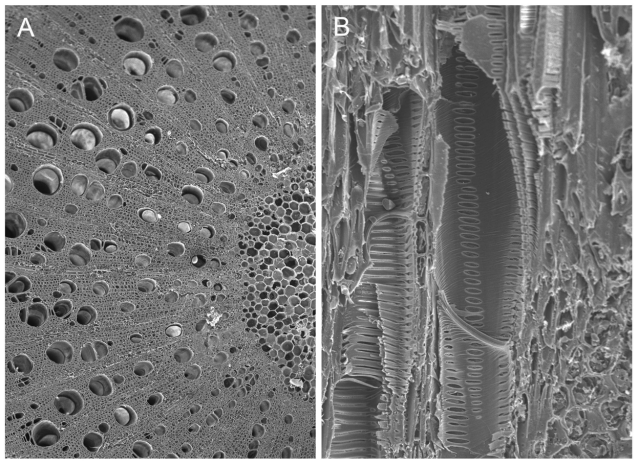


**Figure 4:** Xylem structural features in PBS-inoculated Cabernet Sauvignon vine at the late time point. **A.** Transverse section of stem secondary xylem, showing absence of occluded vessels. **B.** Longitudinal section of stem secondary xylem, showing vessels free of tyloses.

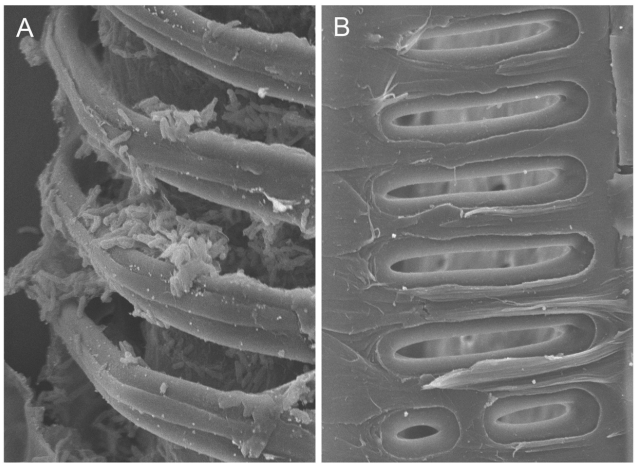


**Figure 5:** Xylem structural features in wild-type Temecula 1-inoculated Cabernet Sauvignon vine at the late time-point. **A.** Longitudinal section of stem secondary xylem, showing abundant presence of wild-type cells in a vessel. **B.** A longitudinally transected vessels, showing that intervessel PMs have completely disappeared.

