

**MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES:  
THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*.  
PROGRESS REPORT NUMBER ONE: COMPARISON WITH SYMPTOMS OF WATER DEFICIT  
AND THE IMPACT OF WATER STRESS**

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**ABSTRACT**

The pathology of diseases such as Pierce's disease (PD) of grapevine (*Vitis vinifera* L.) that are caused by the xylem-limited bacteria *Xylella fastidiosa* (*Xf*) is widely attributed to vessel occlusion and subsequent water deficits. Grapevines (*Vitis vinifera* L. 'Chardonnay') were exposed to water deficits, stem inoculation with *Xf*, and combinations of both to evaluate whether symptoms of PD were a consequence of water deficits. When vines were inoculated with *Xf* and exposed to water deficits, more extensive PD symptoms developed throughout the plant than when + *Xf* vines were well-watered. However, vines infected with *Xf* exhibited symptoms unique to PD that included inhibited periderm development in stems (green islands), leaf blade separation from the petiole (matchsticks), and irregular leaf scorch. Vines exposed to water deficits and not *Xf*, displayed accelerated periderm development, basal leaf abscission at the stem/petiole junction, and uniform leaf chlorosis. Water deficits induced the development of an abscission zone, but PD did not. Pierce's disease symptoms could not be produced with any of several water deficit treatments, including severing all but one secondary vein near the leaf tip. The results indicate that factors other than water deficits are involved producing the symptoms of PD. We conclude that the widely accepted hypothesis that PD-infected plants develop water deficits that cause green islands, matchsticks, localized leaf scorch, and eventual death of vines should be reevaluated.

**INTRODUCTION**

The overwhelming consensus among researchers is that the fatal nature of PD is a result of the *Xf* bacteria becoming systemic and blockage occurring in xylem vessels (due to bacterial accumulation, tyloses, gums, and/or emboli), causing water transport to become progressively impaired until the plant is no longer able to function (Goodwin *et al.* 1988a, b; McElrone *et al.* 2001, 2003; Newman *et al.* 2003, 2004; California Agricultural Research Priorities 2004). Indeed, Pierce's disease has become nearly synonymous with plant water deficit. This view is largely based on correlative evidence. Hopkins (1988) showed a strong association between reduced water conductance in stems of citrus seedlings and *Xf*-caused disease symptoms. Low leaf water potential and turgor, impaired hydraulic conductance, and higher stomatal resistance were correlated with PD symptoms in grapevines (Goodwin *et al.* 1988a). While reduced leaf water potential, stomatal conductance and stem hydraulic conductivity are characteristic of water deficit, it should be noted that these same features also occur in flooded plants (Kramer & Boyer 1995), so correlations are not necessarily indicative of causality.

From our recent work we observed that, although PD symptoms have been attributed to water deficit, the visual symptoms of PD did not appear to be the same as those resulting from water deficit alone. In grapevine, typical visual symptoms of PD are "green islands," patchy or marginal leaf necrosis (often called leaf scorch), and "matchsticks" (petioles that remain attached to the stem after the laminae have fallen off) (Purcell 1986; Goheen & Hopkins 1988, 1989; Stevenson *et al.* 2004). These symptoms are not characteristic of water deficit symptoms in grapevines (Okamoto *et al.* 2004). In addition, the diagnostic symptoms of PD have never been observed in healthy grapevines exposed to water deficits, nor have they ever been reported to develop as a consequence of water deficits.

Interestingly, citrus trees already infected with *Xf* and subjected to drought displayed accelerated symptom development of citrus variegated chlorosis (Gomes *et al.* 2003). Extended water deficit also increased the severity of Pierce's disease in the woody liana, Virginia creeper (McElrone *et al.* 2001, 2003). Thus, extended water deficit (such as drought) may exacerbate the development of PD symptoms in grapevine as well. However, there are no reports describing the effects of water deficit on *Xf*-infected grapevines, nor has there been a detailed comparison of water deficit and PD symptoms. If the visual symptoms of PD are not, in fact, a result of water deficit, then studies relying on the assumption that water stress is the

ultimate killer of plants suffering from PD may result in misleading information and add years to finding solutions to the PD problem. Therefore, it is important that it be determined which PD symptoms, if any, are a result of water stress, and what role water shortage actually plays in symptom development and vine death.

