Grapevine Susceptibility to Pierce’s Disease I:  
Relevance of Hydraulic Architecture  
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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 Xylella fastidiosa, the cause of Pierce’s disease. 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 the movement of stains within the xylem. Four to eight leaf traces diverge from the stele, which may or may not fuse to form a reduced number, and anastomosis of these traces begins before entering the petiole proper. No evidence was observed to support the report that leaf traces are distinct for four nodes prior to divergence and are not considered to be conductively isolated. Lateral divergence of stem xylem into tendrils and lateral shoots creates a repeating pattern of gaps in the node that may preclude long distance movement of bacteria in these sectors of the stem. The hydraulic architecture of the grapevine node is described and implications for the spread of Pierce’s disease within the grapevine shoot are discussed.  

Key words: Pierce’s disease, xylem, hydraulic architecture, Vitis  

INTRODUCTION  

Pierce’s disease (PD) in grapevine is caused by a bacterial infection of Xylella fastidiosa (Xf) within xylem vessels (Davis et al. 1978). The impact of the disease has limited Vitis vinifera production across much of the southeastern U.S. (Hopkins 1989), and currently threatens the lucrative California wine and table grape industry (Varela et al. 2001). Plants are infected with this disease by xylem-feeding suctorial insects commonly known as sharpshooters (Purcell and Finlay 1979). It is unknown whether the mechanism of pathogenesis is a 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), programmed cell death (David Gilchrist personal communication), or a combination of these factors.

The spread of Xf bacteria or bacterial products within the grapevine likely leads to blockage of water within the plant’s hydraulic network (the xylem) and consequently 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 xylem borne bacteria or phytotoxins within grapevine shoots.

The general vegetative anatomy and the primary vascularization of grapevine have been summarized (Fournioux and Bessis 1973, Fournioux and Bessis 1974, Fournioux and Bessis 1979, Gerrath et al. 2001, Mullins et al. 1992, Pratt 1974, Pratt 1979) and certain aspects of anatomical symptoms of Pierce’s disease have been documented (Esau 1948, Hopkins 1989, Tyson et al. 1985). Although developmental patterns of grapevine hydraulic architecture have been proposed, the vascular arrangement of the grapevine stem must be discussed in the context of the spread of Pierce’s disease within the plant from the site of inoculation to a systemic presence. This research is not a study of the development of the vascular system, but instead describes the potential pathways for pathogen movement through the node of a mature grapevine stem from a functional perspective and with a view of the practical consequences of the system.

MATERIALS AND METHODS

Grapevines (Vitis vinifera L. cv. Chardonnay) were grown in a greenhouse at the University of California at Davis. Stem samples were collected between the 10th and 20th nodes of shoots of current year’s growth from three different plants. Overall, six nodes were serially sectioned in a transverse plane, and three in a longitudinal plane. Samples were dissected, fixed in formalin, acetic acid and alcohol (FAA), and dehydrated in a graduated ethanol series (Ruzin 1999). The tissue was then infiltrated with a xylene substitute, Hemo-De (Fisher Scientific, Hampton, NH, USA) and subsequently into Paraplast Extra paraffin (Fisher HealthCare, Houston, TX, USA) using a Leica TP 1020 automatic tissue processor (Leica Microsystems, Bannockburn, IL, USA), embedded with a Leica EG 1160 paraffin embedding center, and then sectioned with a Microm HM 340E rotary microtome (Microm, Walldorf, Germany) at 10 μm. Sections were stained with Johansen’s safranin and fast green protocol (Ruzin 1999). Prepared slides and fresh material were observed with compound and dissecting microscopes, and images were captured on slide film and scanned into digital format. Fresh sections made from the node were stained with phloroglucinol as an indicator for the presence of lignin (Ruzin 1999).