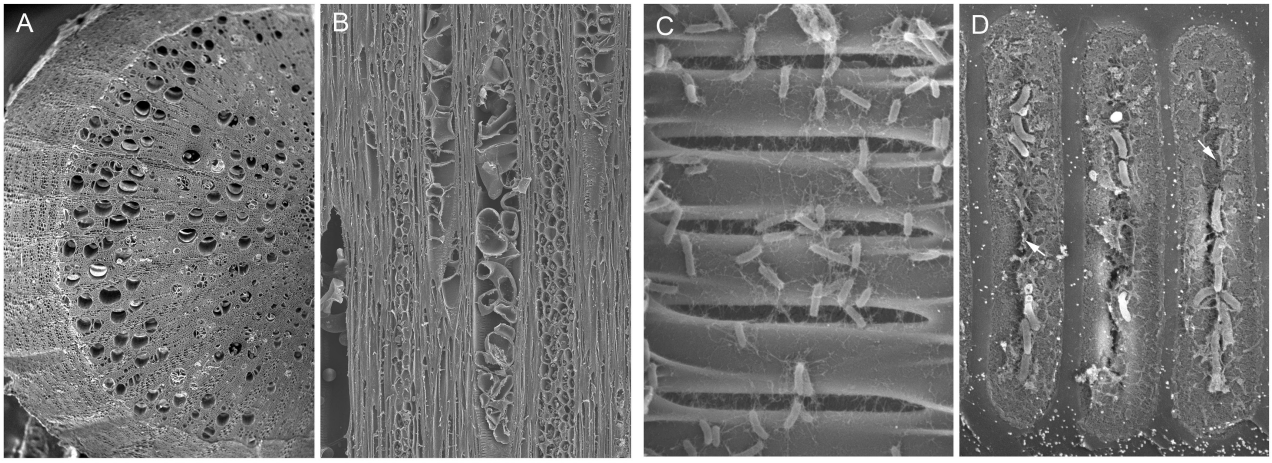


**Figure 6:** Xylem structural features in Δ*engXCA2*-inoculated Cabernet Sauvignon vine at the late time-point of PD symptom development. **A.** Transverse section of secondary xylem, showing occlusion in some vessels. **B.** Longitudinal section of secondary xylem, show two transected vessels fully occluded by tyloses. **C.** A longitudinally transected vessel, showing an abundant presence of Δ*engXCA2* cells. **D.** Δ*engXCA2* cells on partially some degraded intervessel PMs (arrows indicate pores or cracks in the PMs).

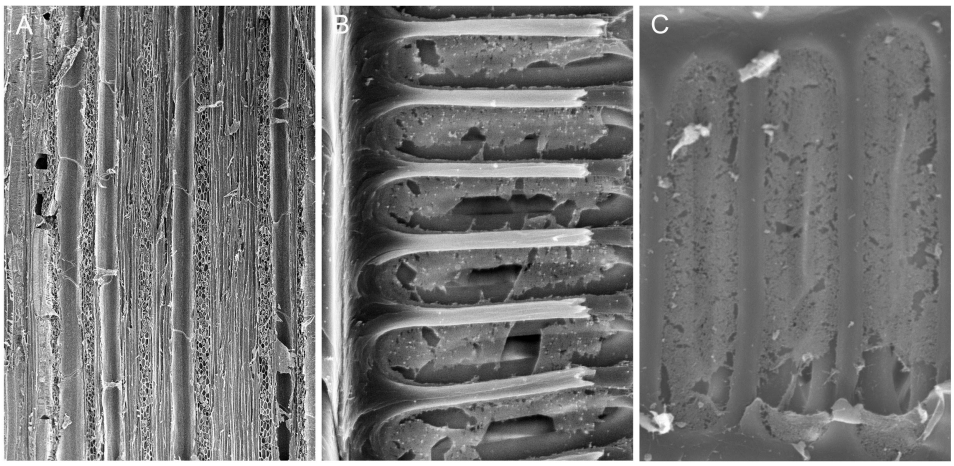


**Figure 7:** Xylem structural features in Δ*engXCA1*-inoculated Cabernet Sauvignon vine at the late time point of PD symptom development. **A.** Longitudinal section of stem secondary xylem, showing open vessels. **B** and **C.** Longitudinally transected vessels, showing intervessel PM degradation.