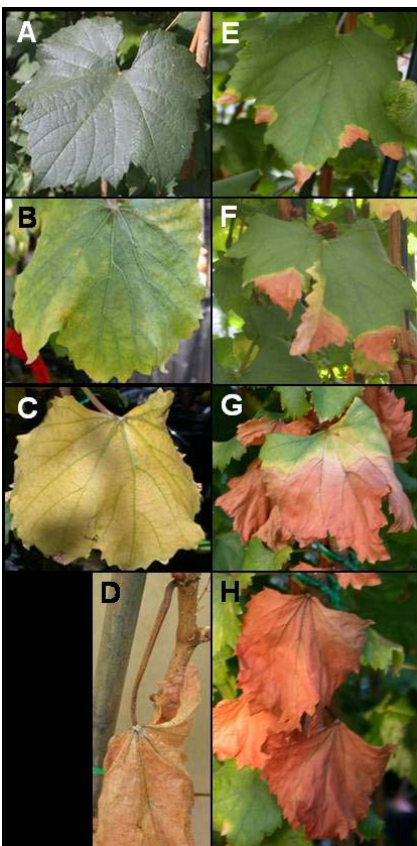
## OBJECTIVES

1. Evaluate the impact of vine water status on the development of the visual symptoms of PD.
2. Determine whether visual PD symptoms are a direct result of water deficits.

## RESULTS

### Objective 1

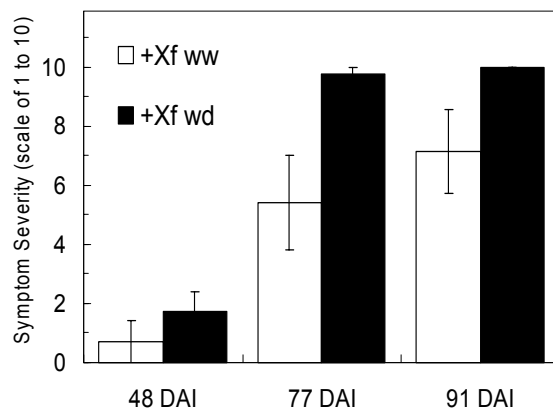
In the field, extended water deficit exacerbates citrus variegated chlorosis in citrus (Gomes *et al.* 2003) and PD in Virginia creeper (McElrone *et al.* 2001, 2003). Thus, it was not surprising that subjecting potted grapevines to extended water deficit also resulted in a faster and more extensive onset of PD symptoms (barring green islands) than in well-watered *Xf*-infected (+*Xf*) vines. The first clear indications of leaf scorch were seen 48 DAI. Water-stressed +*Xf* vines developed more symptomatic leaves with severe symptoms than well-watered +*Xf* vines (Fig. 1). Interestingly, the leaf scorch and matchstick symptoms in the well-watered +*Xf* plants had the same visual characteristics as in the +*Xf* water-stressed plants. There was no significant difference between well-watered +*Xf* and healthy (−*Xf*) vines in stomatal conductance ( $0.86 \pm 0.09$  &  $0.69 \pm 0.06$  cm s<sup>−1</sup>), transpiration ( $6.53 \pm 0.83$  &  $5.66 \pm 0.83$  μg cm<sup>−2</sup> s<sup>−1</sup>), and leaf water potentials ( $-0.60 \pm 0.05$  &  $-0.73 \pm 0.11$  MPa, respectively). Likewise, these parameters were equivalent for water-deficit +*Xf* and −*Xf* vines ( $0.28 \pm 0.04$  &  $0.34 \pm 0.05$  cm s<sup>−1</sup>,  $2.41 \pm 0.31$  &  $2.86 \pm .39$  μg cm<sup>−2</sup> s<sup>−1</sup>,  $-1.07 \pm 0.05$  &  $-1.28 \pm 0.13$  MPa, respectively).



