MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: AN ANALYSIS OF THE MOVEMENT OF *XYLELLA FASTIDIOSA* IN XYLEM PATHWAYS

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INTRODUCTION

Pierce's disease (PD) is a consequence of the spread of xylem-limited bacteria, bacterial products, or plant responses to either leading to blockage of water movement within the grape's hydraulic network (Hopkins and Mollenhauer 1973). The progression of symptoms and movement of PD pathogens from the point of inoculation into the hydraulic network is poorly understood. The development of xylem blockage from the inoculation point to distal or basal organs and the pathways for movement of bacteria within grapevines needs to be determined.

The general vegetative anatomy and the primary vascularization of grapevine have been summarized (Pratt 1974, Mullins et al. 1992, Fournioux 1982) and anatomical symptoms of PD have been documented (Esau 1948, Tyson et al. 1985). Although a general pattern of grapevine hydraulic architecture has been proposed, the vascular arrangement within grapevine must be studied in the context of the spread of PD within the plant from the site of inoculation to a systemic presence. It is unknown whether the mechanisms of pathenogenesis of PD are a direct result of xylem blockage by the bacteria (Hopkins 1981), phytotoxins produced by the bacteria (Lee et al. 1982), resultant gums and tyloses produced by the plant (Esau 1948), or a combination of these factors.

OBJECTIVES

Through an analysis of the vascular system of grape shoots correlate the progression of PD from inoculation to infected organs with the movement of *Xylella* bacteria, the development of tyloses and gums, and the loss of water transport.

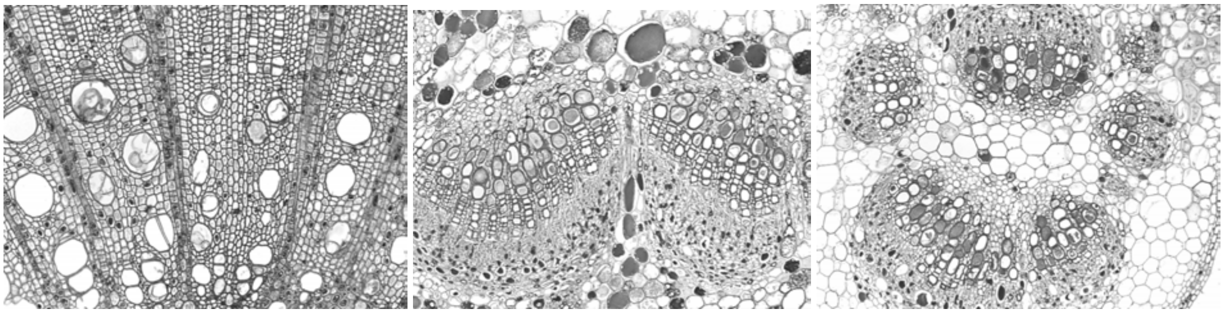
RESULTS AND CONCLUSIONS

Grapevines were inoculated with *Xylella* in one of the two shoots per plant. Six weeks following inoculation, symptoms consistent with PD were observed on the leaves (marginal leaf necrosis) and the stem (incomplete cork formation on stem) of the inoculated shoot, but the opposite, non-inoculated shoot was asymptomatic. Anatomical examination of stem, petiole, and midrib xylem with both light and electron microscopy revealed the internal progression of PD corresponding to external symptoms. The petioles and midribs of leaves displaying external PD symptoms contained tracheary elements with abundant gummosis and accumulation of bacteria, but few tyloses (Figures 1-3). Bacteria observed in symptomatic leaf midrib xylem, and to a lesser extent in petiole xylem, were embedded within a globular matrix (Figures 5-6). Stem xylem proximal to leaves showing PD symptoms included tracheary elements with abundant tyloses, but little gum formation (Figure 4). Bacteria were rare in the proximal stem and bacterial cells were not contained within a matrix. Observations of fully expanded asymptomatic leaves and stem tissue distal to the inoculation site showed similar anatomical pathology to nearby symptomatic leaves. Consequently, internal progression of PD appears to precede external symptoms. Six weeks following inoculation, no anatomical symptoms of PD were found in the basal main shoot subtending the inoculated shoot, nor in the opposite non-inoculated shoot. Eight weeks following inoculation, PD symptoms manifested in the opposite, non-inoculated, shoots. Anatomical examination showed the same pathology as was observed two weeks earlier in the inoculated shoot, including the accumulation of bacteria and embedding matrix in the xylem vessel members of leaf midribs and petioles.