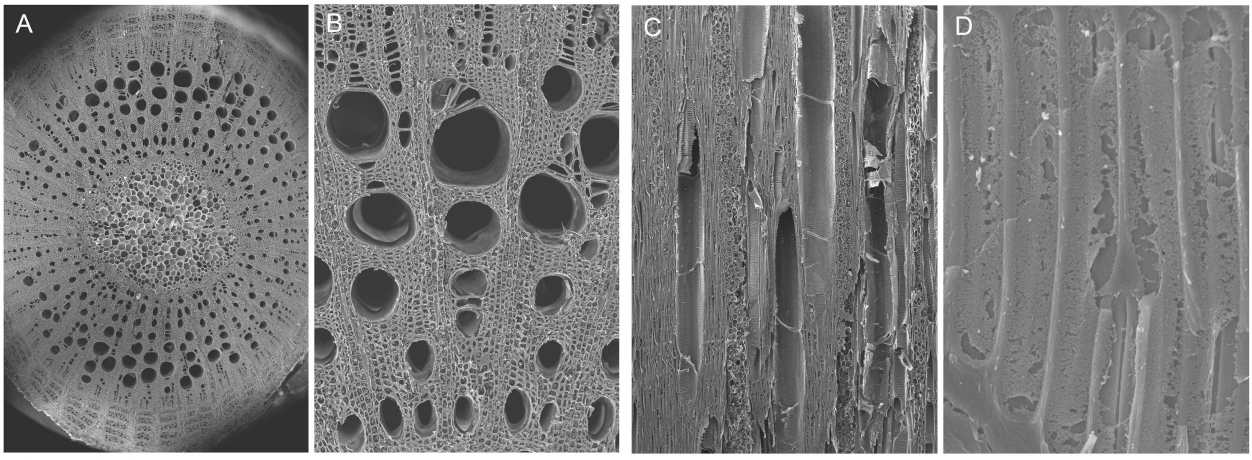


**Figure 8:** Xylem structural features in Δ*engXCA1*/Δ*engXCA2*-inoculated Cabernet Sauvignon vine at the late time point of PD symptom development. **A** and **B.** Transverse section of stem secondary xylem, showing vessels free of occlusions. **C.** Longitudinal section of secondary xylem, showing empty vessels with mostly intact PMs. **D.** A longitudinally transected vessel, showing pores of different sizes in intervessel PMs.

In addition to samples imaged via electron microscopy, samples from inoculated Cabernet Sauvignon have also been analyzed by microCT for all time-points. Singular midslice images were analyzed to determine if tyloses formed in the xylem in response to *Xf* infection (Fig. 9A, B, C). Cabernet Sauvignon vines inoculated with wild-type *Xf*, Δ*engXCA1*, or Δ*engXCA2* exhibited a similar number of vessels containing tyloses and both early and middle time-points. However, at the late time-point, Δ*engXCA2*-inoculated vines had more vessels with tyloses than vines inoculated with wild-type *Xf*, and vines inoculated with Δ*engXCA1* had relatively few vessels with tyloses. Vines inoculated with the Δ*engXCA1*/Δ*engXCA2* double mutant had fewer vessels with tyloses relative to all other treatments across all time-points (Fig. 9D).