**Figure 2.** Progressive development of leaf symptoms of non-infected water-stressed Chardonnay leaves (A-D), and Chardonnay with Pierce's disease (E-H).

In −*Xf* water-stressed plants, two sites of constriction and necrosis developed on petioles, one at the stem/petiole junction (the basal end of the petiole) and the other at the petiole/lamina junction (the distal end of the petiole). At the basal end of the

**Figure 1.** Symptom development during whole plant water deficit and well-watered conditions. Severity of Pierce's disease symptoms in Chardonnay grapevines. Vines infected with *X. fastidiosa* were well-watered (+*Xf* ww; white bars) or subjected to water deficit (+*Xf* wd, black bars). On a scale from 1 to 10, 1 indicates mild symptoms, 10, the most severe PD symptoms. Non-infected values are zero due to the lack of PD symptoms and are not shown.



### Objective 2

The results revealed that visual symptoms of Pierce's disease in grapevine are qualitatively and quantitatively different than those of extended water deficit. Regardless of water status, +*Xf* plants displayed symptoms unique to PD. In general, PD symptoms masked water-deficit symptoms. The PD symptoms manifested in laminae, petioles and stems often revealed an interaction between plant and bacteria in which plant responses to *Xf*-infection seemed to be either elicited or suppressed by the bacteria.

### Comparison of Visual Symptoms of Water Deficit and PD

To determine whether PD symptoms are a direct result of water deficit, the visual characteristics of well-watered and water-stressed grapevines inoculated with *Xylella* (+*Xf*) or water (−*Xf*) were evaluated. Leaves of well-watered −*Xf* grapevines remained green and healthy throughout the course of the experiments (Fig. 2a). Water-stressed −*Xf* vines gradually developed leaf chlorosis in a fairly uniform pattern over the entire leaf lamina (Fig. 2b-c), with the veins staying green until leaves became necrotic. Leaves remained attached to the stem even after the leaves were apparently dead (Fig. 2d). In contrast, the first PD symptom to appear was leaf scorch. Leaf scorch symptoms started with chlorosis at the margins of the leaves and moved towards the petiole in patches such that sections of necrosis were bordered by slim regions of chlorosis (Fig. 2e-f). As symptoms progressed, laminae of +*Xf* vines became completely necrotic, while the petioles remained green (Fig. 2g-h). Eventually laminae fell from the petioles to form “matchsticks.”

petiole, a true abscission zone formed. At the distal end of the petiole where the lamina is attached, the tissue constricted and concurrently became necrotic. Observations at the cellular level suggest that the constriction and necrosis at this junction is not an actual abscission zone (Stevenson *et al.* 2004). Neither the abscission zone at the stem/petiole junction nor the fracture zone at the petiole/lamina junction developed until the lamina was severely chlorotic. In +*Xf* vines, a fracture zone also occurred at the petiole/lamina junction. Comparisons of the anatomy of the fracture zone at the petiole/lamina junction of +*Xf* vines and -*Xf* water-stressed vines showed that these fracture zones were identical. However, abscission zones did not develop at the stem/petiole junction of either well-watered or water-stressed +*Xf* plants.

The canes of both +*Xf* and -*Xf* water-stressed plants matured faster, becoming stiffer and more woody than those of the well-watered plants, based on the extent of periderm development up the canes. Stems of water-stressed +*Xf* plants became woody before the well-watered plants. Interestingly, in +*Xf* plants only the well-watered vines developed green islands, having an average of  $2.1 \pm 0.31$  green islands per plant.

