MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: RELEVANCE OF HYDRAULIC ARCHITECTURE

Project Leaders:

Thomas L. Rost
Section of Plant Biology
University of California
Davis, CA 95616

Mark A. Matthews
Dept of Viticulture and Enology
University of California
Davis, CA 95616

Joshua F. Stevenson
Section of Plant Biology
University of California
Davis, CA 95616

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ABSTRACT

The arrangement of vascular tissue within the nodes of Chardonnay grapevine (*Vitis vinifera*) shoots was studied as an investigation of potential pathways of infection for the bacterium that causes Pierce's disease (*Xylella fastidiosa*). Grapevine stem anatomy of the current year's growth was observed with both light and scanning electron microscopy and xylem conductance was observed by following traces of stains within the vascular tissue. The pattern of vascular divergence to lateral organs is described and implications for the spread of Pierce's disease within the grapevine shoot are discussed.

INTRODUCTION

The spread of *Xylella fastidiosa* (*Xf*) bacteria or bacterial products within the grapevine likely leads to Pierce's disease (Hopkins and Mollenhauer 1973). A thorough understanding of the hydraulic architecture is necessary to predict both the effect of localized xylem blockage on distal or basal organs and the pathways for movement of bacteria or phytotoxins within grapevine shoots. The general vegetative anatomy and the primary vascularization of grapevine have been summarized (Fournioux and Bessis 1973, Pratt 1974, Fournioux and Bessis 1974, Fournioux and Bessis 1979, Mullins et al. 1992). Although patterns of grapevine hydraulic architecture have been proposed, confirmation of this descriptive work is needed.

OBJECTIVE

Analyze the vascular arrangement of the grapevine in the context of the spread of Pierce's disease within the plant from the site of inoculation to a systemic presence.

RESULTS

Grapevine nodes were serially sectioned (Figure 1). In the most basal section below the node, a complete ring of secondary xylem (wood) is present bounding a regular parenchymatous pith. At this location, less than a centimeter from the node proper, there are no visually distinct leaf traces. In the next distal section, five leaf traces are distinct and have begun to diverge from the stele; two dorsal traces, two ventral traces, and a lateral trace (Figure 1B). The number of leaf traces at each node was variable; ranging from four to eight traces, with five being the most common. Anastomosis of leaf traces may begin while the trace is still in the stem cortex before it enters the petiole. As a consequence of leaf trace divergence from the stele a leaf gap in the vascular cylinder is created. Once the leaf traces have diverged from the stele in an axial orientation, their pathway quickly bends perpendicular from the vertical axis and they become radially oriented. Within a few millimeters after the leaf traces have diverged into the petiole, the parenchymatous gaps in the stele are no longer present and no leaf traces are distinct for further distal nodes. Large portions of the lateral regions of the node are void of conductive tissue (gap) due to vascular divergence into the tendril on one side and to the developing summer lateral shoot and compound bud on the other side (Figure 1E). Within one lateral gap, vascular differentiation occurs between the developing summer lateral shoot and each of the compound buds to the existing axial hydraulic network. Integration of the developing xylem of the summer lateral shoot to the axial system occurs through differentiated branch traces connecting the base of the summer lateral to the xylem of the main shoot (Figure 1F). The common summer lateral shoot and compound bud gap and the tendril gap close in the stele within a few millimeters distal to these respective organs (Figure 1H). Additionally, a sclerified parenchymatous diaphragm present in the node has been replaced by a regular unsclerified parenchymatous pith. Acropetal to the node, the stele is again complete with no gaps in the stele or distinct traces present.