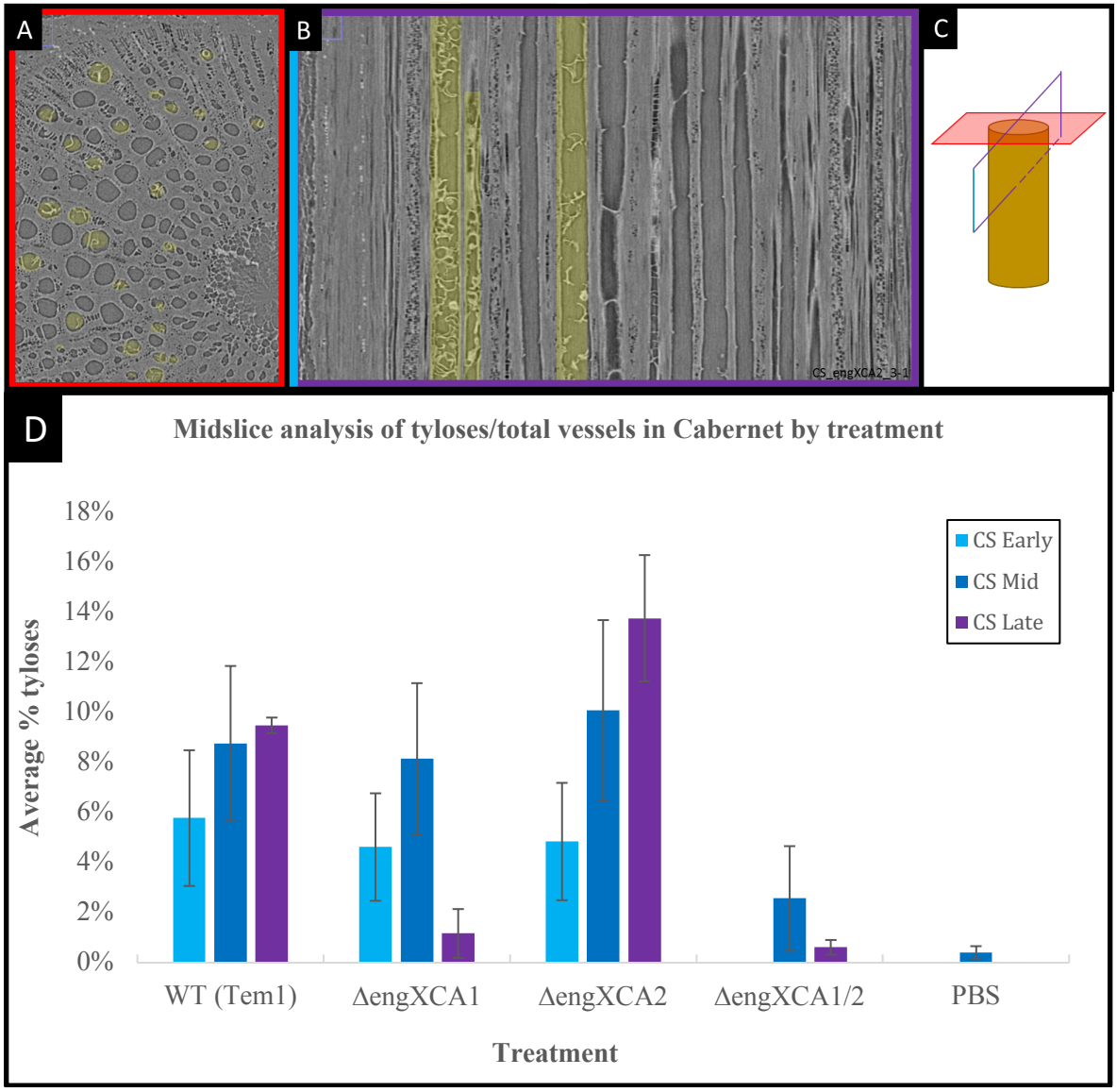


**Figure 9:** Improved tyloses detection/quantification. Colored outlines in **A** (xy-axis) and **B** (yz-axis) correspond with **C** to help orient the viewer. Tyloses (highlighted in yellow) are small and rare features relative to empty vessels on the xy-axis, and can easily be confused with interconnected vessels, yet appear more distinctly in the yz-axis. **D.** Manual midslice analysis of %-tyloses (occluded vessels/total vessels) per treatment in Cabernet Sauvignon. Vessels with tyloses were manually counted on midslices of microCT scans.

The McElrone lab recently developed a method to measure starch content in ray and axial parenchyma (RAP) *in vivo* using microCT and machine learning algorithms (Earles 2018). In microCT images, x-ray absorption corresponds to the distinct molecular structure of air, water, starch and cell wall material, which enables the

visualization of RAP, which are located in xylem tissue between radial files of vessels. While microCT images pictured here are of dried stems, patterns of full/empty RAP reflect those found *in vivo* in grapevine rootstocks and the method has implications for tracking starch utilization over the course of *Xf* infection. RAP in Cabernet Sauvignon vines inoculated with wild-type *Xf* show patterns of starch depletion at the early time-point with significant depletion at the late time-point. RAP in Δ*engXCA1*-inoculated vines are full of starch at the early time-point and moderately depleted at the late time-point (Fig. 10). RAP in PBS-inoculated vines remain full of starch at all time-points.

