MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*. XYLEM STRUCTURE AND CONNECTIVITY IN GRAPEVINE SHOOTS SUGGESTS A PASSIVE MECHANISM FOR THE SYSTEMIC SPREAD OF BACTERIA IN GRAPE PLANTS INFECTED WITH PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted September 2004 to June 2005.

ABSTRACT

Grapevine xylem is composed of vessels connected by intervessel bordered pits with pit membranes that prevent the passive movement of particles, especially at the stem-leaf junction where most vessels end. The traditional view of *Xylella fastidiosa* (*Xf*) movement within the xylem requires the digestion of the intervessel pit membrane to move from one vessel to another. However, bacteria such as *Yersinia enterocolitica* (*Ye*) and fluorescent beads have been observed moving rapidly within the grapevine xylem, suggesting a pathway for passive movement. In this report, we used air and latex paint to confirm the existence a xylem vessel pathway from stems into the leaf lamina. Anatomical investigation of the leaf xylem revealed a switch from vessels to tracheids at about 50-60% the length of the leaf lamina. In addition, inoculations of gfp-*Xf* showed that bacteria never reached the leaf margin where the symptoms appear, suggesting that tracheids inhibited the free movement of *Xf*.

INTRODUCTION

Particle movement is limited by the frequency of vessel endings, especially at the stem-leaf junction, where most vessels have been thought to end, with a few exceptions (Andre, 2002; Larson and Isebrands, 1978; Tyree and Zimmermann, 2002). Indeed a bacterium such as *Xf* (0.1-0.5 x 1-5 µm, Nyland et al., 1973) is too big to move through the intervessel pit membrane pores (<0.2 µm, Siau, 1984) with water flow. The colonization of a plant by *Xf* requires that the bacteria move within the xylem between vessels across pit membranes and from one organ to another (Stevenson et al., 2004). A favored hypothesis to explain how bacteria become systemic is that the bacteria digest the pit membrane cell wall (Roper et al., 2002; Stevenson et al., 2004). Another more recent twist in the mechanism is that bacteria might also move through torn or remnant pit pore membranes (Carlquist and Schneider, 2004; Stevenson et al., 2004). This propagation by digestion could be rather slow if vessels are short and if numerous membranes have to be crossed. Bacterial movement in grape stem can be relatively easy because vessels can be very long, up to 1m (Sperry et al., 1987). However, there is still the problem of bacterial passage into leaves if most of the vessels end at the stem-petiole and petiole-lamina junctions.

Recent experiments on the passive movement of *Ye* and fluorescent beads showed the existence of open, continuous xylem conduits (one or more xylem vessels allowing free movement of particles of at least one micrometer in size) from the stem to the leaf lamina of grapevine (Thorne et al., personal communication). They found that *Ye* and beads were moving freely with the transpiration stream from the stem into primary and secondary veins of the leaf blade in three leaves above the loading point. In addition, they showed that *Ye* and beads traveled to about 50-60% of the length of the leaf lamina. This shows an open xylem conduit all the way from the stem through the petiole and into the leaf blade without the need to digest a pit membrane, but also suggests that a feature of xylem structure precluded the movement of bacteria all the way to the leaf blade periphery.

In this study, we verified the presence of the open xylem conduits by studying the movement of air and latex paint. We also looked at the xylem anatomy of the leaf blade to identify the change in the vascular structure causing the halt of *Ye*, beads, air and paint within 50-60% of the leaf blade length. Leaves were also inoculated with *Xf* engineered by the addition of the green fluorescent protein (gfp-*Xf*) to check its movement within the leaf at different times after inoculation. The question was whether *Xf* would be affected by the change in xylem structure. Since *Xf* possess the ability to digest cell walls, we would theoretically expect the bacteria to be able to move farther than 50-60% of the leaf blade length and eventually be found at the leaf margin.

OBJECTIVES

1. Confirm the presence of open, continuous vessels from stem to leaf by using air and latex paint.
2. Identify the change in the leaf xylem structure that inhibits movement of air, paint and bacteria.
3. Study the effect of the leaf xylem structure on gfp-*Xf* movement.

RESULTS

Objective 1: Open, continuous vessels

When air or paint was loaded in the petiole base, the distance traveled ranged from 40 - 60% of the total length of the xylem path from petiole base toward the leaf margin (Figure 1). Since neither air nor paint can move from one intact vessel to another across intervessel pit membrane, the similar results obtained with air and paint suggest that both moved through open, continuous conduits until they reach a zone in the leaf blade where a change occurs in leaf xylem structure (Figure2).