### ***Vessel Blockage in Relation to Leaf Scorch Symptoms***

Leaf scorch symptoms, in particular, have been considered a direct result of water deficits within the leaf, specifically due to clogged vessels limiting water transport. If leaf scorch is simply a matter of reduced water availability to the leaf margins, then we should be able to induce leaf scorch symptoms by selectively severing veins to simulate xylem vessel blockage. To this end, experiments were conducted in which all veins but one were severed such that a single secondary leaf vein connected the two halves of a lamina and was the sole water source for the nearly-severed portion of the leaf. Nearly-severed leaf halves of vines experiencing low transpirational demand in the laboratory appeared turgid and showed no signs of necrosis for up to 36 days. In the greenhouse, under medium to high transpirational conditions, sections of leaves which received water via a single vein remained green and turgid (Fig. 3) for at least 30 days after the veins were severed. This was true for leaves of +*Xf* and -*Xf* grapevines alike. Significantly, leaf scorch symptoms of PD did not develop on any of the -*Xf* nearly-severed leaves. Even when these leaf sections did eventually dehydrate after approximately two months, the symptoms were similar to water deficit, not PD.



**Figure 3.** Turgid leaf of non-infected Chardonnay under moderate to high transpiration 13 days after all but one vein was severed, resulting in a single leaf vein connecting and supplying water to half of the leaf. Black arrow shows secondary vein supplying water to the nearly-severed leaf half.

## **CONCLUSIONS**

In summary, water deficit clearly had an exacerbating effect on the symptom development of PD. Water-stressed +*Xf* vines displayed more extensive PD symptoms throughout the plant than did well-watered vines. Matchstick and leaf scorch symptoms moved up the canes more rapidly than in well-watered vines implying that the bacteria spread more rapidly throughout the plant under water deficit conditions, assuming bacterial proximity is necessary for symptom development. Importantly, with the exception of green islands, extended water deficit did not affect the nature of the PD symptoms. Indeed, in water-stressed +*Xf* plants, PD masked all of the symptoms of water deficit, except green islands, which occurred only in well-watered +*Xf* vines.

Detailed comparisons of the visual symptoms of PD and water deficit revealed that conclusions reached from earlier work, stating that water deficit causes PD symptoms, were not completely correct. The visual characteristics of +*Xf* vines were unique to PD and distinctly different from -*Xf* vines experiencing extended water deficit. The fracture zone at the petiole/lamina junction, common to all treatments, appears to be a plant response to stress and not specifically induced by bacterial infection. In contrast, the lack of an abscission zone in +*Xf* plants implies that the bacteria were in some way suppressing development of an abscission zone. Conversely, water deficit overcame the influence of *Xf* to prevent the occurrence of green islands, possibly by hastening periderm development. Considering that only well-watered +*Xf* vines developed green islands, water deficit could have masked the green island symptom of PD by inducing the periderm of +*Xf* water-stressed canes to develop faster than could the conditions necessary to impair periderm activity leading to green islands. This suggests that the bacteria are in some way inhibiting periderm activity at seemingly random locations.

Finally, based on the dramatic and sudden increase in the number of nonfunctional vessels which was caused by severing leaf veins, it seems clear that xylem vessel blockage, whether due to gums, tyloses or bacterial accumulation, is not responsible for leaf scorch symptoms and that *Xf* bacteria are able to affect plant responses in ways not involving altered vine water status. While occluded xylem vessels may worsen leaf scorch symptoms, several other factors, or combination of factors, may contribute. Ultimately, however, comparison of the leaf scorch symptoms of PD and the chlorosis of extended water-stressed leaves shows that *Xf* bacteria are able to produce, alter or eliminate signals that result in leaf scorch symptoms and that these signals can, to some degree, override signals controlling plant responses to water deficit. (A manuscript containing the completed study will be submitted to a peer-reviewed journal shortly.)

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