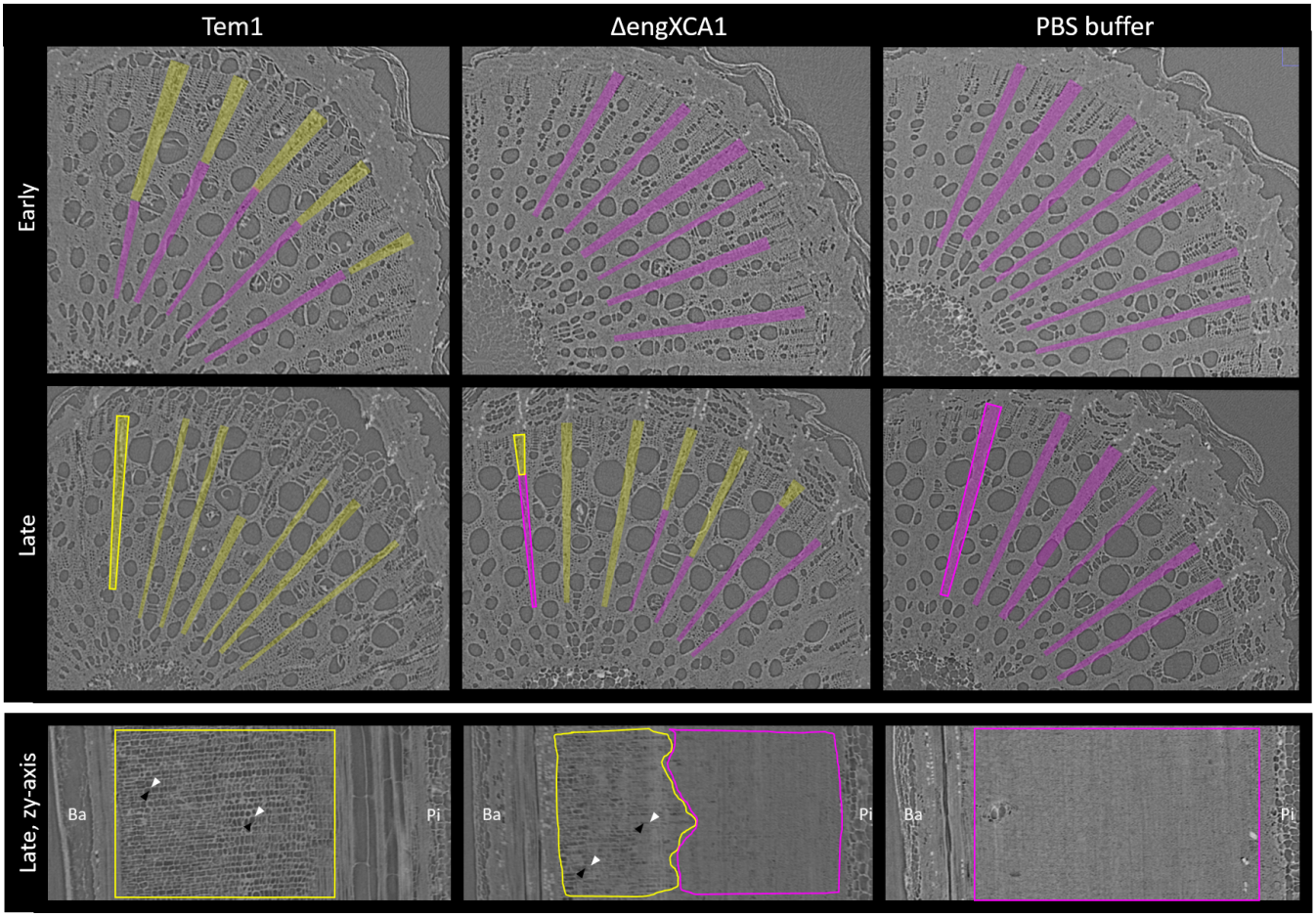


**Figure 10:** Visual classification of ray and axial parenchyma (RAP) regions as full (magenta) or empty (yellow) in Cabernet Sauvignon vines inoculated with either wild-type Temecula 1, Δ*engXCA1*, or PBS (negative control).. Longitudinal slices of outlined, late-timepoint RAP emphasize a spatial pattern of starch depletion with empty cells (dark airspace and light cell walls indicated with corresponding triangles) near the periphery bark (Ba) layer progressing towards the pith (Pi).