For dye movement experiments, stain was introduced to the xylem of plants exposed to full sunlight by using 27 gauge Monoject hypodermic needles (Sherwood Medical, USA) attached to 1 cc tuberculin syringes. Basic fuchsin and crystal violet (0.1 % aqueous) were decolorized with sodium metabisulfite and used as stain tracers (Talboys 1955). The syringe plunger was removed and the syringe body was used as a stain reservoir. Two syringes, each with a different stain, were inserted simultaneously into the wood of stem internodes and taped in place; one syringe was inserted into the
dorsal side and one syringe was inserted into the ventral side, both perpendicular to the plane of the leaves and tendrils. Consequently, stain was not forced into the xylem under positive pressure as with a typical injection, but was allowed to passively flow into the xylem.

Macerations of xylem tissue were made from samples dissected from the stem internode (wood and pith), stem node (wood and diaphragm), leaf traces, petiole, young summer lateral, and tendril. Macerations were prepared using Schmid’s improved Jeffrey’s method (Ruzin 1999) and stained with safranin O.

For scanning electron microscopy, blocks of grapevine wood were dissected with razor blades and air-dried. A resin-casting technique utilizing Mercox resin (Structure Probe Inc., West Chester, PA, USA) was used to study the internal morphology of vascular conduits (Mauseth and Fujii 1994). Wood preparation consisted of complete dehydration and trimming a shoot segment to fit into the perfusion tubing. Digestion of organic material followed polymerization of casts by initially immersing the sample in a solution of equal parts hydrogen peroxide and glacial acetic acid incubated at 60°C for a minimum of 24 hours followed by treatment with concentrated sulfuric acid. Blocks of wood and resin casts of grapevine vessels were sputter coated with gold and observed at 5 kHz with a Hitachi S-3500N scanning electron microscope (Hitachi High-Technologies America, San Francisco, CA, USA).

RESULTS

Morphology

The external morphology of a grapevine node with a petiole, summer lateral, and compound bud on one lateral side, and a tendril on the other is shown in Figure 1. Protrusions on the dorsal and ventral surfaces of the node created by leaf traces diverging from the stele are clearly visible on the stem surface. The dorsi-ventral plane of the stem is perpendicular to the lateral plane of the leaves and tendrils. The dorsal and ventral surfaces are superficially identical, however, the ventral side of the stem, as defined by Fournioux and Bessis, is the side towards which lateral leaf traces are slightly biased (i.e. lateral traces of successive nodes, on opposite sides of the stem, diverge at less than 180° from each other) (Fournioux and Bessis 1979). A similar node bisected longitudinally is shown in Figure 2. Following this section from bottom to top, the internodal arrangement of a complete ring of wood continues to the node where vascular divergence into organs creates lateral gaps in the hydraulic network (arrows). In the successive internode a complete ring of wood is again present. The pith in the internodal regions consists of regular unsclerified parenchyma, whereas in the node, a diaphragm of parenchyma with sclerified cell walls is present. The diaphragm region stained positively for the presence of lignified cell walls with phloroglucinol. The diaphragm does not contain any medullary vascular bundles and consequently no vascular connections are made transversely across the pith.

A serially sectioned grapevine node with a petiole, developing summer lateral shoot, compound bud, and tendril is depicted in a series of images ordered from below the node to above the node as outlined in Figure 3. In the most basal section below the node, a complete ring of wood is present bounding a regular parenchymatous pith (Figure 4A). Consequently, 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
4B). In this paper, leaf traces are described from their point of origin within the stem, for
example, the dorsal leaf trace(s) originates from the dorsal side of the stem, the lateral
leaf trace originates from the lateral side of the stem. This is somewhat unconventional,
as traces that originate on the dorsal and ventral sides of the stem are typically described
as lateral, and traces that originate from the lateral side are described as central or
median. As conventional descriptors may be confusing to non-anatomists, they were
abandoned.

The number of leaf traces at each node was variable; ranging from four to eight
traces, with five being the most common. Anastomosis, or branching, of leaf traces may
begin while the trace is still in the stem cortex before it enters the petiole. In this
example, the single lateral trace has already begun to segregate into multiple bundles
from the original single trace (Figure 4B). As the leaf trace diverges completely from the
stele, a gap of parenchyma cells appears in the vascular cylinder; these areas are
traditionally called leaf gaps. Once the leaf traces have diverged from the stele in an
axial orientation (Figure 4C), their pathway quickly bends perpendicular from the vertical
axis and they become radially oriented (Figures 4D). 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. Additionally, in the
region of trace divergence, the pith is a lignified parenchymatous diaphragm (Figure 4D).