A transverse section through the shoot shows an early stage of leaf trace divergence similar to that depicted in Figure 1B (Figure 2). There is a difference in vessel diameter between the lateral and dorsiventral sectors of the grapevine stem; laterally sectored vessels typically have narrow diameters and dorsiventral vessels have wider diameters. From this particular sample, the dorsi-ventral sector vessel diameters were 65 ± 21 microns (mean ± sd), whereas lateral sector vessel diameters were 31 ± 12 microns. In this case there are five leaf traces; two in the dorsal sector, two in the ventral sector, and one in a lateral sector. The leaf traces are identifiable by tracheary elements of a comparatively narrow diameter. The leaf traces each originate from a single lamella, and as with all the lamellae of the stem, are delimited by very tall rays. The exact lammelar location of trace origin is variable for all traces from node to node, with the lateral trace(s) sometimes originating nearly from the ventral side. The leaf traces diverge into the petiole at nearly a right angle to the axial system. Each pair of dorsal and ventral leaf traces may appear to fuse (Figure 2 upper right) or remain distinct (Figure 2 lower right).

Figure 1. Representative images from serially sectioned node (left). Images B, E, F, and H correspond to sections through locations (D1, D2–dorsal traces, V1, V2-ventral traces, L-lateral trace, TG-tendril gap, CG-common lateral shoot and compound bud gap, T-tendril, P-petiole, Pi-unsclerified pith, SL-summer lateral, arrow in F indicates branch traces, arrow in H indicates closing lateral gap).

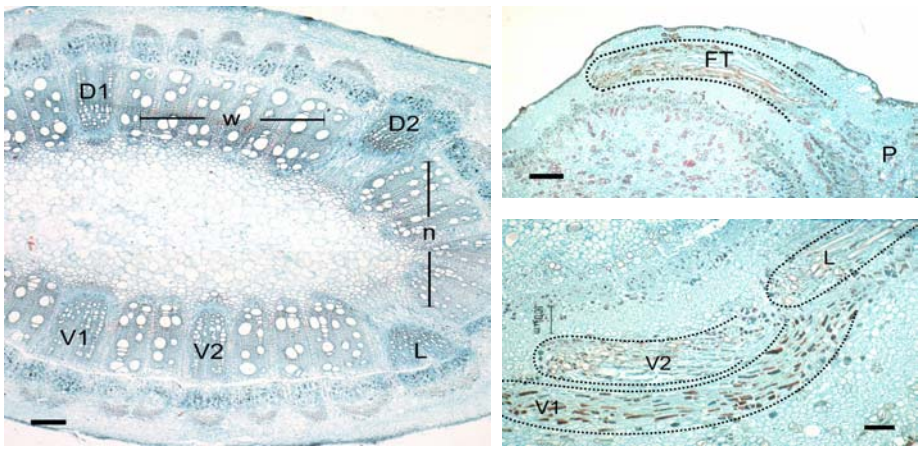

Figure 2. Cross sections displaying diverging leaf traces, stem vessel dimorphism, and subjective fusion of traces (D1, D2–dorsal traces, V1, V2-ventral traces, L-lateral trace, w-dorsal sector with wide vessels, n-lateral sector with narrow vessels, FT-dorsal traces that appear fused, P-petiole)

Multiple stains were used as tracers to follow potential pathways of water and bacterial movement through the node allowing each set of dorsiventral traces and subsequent anastamoses to be followed independently (Figure 3). As the stains moved through the node region, no stains were observed across the sclerified parenchyma diaphragm indicating an absence of medullary vascular connections. The stains also allowed visualization of trace divergence from the stele over a distance of roughly a millimeter and the leaf gap that was created.

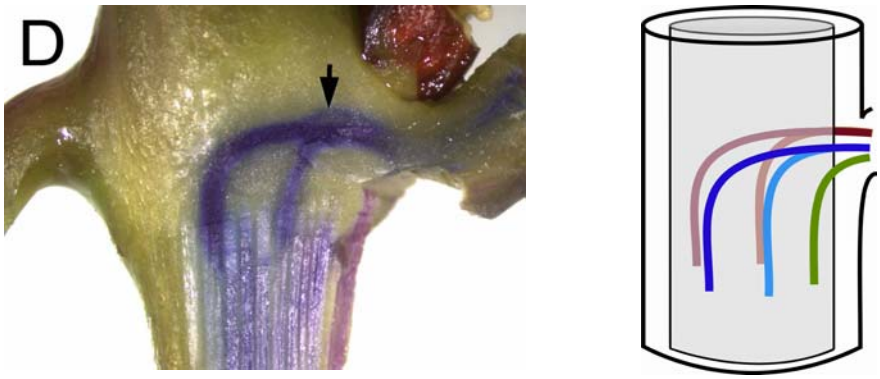

Figure 3. Stain tracer initiated in stem tissue following the conductive stream within leaf traces into the petiole (left). Model of leaf trace divergence into petiole based on serial sectioning and stain tracers (right). Arrow indicates distinct leaf trace.