**Quantitative analysis of plant defense pathways induced by *Xf* cell wall degrading enzyme activity: biochemical and transcriptional studies.**

Pit membrane degradation by *Xf* CWDEs likely results in the release of small chain carbohydrates into the xylem. These oligosaccharides have been known to act as elicitors of plant immunity (i.e. damage-associated molecular patterns). It is possible that oligosaccharides released from pit membrane degradation are being recognized by associated parenchyma cells, triggering defense responses such as tylose production. To test this hypothesis, we used RNAseq to analyze the Cabernet Sauvignon transcriptome to determine if pit membrane degradation products act as elicitors of plant immunity and trigger tylose production. So far, we have counts of differentially expressed genes (DEGs, p-value < 0.05) from the early and middle time-points in 2016 and the early time-point in 2017. When compared to PBS-inoculated vines, the transcriptomes of vines inoculated with either wild-type *Xf* or any of the endoglucanase mutant strains differed significantly (Table 1). When compared to wild-type *Xf*-inoculated vines, the transcriptomes of all vines inoculated with any of the *Xf* endoglucanase mutant strains

differed significantly, though there were less DEGs in Δ*engXCA1*- and Δ*engXCA2*-inoculated vines and more in Δ*engXCA1*/Δ*engXCA2*-inoculated vines (Table 2).

**Table 1:** Summary of the differentially expressed genes (DEGs; *P*-value < 0.05) between the Cabernet Sauvignon vines inoculated with *Xf* strains (wild-type, Δ*engXCA1*, Δ*engXCA2*, or Δ*engXCA1*/ Δ*engXCA2*) and PBS.

| Year | Time point | Number of DEGs | Wild-type vs. PBS | Δ*engXCA1* vs. PBS | Δ*engXCA2* vs. PBS | Δ*engXCA1*/ Δ*engXCA2* vs. PBS |
|---|---|---|---|---|---|---|
| 2016 | Early | Up-regulated | 2,831 | 2,335 | 469 | - |
| | | Down-regulated | 1,805 | 1,446 | 240 | - |
| | | Total | 4,636 | 3,781 | 709 | - |
| | Middle | Up-regulated | 1,791 | 4,495 | 1,263 | - |
| | | Down-regulated | 471 | 2,566 | 325 | - |
| | | Total | 2,262 | 7,061 | 1,588 | - |
| 2017 | Early | Up-regulated | 4,567 | 1,356 | 3,272 | 449 |
| | | Down-regulated | 3,114 | 638 | 1,789 | 259 |
| | | Total | 7,681 | 1,994 | 5,061 | 708 |

**Table 2:** Summary of the differentially expressed genes (DEGs; *P*-value < 0.05) between the Cabernet Sauvignon vines inoculated with the endoglucanase mutant strains and the wild-type *Xf* strain.

| Year | Time point | Number of DEGs | Δ*engXCA1* vs. WT | Δ*engXCA2* vs. WT | Δ*engXCA1*/Δ*engXCA2* vs. WT |
|---|---|---|---|---|---|
| 2016 | Early | Up-regulated | 215 | 1,214 | - |
| | | Down-regulated | 260 | 1,695 | - |
| | | Total | 475 | 2,909 | - |
| | Middle | Up-regulated | 486 | 29 | - |
| | | Down-regulated | 255 | 89 | - |
| | | Total | 741 | 118 | - |
| 2017 | Early | Up-regulated | 1,717 | 300 | 2,866 |
| | | Down-regulated | 2,965 | 507 | 4,068 |
| | | Total | 4,682 | 807 | 6,934 |