Large portions of the lateral regions of the node lack conductive tissue 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 4E). As the xylem supplying these
organs diverges, parenchymatous gaps are created that lack any vascular tissue and
consequently conduction cannot occur across the node in these regions. Within one
lateral gap, vascular differentiation occurs separately 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 is
evident by the differentiated branch traces connecting the base of the summer lateral to
the xylem of the main shoot (Figure 4F). No branch traces are visible for the dormant
shoots in the compound bud, as those shoots have not yet begun to elongate (Figure 4G).
If further shoot development occurs in some or all of the many meristems within the
compound bud, then a set(s) of branch traces would partially fill the common branch and
bud gap in a similar fashion as the summer lateral branch traces. 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 4H). Additionally, the sclerified
parenchymatous diaphragm of the node is no longer present, instead it is replaced by a
regular unsclerified parenchymatous pith. In the most acropetal of the serial sections, in
the successive internode, the stele is once again complete with no gaps in the stele or
distinct traces present (Figure 4I).

Anatomy

A higher magnification micrograph of a transverse section through the shoot
shows an early stage of leaf trace divergence similar to that depicted in Figure 4b (Figure
5). There is a great 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 (Figure 5). From this particular sample, the dorsi-ventral sector vessel diameters were $65 \pm 21 \mu m$ (mean ± sd, n = 70), whereas lateral sector vessel diameters were $31 \pm 12 \mu m$ (n = 45). 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 (Figure 6). Although the number of leaf traces ranged from four to eight, the pathways of individual leaf trace divergence were similar no matter the total trace number. The exact lamellar location of trace origin within the stem’s vasculature is variable for all traces from node to node, with the lateral trace(s) sometimes originating nearly from the ventral side. As a leaf trace diverges completely from the stele, a leaf gap of parenchyma tissue is created in the vascular cylinder (Figure 7). One leaf gap is created for each trace.

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 8) or remain distinct (Figures 9 and 10), but nevertheless are usually in close proximity to one another. Viewed in transverse section, the traces may appear to merge into a single vascular bundle (Figure 8), however, a closer inspection will usually reveal some parenchyma cells remain between the traces (Figure 9). Additionally, even though these traces may appear to fuse in transverse section, when viewed in tangential sections, parallel to the stem axis, they often appear to be layered on top of another (Figure 10). Distal from the point that leaf traces have diverged into the petiole, the parenchymatous gaps in the dorsiventral sectors are no longer present and no leaf traces are distinct for further distal nodes.

Vascular differentiation occurs independently between the developing summer lateral shoot and each of the compound buds to the axial hydraulic network based on the separation between respective vascular bundles (Figure 11) and procambial strands (Figure 12). The vascular system of the developing summer lateral shoot is continuous with the existing axial xylem creating a seamless series of lamellae uninterrupted at the junction (Figure 11). Similar connections will ultimately be made between the dormant shoots in the compound buds and the axial system upon their further development. These connections will be made upon the tangents of the procambial strands towards existing vascular lamellae on the margins of the lateral sector (Figure 12).

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. Stem internode, stem node, and summer lateral macerations were dominated by similar proportions of fibers, parenchyma, and tracheary elements. Petiole and leaf traces had higher proportions of parenchyma and tracheary elements and fewer fibers. Tendril macerations displayed a greater proportion of fibers, a moderate amount of parenchyma, and few narrow tracheary elements. The fibers observed in all macerations were primarily septate fibers, however simple fibers were also present. Parenchyma cells in the stem internode, summer lateral, leaf traces, petiole, and tendril were unsclerified, whereas parenchyma cells of the stem node sample possessed either darkly-staining lignified or lighter unlignified cell walls. Vessel elements in the stem internode, stem node, summer lateral, and tendril typically had scalariform lateral wall pitting and simple
perforation plates. 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.