Following these observations, a working hypothesis of the progression of PD within a young grapevine shoot can be proposed. *Xylella* inoculation of stem xylem precedes a relatively rapid movement of bacteria through the hydraulic network to distal stem regions, petioles, and leaf vascular tissue. The rapid movement is potentially facilitated by one, or a combination, of three mechanisms:

1. Grapevine vessels are long and few vessel-vessel transitions are needed to reach distal tissues,
2. Pit membranes of grapevine are frequently damaged, either in development, or as a result of frequent cavitation/refilling cycles, or
3. Bacteria are able to quickly digest pit membranes of terminal vessel elements.

Once bacteria moving in the transpirational stream enter regions of the hydraulic network that contain many narrow tracheary elements and more frequent terminal tracheary elements (i.e. shorter vessels in petioles and leaves), bacteria are 'filtered out' and accumulate, and become embedded in a surrounding matrix which effectively blocks water flow in that conduit. It is unknown whether this matrix is secreted from the bacteria itself, from the plant either as a defense reaction or responding to bacterial stimulus, or a combination of the two. Tylose formation in the stem coincides with bacterial infection, but at least initially, is not present to such a degree that bacterial movement is prevented or that the water supply to distal tissues is restricted to levels causing visual symptoms. Additionally, bacteria can move from an inoculated shoot to another shoot via the subtending trunk relatively quickly. Consequently, in can be proposed that the PD symptoms observed in multiple shoots of a grapevine are not a symptom of a whole-plant response to a localized infection, but rather are an indication of a systemic *Xylella* presence. For this to occur, bacteria must move basipetally from the site of inoculation, into the basal stem and then acropetally into the opposite shoot. Whether bacteria are moving against the transpirational stream in an intact water column, or whether the downward bacterial movement is facilitated by the release of tension in a cavitated water column is unknown.



Figures 1-3. Light micrographs of grapevine stem, petiole, and leaf midrib (l-r) six weeks after inoculation. Tyloses are frequent in vessels of stem sections, whereas xylem is occluded by gums in petioles and midribs.

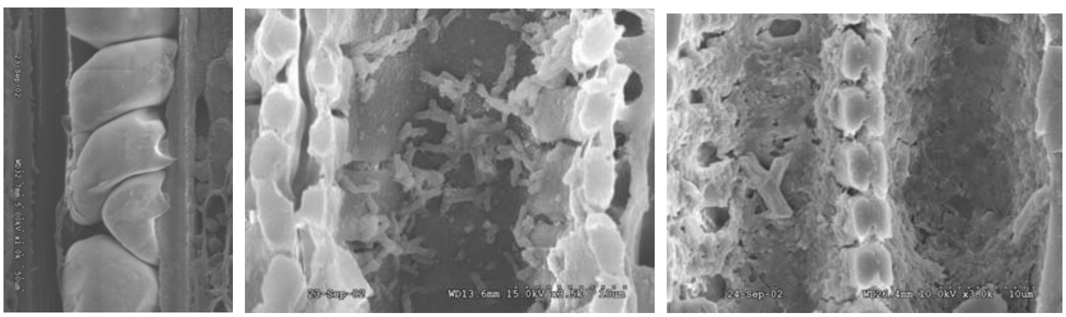


Figure 4-6. Scanning electron micrographs of grapevine stem, petiole, and leaf midrib (l-r) six weeks after inoculation. Symptoms of PD in stem xylem were typically tyloses, whereas petioles and midrib symptoms were associated with an abundance of bacteria and surrounding matrix increasing from petiole to leaf.

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