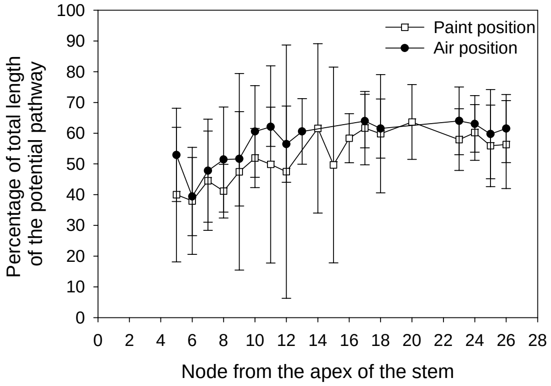

Figure 1. Air and paint position in the leaf veins calculated as percentage of the total length of the vascular pathway. Air and paint were loaded at the base of the petiole of leaves from different nodes. For each node, the farthest position of the air or paint inside the five major veins and ten secondary veins of five leaves was recorded.

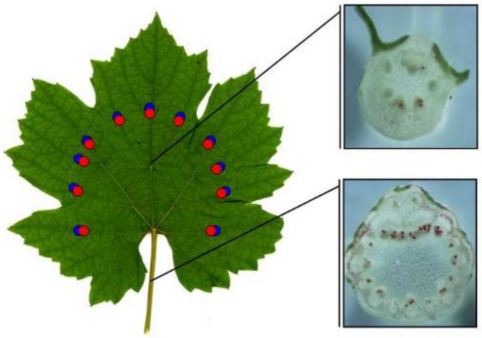

Figure 2. Map of a leaf showing the longest distance traveled by air and paint in the primary and secondary veins when loaded into the petiole base. Images on the right are cross sections of the petiole (bottom) and of the central vein (top) showing paint-filled vessels. Notice the decrease in the number of paint-filled vessels as we get closer to the margin of the leaf.

When air and paint were loaded in the stem, only the three leaves immediately apical to the loading point had air or paint in their veins (Figure 3). No air or paint was observed going into petioles beyond these three leaves, but both could be observed up to 1m past the loading point within the stem axis. The progression of air and paint inside the three leaves was comparable to that seen when air or paint were loaded into an individual isolated leaf via the petiole (Figure 2). This suggests that the open, continuous conduits present from petiole to leaf blade are also continuous from stem to leaf blade. This also shows that these xylem conduits are open for three internodes. However these open conduits connecting stem to leaves were not present at all stages of development of the vines. When air and paint were loaded in the stem below the nodes 10-12, air and paint was observed in the first three leaves above the loading point (Figure 3). But when air and paint was loaded in the stem above these nodes, nothing moved into any leaves but paint and air did continue to move up the stem. The open conduits from stem to petiole connected around nodes 10 to 12.

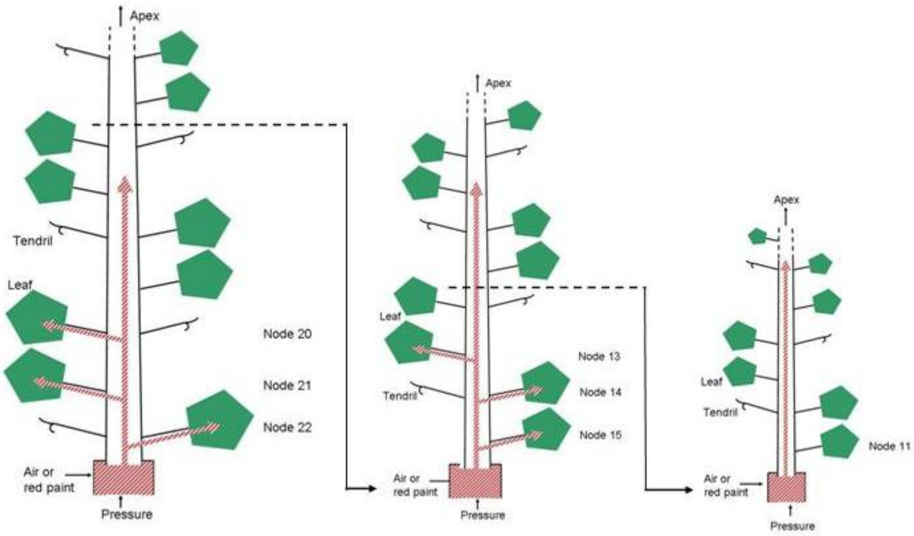

Figure 3. Diagram representing the movement of air or paint within the stem and into the leaves when loaded at different internodes. Below node 10 to 12, air and paint moved into the three leaves above the loading point and into the stem. From node 10-12, air and paint moved only in the stem. A pressure of 35 KPa was used and the presence of air and paint was first checked into the leaves then into the stem starting from the apex of the stem toward its base.