Scalariform lateral pitting patterns and simple perforation plates present in vessel members in wood were verified by resin casting and electron microscopy (Figure 13). Tracheids were not definitively observed in macerations or sections with either light or electron microscopy. Narrow tracheary elements that commonly bordered wide vessels are likely vasicentric tracheids (Metcalf and Chalk 1950). When observed with SEM, these vasicentric cells possessed tracheid-like qualities including delicate spiral thickenings and bordered pits, however, absence of perforations could not be verified.

Stain Tracers

Stains were used as tracers to follow potential pathways of water and bacterial movement through the node. Trials occurred in which roughly half the stem vascular tissue conducted and stained red with basic fuchsin and the other half stained violet with crystal violet (Figure 14a). The bilateral staining allowed each set of dorsiventral traces and subsequent anastamoses to be followed independently (Figure 14b). As the stains moved through the node region, no stains were observed across the sclerified parenchyma diaphragm indicating an absence of medullary vascular connections (Figure 14c). 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 14c). Additionally, longitudinal dissection of the node showed that the separate ventral leaf traces may not necessarily fuse, but may only be in close proximity, as individual traces were visible to the point of petiole anastomosis (Figure 14d).

DISCUSSION

The vascular structure of the node of *V. vinifera* Chardonnay grapevine was studied and largely confirmed prior investigations (Fournioux and Bessis 1973, 1974, 1979). However, based on sectioned material, no evidence was found in this study that the traces of each leaf might be functionally distinct for up to four internodes before they leave the stele, or that trace fusion necessarily occurs between pairs of dorsal and ventral leaf traces (Fournioux and Bessis 1974). Additionally, the variability in the number of leaf traces at each node shown in this study had not been suggested in previous published reports (Fournioux and Bessis 1973, 1974, 1979). Gerrath et al. (2001) did observe some variability in the number of leaf traces for *Vitis riparia*. The Fournioux and Bessis studies used stem clearings of the youngest nodes of developing Pinot noir shoots and described the differentiation of the first few tracheary elements, whereas this study examined older stem tissue that had undergone significant secondary growth. Although leaf traces four nodes near the tip of an elongating shoot may only be separated by a few centimeters and be only a cell or two wide, four nodes of a mature cane can be separated by well over 30 cm and the traces consist of a hundred cells. It is possible that these aforementioned contradictions are simply differences of vascular organization between tissues of different maturity, or differences in the cultivar of *Vitis vinifera* examined.

Fournioux and Bessis have shown that the very first elements of the primary xylem could be developmentally distinct for four nodes (Fournioux and Bessis 1979), but we found no evidence to support a proposal that these traces remain functionally discrete
in mature vasculature. No distinct traces were observed in serial sections either immediately apical to or basal to 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 spatially distinct across even one node, there is little support for the idea that traces are conductively distinct for four nodes of mature tissues. This is significant 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 very short length many more sources of inoculation are possible. While Fournioux and Bessis are describing developmental distinction, the goal of this study was to describe practical conductive pathways. Leaf traces that are distinct during primary growth of the stem are not distinct when secondary xylem is produced proximal to the primary traces. When secondary xylem is formed, a conductive continuum is created between the primary xylem of the trace and secondary xylem of the stem. The traces only become functionally distinct when they depart the stele immediately below the node to supply the respective petiole.

Trace fusion may occur during the initial differentiation of vascular strands (Fournioux and Bessis 1974), but 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 occur, 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 vascular structure within the node of PD susceptible Chardonnay may allow for predictions to be made about the movement of bacteria through the hydraulic network. Numerous traits appear to be consistent in the nodal anatomy including sectoring of the stem created by positioning of lateral gaps, variability in leaf trace number and origin, and tracheary element properties. These properties will affect where and how far bacteria are able to move depending on their inoculation point into the stem or petiole.