## CONCLUSIONS

Excessive tylose production has been well-documented in grapevines displaying PD symptoms, and is likely one of the factors causing these symptoms. However, the mechanism by which *Xf* triggers tyloses has not been elucidated. Our SEM and microCT data indicate that tylose production differs in vines inoculated with *Xf* endoglucanase mutants when compared to vines inoculated with the wild-type *Xf* strain. Tylose production increases in vines inoculated with Δ*engXCA2*, while it decreases in vines inoculated with Δ*engXCA1*. Interestingly, tylose production is severely reduced in vines inoculated with the endoglucanase double mutant, Δ*engXCA1*/Δ*engXCA2*. The DEG counts from our RNAseq analysis also show that vines inoculated with either Δ*engXCA1* or Δ*engXCA2* behave somewhat similarly to vines inoculated with wild-type *Xf*. Conversely, vines inoculated with the Δ*engXCA1*/Δ*engXCA2* double mutant behave similarly to vines inoculated with PBS. Therefore, we propose that *Xf* endoglucanases play a role in facilitating tylose production in grapevines. How they facilitate tylose production remains unclear, though we hypothesize that the oligosaccharide byproducts of pit membrane degradation trigger a DAMPs response that culminates in the sealing of xylem vessels. We are

currently testing this hypothesis by analyzing the specific genes that are differentially expressed in vines inoculated with wild-type *Xf* and vines inoculated with the *Xf* endoglucanase mutants, and we suspect that several of these genes will be linked to DAMPs signaling pathways. Additionally, we are analyzing the xylem sap of vines inoculated with all *Xf* strains to determine if oligosaccharide profiles differ in vines inoculated with wild-type *Xf* and vines inoculated with the *Xf* endoglucanase mutants.

In light of these findings, it appears that *Xf* CWDEs may be triggering host defense responses that exacerbate PD symptoms. For this reason, the inhibition of these CWDEs may alleviate excessive tylose production, allowing more xylem vessels to remain open, and minimize drought-stress symptoms. However, the inhibition of each individual CWDE is neither practical, nor economical. As these CWDEs are predicted to be Type II-secreted, inhibition of this secretion system will likely prevent both pit membrane degradation (and subsequent systemic colonization) and minimize excessive host defense responses. Therefore, we will continue on into the final phase of this project, looking for natural products that can inhibit the T2SS and block the proliferation of *Xf* CWDEs.

**REFERENCES**


Aleemullah M, Walsh KB (1996) Australian papaya dieback: Evidence against the calcium deficiency hypothesis and observations on the significance of laticifer autofluorescence. Australian Journal of Agricultural Research 47: 371-385

Benedetti M, Pontiggia D, Raggi S*, et al.*, 2015. Plant immunity triggered by engineered in vivo release of oligogalacturonides, damage-associated molecular patterns. *Proc Natl Acad Sci U S A* **112**, 5533-8.

Boller T, Felix G, 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu Rev Plant Biol* **60**, 379-406.

Bonsen KJM, Kučera LJ (1990) Vessel occlusions in plants: morphological functional and evolutionary aspects. International Association of Wood Anatomists Bulletin 11: 393-399

Buchanan BB, Gruissem, W., and Jones, R.L. , 2000. Biochemistry and Molecular Biology of Plants. *American Society of Plant Physiologists. Maryland.* **Chapter 2: The Cell Wall**, 52-100.

Chatterjee S, Almeida RPP, Lindow S, 2008. Living in two worlds: The plant and insect lifestyles of *Xylella fastidiosa*. *Annual Review of Phytopathology* **46**, 243-71.

Cianciotto NP, 2005. Type II secretion: a protein secretion system for all seasons. *Trends Microbiol* **13**, 581-8.

Collins BR, Parke JL, Lachenbruch B, Hansen EM (2009) The effects of Phytophthora ramorum infection on hydraulic conductivity and tylosis formation in tanoak sapwood. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 39: 1766-1776

Dimond AE (1955) Pathogenesis in the Wilt Diseases. Annual Review of Plant Physiology and Plant Molecular Biology 6: 329-350

Earles, J. M., Knipfer, T., Tixier, A., Orozco, J., Reyes, C., Zwieniecki, M. A., ... & McElrone, A. J. (2018). In vivo quantification of plant starch reserves at micrometer resolution using X-ray microCT imaging and machine learning. *New Phytologist*.