Tissue macerations were made of samples dissected from a stem internode (wood and pith), stem node (wood and diaphragm), leaf trace, petiole, young summer lateral, and tendril. Vessel elements in the stem typically had scalariform

lateral wall pitting and simple perforation plates verified by resin casting and electron microscopy (Figure 4). Vessel elements with helical secondary walls and simple perforation plates were predominant in the leaf trace and petiole macerations, but were also visible in all other samples likely representing the primary xylem component of these tissues. Narrow tracheary elements commonly bordered wide vessels and are likely vasicentric tracheids (Metcalf and Chalk 1950). These vasicentric cells possessed tracheid-like qualities including delicate spiral thickenings and bordered pits, however, absence of perforations could not be verified.

CONCLUSIONS

Examination of the vascular structure of the node of *Vitis vinifera* Chardonnay grapevine confirmed many aspects of prior investigations, however, no evidence was found that the traces of each leaf are distinct for four internodes before they leave the stele (Fournioux and Bessis 1979), or that trace fusion necessarily occurs between pairs of dorsal and ventral leaf traces (Fournioux and Bessis 1974). Additionally, variability in number of leaf traces present at each node presented here was not suggested in previous published reports (Fournioux and Bessis 1979).

No distinct traces were observed in serial sections either immediately before a node, or immediately after a node. Leaf traces of fully expanded leaves were visibly and conductively distinct only a few millimeters before divergence from the stele. Leaf gaps closed within a few millimeters of trace divergence, and after gap closure no distinct traces for successive nodes were observed. If traces are not visibly distinct across even one node, there is little support for the idea that traces are distinct for four nodes of mature tissues. This is a significant finding for the study of the spread of Pierce's disease within a grapevine as bacteria must move through leaf traces to colonize the leaf lamina and vice versa. If leaf traces are distinct for four nodes then *Xf* present in a specific leaf would have to be directly inoculated, inoculated within a trace that supplies that leaf, or enter a trace four nodes basal to a leaf from adjacent stem xylem. If leaf traces are only distinct for a short length many more sources of inoculation are possible.

Fusion between mature traces is subjective based on the juxtaposition of two or more adjacent traces. Dorsal and ventral traces were observed from the point of single traces diverging from the stele and progressing into the base of the petiole in both transverse and tangential planes. Frequently these traces did not appear to fuse at all, and when the case for fusion could be argued it was likely that traces were simply juxtaposed with little or no ground tissue between them. If leaf trace fusion does not, or rarely occurs then bacterial colonization of the petiole and perhaps subsequently the leaf lamina may be segregated depending on the location of the source of bacteria from the stem. If specific leaf traces supply specific regions of the leaf lamina then a lack of fusion between traces may make a uniform dispersion of bacteria throughout the leaf unlikely.

The stem of the grapevine can be described as sectored based on the consistent location of large parenchymatous gaps, separate from leaf gaps, in the vascular cylinder. These gaps are created by the regular divergence of xylem into the tendril on one side, and into the summer lateral shoot and compound bud on the other. The result of the regular repeating gap pattern creates 4 sectors in the stem; a dorsal sector, a ventral sector, and two opposite lateral sectors (Figure 4). The consequence of having the large gaps lacking vascular tissue in the lateral sector(s) of each node is that long distance conductance beyond one internode may only occur in the dorsal and ventral sectors of the stem. Consequently, depending on the location of sharpshooter feeding, bacteria introduced within dorsal and ventral sectors would be more likely to move to distal regions of the shoot than bacteria introduced to lateral sectors. Additionally, the wider vessels of the dorsi-ventral sectors would provide a lower resistance pathway for long distance movement of bacterial aggregations.