The vascular cylinder of the grapevine stem can be described as sectored based on the consistent location of large parenchymatous gaps, which are separate from leaf gaps. 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 (Pratt 1978). As vascular divergence occurs in both summer lateral branch and compound bud traces in the same vertical file, a common summer lateral branch and compound bud gap is formed. In many plants the diverging leaf traces will also contribute to the common gap created by the lateral shoot (Esau 1977), however in grapevine it appears that the leaf
gaps close before another separate gap is created by the summer lateral shoot and the compound bud. Vascular divergence to the tendril is simpler as there is only one organ on its side of the node versus the three on the other. It is expected that vascular divergence to an inflorescence and subsequent berry cluster is similar to the tendril based on their developmental homology (Mullins et al. 1992) and would create a gap similar to that of the tendril. The result of the regular repeating gap pattern creates four sectors in the stem, a dorsal sector, a ventral sector, and two opposite lateral sectors (Figure 15). However, the tendril or inflorescence gap would be absent when these organs are not present at a given node.

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. Unless there is significant lateral movement between sectors in the internode, it would seem logical that any bacteria present in lateral sectors would be diverted into the tendril on one side, or into the leaf, summer lateral, or bud on the other. Lateral movement between sectors would require transfer between adjacent vessels or the pathway of an intact vessel transecting two sectors. However, the xylem of grapevine is split up into lamellae, or radial blocks of secondary tissue, by the broad and tall primary (medullary) and secondary rays (Metcalf and Chalk 1950). Depending on the height of these rays, lateral transfer of xylem contents even between adjacent lamellae may be severely limited. Lateral conductance is further restricted by the lack of a vascularized pith that is occasionally found in dicotyledonous families (Fahn 1990). This prevents any vascular connection between sectors on opposite sides of the stem. Consequently, depending on the location of bacterial inoculation, or 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. As a correlation exists between vessel width and vessel length (Carlquist 1988), longer vessels in the dorsi-ventral section may also compound to the speed of bacterial migration by having fewer terminal vessel elements requiring bacterial breaching of pit membranes.

The location of origin and number of leaf traces is variable, which contributes some unpredictability to the hydraulic network. When there are five traces, two arise from the dorsal sector, two from the ventral sector, and one from the lateral sector, but where the traces arise within those sectors varies from node to node. Traces within a dorsal or ventral sector may be close together, or may be separated by several lamellae, and in many cases the lateral trace is skewed nearly into the ventral sector. The number of leaf traces diverging from the stele is also variable from node to node; nodes were observed with trace numbers ranging from four to eight, with five being the most common. The number of traces, although usually five, appears to be random as no pattern in the number of leaf traces was found in successive nodes. When even numbers of traces were observed, traces were evenly divided into the dorsal and ventral halves of the stem (including the lateral traces on each respective half of the stem). When odd numbers of traces were observed, the ventral side of the stem had one additional trace than the dorsal side. This makes the portion of stem which donates the xylem of the petiole somewhat unpredictable and creates a variable source for following the
progression of the spread of PD into the leaf lamina. Additionally, if basipetal movement of bacteria from lateral organs occurs, it would be difficult to predict the exact location within the stem that contains any bacteria introduced from the leaf and petiole.

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 grapevine 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 13) within the terminal vessel elements create a large pit membrane surface area which may be weaker and susceptible to bacterial breach by digestion (Hopkins 1985), or physical damage due to the physical stress of cavitation and refilling cycles within the vessel (Hacke et al. 2001). 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 (Figure 16). Short and narrow vessels provide a higher resistance pathway to bacterial movement as decreased vessel diameter would restrict the movement of large bacterial aggregations and more frequent terminal vessel elements would require more repeated breaching of intervessel pit membranes. Bacterial movement would be even slower through a tracheid network as no perforations are present between consecutive xylem cells and pit membranes would have to be breached by bacteria to move through every single tracheary element.

Some features of Chardonnay grapevine stem wood may alleviate the conductive consequences of xylem occlusion associated with PD. It seems logical that the formation of gums and tyloses developing within vessels of infected plants would decrease water conductance within those cells (Hopkins 1981). However, grapevine vessels commonly occur in radial clusters, or chains, and have associated vasicentric tracheids (Metcalf and Chalk 1950); both of which may provide redundancy in the conductive network. Vessel clusters provide multiple pathways for conductance around blocked tracheary elements allowing alternate routes for water movement to bypass occlusions (Tyree et al. 1994). Tracheids may provide an auxiliary network of conductive cells (Carlquist 1988) much less susceptible to blockage due to their lateral wall pitting characteristics decreasing both the likelihood of tylose formation and providing increased resistance to bacterial colonization. Additionally, sparse paratracheal parenchyma and living septate fibers found in stem wood may slightly decrease the susceptibility of grapevine vessels to cavitation and embolism formation as they may be able to donate water to xylem vessels and alleviate water stress (Carlquist 1980).