Objective 2: Tracheary elements of the leaf blade

Air and paint are not able to move past about 50-60% of the leaf blade (Figure 2). Since air and paint cannot cross the pit membrane between vessels, this means that the open, continuous conduits must end at this boundary (see dotted line in Figure 4). Consequently, the type of tracheary element was studied before this limit, at this limit and past this limit. Vessel elements with helical secondary walls and simple, open perforation plates were predominant in the first 50-60% of the leaf blade (Figure 4). However, past the limit, closer to the leaf margins, no open perforation plates were observed in any tracheary elements. Macerations revealed that the xylem was composed of short, close-ended tracheids (Figure4).

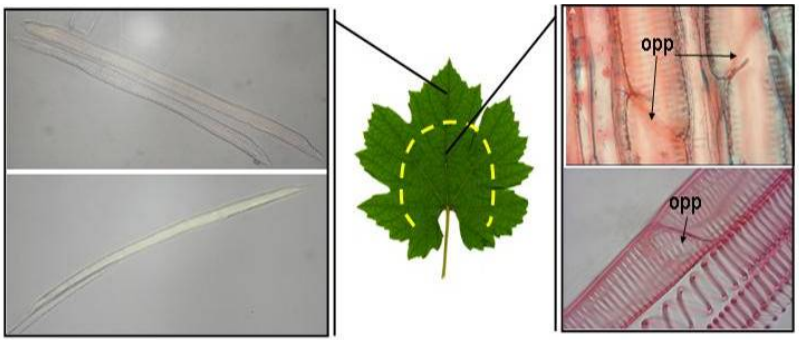

Figure 4. Map of a leaf showing the change in its xylem structure in relation to the limit set by air and paint movement. Images on the right show the presence of open perforation plates (OPP) within the mid vein xylem. The images on the left show the presence of tracheids in the central vein near the margin of the leaf.

Objective 3: Movement of Gfp-*Xf* fastidiosa in leaves

In all examined leaves, the bacteria was observed at 50-60% of the leaf blade, but never closer to the leaf margin (Figure 5). The bacteria reached this limit at five weeks and were not found closer after eleven weeks when symptoms developed at the margins. In addition, bacteria were loaded at different places on leaves to determine if the loading site influenced the distance the bacteria could travel or the outer limit of its travel. The results showed that gfp-*Xf* could not be found past this limit in leaves infected in the petiole or at mid-distance of the mid vein, regardless of the presence of symptoms or not. But the green fluorescence was observed in the petiole of leaves infected in the mid vein, signifying that the bacteria moved against the transpiration stream. However, the green fluorescent bacteria could not be observed in leaves that had been infected near the leaf blade margin. The bacteria may not have survived as their propagation was hindered by the tracheids, or possibly no xylem elements were infected during the needle inoculation as there are few of them near the margin.

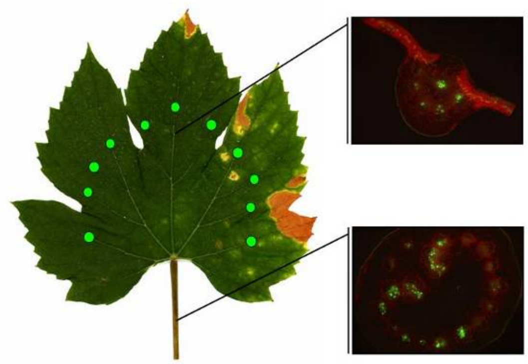

Figure 5. Map of a leaf showing the distance traveled by gfp-*Xf*t in the xylem vessels when injected into the petiole base. Images on the right are cross-sectional views of the infected petiole (bottom) and the central vein (top).

CONCLUSIONS

1. The presence of open xylem conduits from petiole base to 50-60% of the leaf lamina is confirmed.
2. These conduits are present in the stem for three internodes before diverging into mature leaves. However, the conduits present in young leaves are not continuous in the stem, although the stem possesses some.
3. Tracheids replace vessels at 50-60% of the lamina.
4. gfp-*Xf* movement is similar to the movement of air and paint.
5. gfp-*Xf* is stopped or slowed by the tracheids and never reached the leaf margins where necrosis appears. Therefore, necrosis could result from a signal originating from the bacteria and targeting some specific cells near the margin.
6. The open conduits offer easy long distance pathways bridging stem to leaves without interruption, which could facilitate the systemic spread of the bacteria in the plant.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.