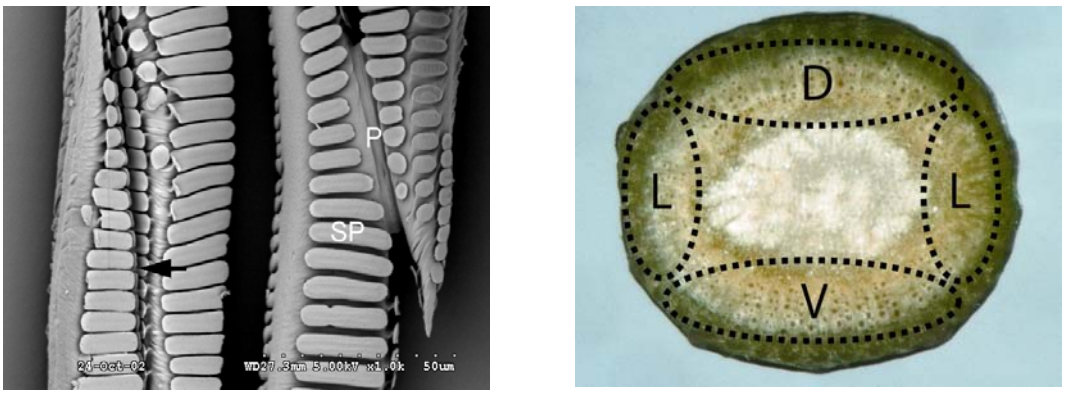

Figure 4. Resin-casts of the interior surface of vessel elements showing frequent scalariform pitting (left) and proposed stem sectoring based on regular patterns of lateral vascular divergence. SP-scalariform pit, P-simple perforation, D-dorsal sector, V-ventral sector, L-lateral sectors.

The characteristics of the tracheary elements within grapevine wood and primary tissues may contribute to the level of susceptibility to PD. The relatively wide vessels found within stem wood possess simple perforation plates with scalariform intervascular pitting (Metcalf and Chalk 1950). Simple perforation plates likely provide a low resistance pathway for bacterial cells between consecutive vessel elements allowing the bacteria to move relatively unimpeded through a single vessel. Resistance to bacterial movement would occur at the end of a vessel within the terminal vessel element. The very

wide scalariform lateral wall pits (Figure 4) within the terminal vessel elements create a large pit membrane surface area which may be weaker and susceptible to bacterial breach by digestion, or physical damage due to the physical stress of cavitation and refilling cycles within the vessel. The combination of these traits may allow bacteria to move through the stem system very quickly until the vessel lamellae in which they are located diverges into a lateral organ. Once bacteria are in a leaf trace or petiole, much narrower and shorter vessels and tracheids may act to filter bacteria from the conductive stream.

REFERENCES

Fournioux, J.C., and R. Bessis. 1979. Etude des relations criblo-vasculaires entre les differents organes de la tige de la vigne (*Vitis vinifera* L.). Connaissance Vigne Vin 13: 91-114.

Fournioux, J.C., and R. Bessis. 1974. Inegalites dans la distribution et la differenciation des traces foliaires en rapport avec la dorsiventralite de la tige chez le *Vitis vinifera* L. Revue Generale de Botanique 81: 41-53.

Fournioux, J.C., and R. Bessis. 1973. Etude du parcours caulinaire des faisceaux conducteurs foliaires permettant la mise en evidence d'une rythmicite anatomique chez la vigne (*Vitis viniferia* L.). Revue Generale de Botanique 80: 177-185.

Hopkins, D.L., and H.H. Mollenhauer. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179: 298-300.

Metcalf, C.R., and L. Chalk. 1950. Anatomy of the Dicotyledons. 2 vols. Oxford at the Clarendon Press, London.

Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. Biology of the Grapevine. Cambridge University Press, New York.

Pratt, C. 1974. Vegetative anatomy of cultivated grapes -- a review. American Journal of Enology and Viticulture 25: 131-150.

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