CONCLUSIONS

The morphology and anatomy of the nodal region of Chardonnay grapevine stem was studied to analyze the hydraulic architecture in the context of the pathology of Pierce’s disease. Comparisons to the prior publications concerning the vasculature of the grapevine node revealed confirmations, but also some significant differences. The
elucidation of the nodal structure also provided insight into the relevance that xylem characteristics may have on the movement of Xf and subsequent progression of PD within infected plants.

Future studies are required to study hydraulic architecture in older grapevine xylem to determine vascular pathways within stems without leaves or within frequent summer lateral shoot and branch junctions. The degree of vascular divergence within more mature tissue will affect long distance movement of bacteria within older grapevine trunks and cordons. Additionally, the extent to which the tall rays segregate individual lamellae and restrict the transfer of xylem contents between sectors of the stem may also determine variability in long distance xylem transport and needs to be examined. Furthermore, anatomical comparisons of hydraulic architecture and xylem cell characteristics between susceptible and resistant varieties may help explain tolerance or avoidance of PD symptoms.
LITERATURE CITED


Legends for Figures

Figure 1. External morphology of grapevine node. LT – leaf trace, P – petiole, SL – summer lateral, CB – compound bud, T – tendril. Scale bar = 1 mm.

Figure 2. Longitudinal section of grapevine node. P – petiole, SL – summer lateral, CB – compound bud, W – wood, Pi – pith, D – diaphragm, T – tendril. Arrows indicate summer lateral/bud and tendril gaps. Scale bar = 1 mm.

Figure 3. Longitudinal section of grapevine node indicating the general location of serial sections for figures 4A-I respectively.

Figure 4. Serial sections of a grapevine node in order from most basal (below node) to distal (above the node). A) Most basal section of grapevine (pre)node. Wood is a complete ring with no distinct leaf traces W- wood, Pi – pith. B) Section showing leaf trace divergence. Arrows indicate five leaf traces (two dorsal, two ventral, one lateral) with the lateral leaf trace anastomosing. C) Serial section in which leaf trace anastomosis and early transition from axial orientation to transverse orientation is evident. Arrows indicate distinct traces, AT – anastomosing lateral leaf trace. D) Section showing leaf traces transversely oriented, anastomosis into base of petiole visible, and presence of sclerified parenchymatous diaphragm. FT – potentially fused traces, P – petiole, D – diaphragm. E) Section in which leaf traces and leaf gaps are absent. Vascular divergence into the tendril and compound buds/summer lateral has created gaps in axial hydraulic network. TG – tendril gap, CG – common summer lateral shoot and compound bud gap. F) Serial section showing summer lateral and tendril gaps, developing vascular differentiation from summer lateral to existing axial vasculature. P- petiole, T – tendril, arrow indicates developing summer lateral branch traces. G) Section above the level of summer lateral divergence showing continuation of gaps. CG - common summer lateral shoot and compound bud gap. H) Serial section nearly above tendril and petiole, tendril and common gaps almost closed, regular parenchymatous pith. Region where common gap was present in previous sections now contains axial vascular tissue. Arrow indicates closure of common gap, Pi – pith, CB – compound bud, SL – summer lateral. I) Most distal of the serial sections showing complete ring of wood with no distinct leaf traces or gaps. Scale bars = 1mm.

Figure 5. Transverse section through the basal region of node showing five distinct traces in an early stage of divergence (two dorsal, two ventral, one lateral) with the lateral trace skewed to the ventral side of the stem. Note the predominantly wide diameter of vessels in the dorsal and ventral sectors and the predominantly narrow vessels in the lateral sector. D1 and D2 – dorsal leaf traces, V1 and V2 – ventral leaf traces, L – lateral leaf trace, w – dorsal sector with predominantly wide vessels, n – lateral sector with predominantly narrow vessels. Scale bar = 250 µm.