Esau K (1977) Anatomy of Seed Plants. 2nd edition. Wiley, New York, NY

Fritschi FB, Lin H, Walker MA (2008) Scanning electron Microscopy reveals different response pattern of four Vitis genotypes to Xylella fastidiosa infection. Plant Disease 92: 276-286

Gough CL, Dow, M.J., Barber, C.E. And Daniels, M.J., 1988. Cloning of two endoglucanase genes of *Xanthomonas campestris* pv. *campestris*: analysis of the role of the major endoglucanase in pathogenesis. *Molecular Plant Microbe Interactions* **1**, 275-81.

Guilhabert MR, Kirkpatrick BC, 2005. Identification of *Xylella fastidiosa* antivirulence genes: hemagglutinin adhesins contribute to *X. fastidiosa* biofilm maturation and colonization and attenuate virulence. *Molecular Plant-Microbe Interactions* **18**, 856-868.

Hopkins DL, Purcell AH, 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. *Plant Disease* **86**, 1056-66.

Jha G, Rajeshwari R, Sonti RV, 2005. Bacterial type two secretion system secreted proteins: double-edged swords for plant pathogens. *Molecular Plant Microbe Interactions* **18**, 891-8.

Korotkov KV, Sandkvist M, Hol WG, 2012. The type II secretion system: biogenesis, molecular architecture and mechanism. *Nature Reviews Microbiology* **10**, 336-51.

Mohammadi M, Burbank L, Roper MC, 2012. *Pantoea stewartii* subsp. *stewartii* produces an endoglucanase that is required for full virulence in sweet corn. *Molecular Plant Microbe Interactions* **25**, 463-70.

Mollenhauer HH, Hopkins DL, 1974. Ultrastructural study of Pierce's disease bacterium in grape xylem tissue. *J Bacteriol* **119**, 612-8.

Parameswaran N, Knigge H, Liese W (1985) Electron microscopic demonstration of a suberized layer in the tylosis wall of beech Fagus sylvatica and oak Quercus robur. International Association of Wood Anatomists Bulletin 6: 269-271

Parke JL, Oh E, Voelker S, Hansen EM, Buckles G, Lachenbruch B (2007) Phytophthora ramorum colonizes tanoak xylem and is associated with reduced stem water transport. Phytopathology 97: 1558-1567

Perez-Donoso AG, Sun Q, Roper MC, Greve LC, Kirkpatrick B, Labavitch JM, 2010. Cell Wall-Degrading Enzymes Enlarge the Pore Size of Intervessel Pit Membranes in Healthy and Xylella fastidiosa-Infected Grapevines. *Plant Physiology* **152**, 1748-59.

Purcell AH, Hopkins DL, 1996. Fastidious xylem-limited bacterial plant pathogens. *Annu Rev Phytopathol* **34**, 131-51.

Roberts DP, Denny TP, Schell MA, 1988. Cloning of the egl gene of *Pseudomonas solanacearum* and analysis of its role in phytopathogenicity. *J Bacteriol* **170**, 1445-51.

Roper MC, Greve LC, Warren JG, Labavitch JM, Kirkpatrick BC, 2007. Xylella fastidiosa requires polygalacturonase for colonization and pathogenicity in Vitis vinifera grapevines. *Molecular Plant Microbe Interactions* **20**, 411-9.

Saile E, Mcgarvey JA, Schell MA, Denny TP, 1997. Role of Extracellular Polysaccharide and Endoglucanase in Root Invasion and Colonization of Tomato Plants by Ralstonia solanacearum. *Phytopathology* **87**, 1264-71.

Simpson AJ, Reinach FC, Arruda P*, et al.*, 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. The Xylella fastidiosa Consortium of the Organization for Nucleotide Sequencing and Analysis. *Nature* **406**, 151-9.

Slonczewski J.L. and Foster, J.W., 2014. Microbiology: An Evolving Science. *W.W. Norton and Company, New York, NY.*

Sun Q, Sun Y, Walker MA, Labavitch JM, 2013. Vascular occlusions in grapevines with Pierce's disease make disease symptom development worse. *Plant Physiol* **161**, 1529-41.

Tyree MT, Zimmermann MH (2002) Xylem Structure and The Ascent of Sap. 2nd Edition. Springer, Berlin, Germany

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