Figure 6. Tangential section through grapevine stem depicting a vessel and fiber matrix in the axial system and very tall rays in the radial system delimiting four lamellae. R1, R2, R3 – separate rays, F – wood fibers, P – lateral vessel wall scalariform pitting, V –
vessel lumen with three vessel members separated by simple perforation plates. Range of scale dots = 250 µm.

Figure 7. Transverse section showing a dorsal leaf trace diverging and the parenchymatous leaf gap in the stele. T – leaf trace, G – leaf gap. Scale bar = 250 µm.

Figure 8. Transverse section through a young node depicting either a single dorsal leaf trace or fused dorsal leaf traces diverging from the stem into the petiole in a transverse orientation. FT – fused leaf traces, P – petiole. Scale bar = 250 microns.

Figure 9. Transverse section showing two ventral leaf traces and the lateral leaf trace. Section illustrates the subjective nature of whether leaf trace fusion occurs in transversely oriented leaf traces, or whether leaf traces are simply juxtaposed. V1 – ventral leaf trace originating to the left of the field of view, V2 – second ventral leaf trace in transition to transverse orientation, L – lateral leaf trace. Scale bar = 100 µm.

Figure 10. Longitudinal section showing two radially oriented dorsal leaf traces separated by parenchymatous ground tissue. Image shows lack of fusion between traces just prior to anastomosis into the petiole. T1 – first dorsal leaf trace, T2 – second dorsal leaf trace. Scale bar = 250 µm.

Figure 11. Transverse section through a node illustrating differentiating summer lateral branch traces to the existing axial vasculature. SL – summer lateral, W – wood of the main shoot, arrow shows differentiating summer lateral branch trace. Scale bar = 250 µm.

Figure 12. Transverse section through node showing differentiating procambium associated with one of the compound buds. When bud elongation occurs, vascular development between the new shoot and existing axial network will occur along this tangent. Common summer lateral and compound bud gap without any developed vasculature is clearly visible. Arrow indicates bud procambium, B – young dormant shoot enclosed with bud scales, CG – common summer lateral shoot and compound bud gap, W – wood of main shoot. Scale bar = 250 µm.

Figure 13. SEM micrograph of vessel resin casts. Casts of scalariform pitting are visible on the lateral walls and a simple perforation is visible between two vessel elements of the right-hand vessel cast. SP- scalariform pit, arrow indicates scalariform pits between two adjacent vessel elements, P - simple perforation. Scale dot range = 50 µm.

Figure 14. A) Transverse section through grapevine internode that has had basic fuchsin (lighter) and crystal violet (darker) introduced to the dorsal and ventral xylem respectively. B) Stem vasculature maintains orientation into petiole as indicated by consistent position of stain. Node is viewed from below the petiole base. Arrows indicate stained anastomosing leaf traces into base of petiole. C) Longitudinal section through node showing stain running axially through the dorsal and ventral sectors. Leaf trace divergence and gap is illustrated on the ventral sector (violet or dark) and there is no
staining visible in the diaphragm. Arrow indicates diverging leaf trace, D – diaphragm of sclerified parenchyma. D) Longitudinal section just below the epidermis depicting ventral leaf traces in transition from axial to transverse orientation. Traces are visibly distinct into the base of the petiole. Arrow indicates region where leaf traces are juxtaposed, but appear to be nonetheless distinct.

Figure 15. Grapevine stem transverse section showing proposed wood sectoring. D – dorsal sector, V – ventral sector, L – lateral sector.

Figure 16. Transverse section of leaf trace tracheary elements from a grapevine infected with *Xylella fastidiosa* bacteria. Arrow indicates scalariform lateral pitting between two adjacent tracheids and bacteria that have accumulated against the terminal wall face. Scale bar = 25 µm.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.
Figure 9.
Figure 10.
Figure 12.
Figure 13.
Figure 14.
Figure 15.
Figure 16.