

PRUNING-INDUCED TYLOSE DEVELOPMENT IN STEMS OF CURRENT-YEAR SHOOTS OF *VITIS VINIFERA* (VITACEAE)¹

QIANG SUN,^{2,4} THOMAS L. ROST,³ AND MARK A. MATTHEWS²

²Department of Viticulture and Enology and ³Section of Plant Biology, University of California, One Shields Avenue, Davis, California 95616 USA

Tyloses form in xylem vessels in response to various environmental stimuli, but little is known of the kinetics or regulation of their development. Preliminary investigations indicated that wounds seal quickly with tyloses after pruning of grapevine shoots. In this study, tylose development was analyzed qualitatively and quantitatively at different depths and times from pruning cuts along current-year shoots of grapevines at basal, middle, and apical stem regions. Tyloses developed simultaneously within a single vessel but much separated in time among vessels. Pruning caused prodigious tylosis in vessels of grape stems, extending to approximately 1 cm deep and to 7 d after wounding, but about half of the vessels did not become completely occluded. The fraction of vessels forming tyloses was greatest in basal (85%) and least in apical (50%) regions. The depth of maximum density of tyloses was 4 mm from the cut in the basal region and 2 mm from the cut in the middle and apical regions. Tylose development was faster in the basal and middle than in the apical region. The pattern of tylose development is discussed in the context of wound repair and pathogen movement in grapevines.

Key words: current-year shoot; grapevine; pruning; tylose; Vitaceae; *Vitis vinifera*; wood anatomy; wounding; xylem.

Tyloses are outgrowths of parenchyma cells through vessel-parenchyma pit pairs into the lumen of tracheary elements (Esau, 1977). Tyloses occur in a wide range of species (Saitoh et al., 1993), and in plants of some genera (e.g., *Quercus* and *Robinia*) there is genotypic variation in the propensity for tylosis (Biggs, 1987; Saitoh et al., 1993). Tyloses form naturally in heartwood (Parameswaran et al., 1985; Ranjani and Krishnamurthy, 1988) and are observed in xylem of leaves during senescence (Chaffey and Pearson, 1985; Dute et al., 1999). Investigations of tylose development have been based mostly on plants infected by pathogens and in response to wounding. These studies have made clear that in many species the formation of tyloses is a common response to infection by vascular fungi (e.g., *Fusarium*; Beckman and Talboys, 1981) or by bacteria (e.g., *Pseudomonas*; Wallis and Truter, 1978), flooding (Davison and Tay, 1985), freezing (Cochar and Tyree, 1990), and mechanical injury (Schmitt and Liese, 1993).

Although tyloses have long been recognized (Boehm, 1867; von Reichenbach, 1845, described in Zimmermann, 1979), little has been resolved regarding the dynamic process, spatial and temporal details, and functional significance of tylose formation (Bonsen and Kučera, 1990; Pearce, 1991; Canny, 1997). In cultivated grapevines, pruning at different locations along the shoot is a normal part of viticulture (Galet, 2000), and in grapevines infected with Pierce's disease (caused by *Xylella fastidiosa*), tyloses are observed in primary and secondary xylem (Esau, 1948; Mollenhauer and Hopkins, 1976; Stevenson et al., 2004), but the role of tylosis in Pierce's disease of grapevine is similarly not clear.

In our preliminary investigations, wounds sealed quickly

after pruning of a grapevine stem in association with the appearance of tyloses in xylem vessels. Our objective in this study was to show the dynamic process of tylose development from xylem parenchyma and the differences in wounding repair in different ages of wood in a single cane in its first year of growth. Therefore, we used current-year shoots of grapes to clarify unrevealed details of wounding-induced tylosis by investigating (1) xylem structure of current-year shoots with special regard to the types of pits and tyloses, (2) tylose-forming capacity of xylem tissue along the axis of current-year shoots, and (3) the temporal progress and spatial distribution of wound-induced tylose development in xylem tissue and in single vessels. We expect that the information revealed by this study can provide a basis for an analysis of any possible roles of tylosis in wound healing and defense against bacterial diseases such as Pierce's disease in grapevines.

MATERIALS AND METHODS

Pruning treatments of current-year shoots—Experiments were conducted on 1.5–2.5-m long current-year shoots of eight-year-old *Vitis vinifera* cv. Chardonnay plants at the University of California, Davis, California, USA. Pruning treatments were imposed by cutting through an internode at one of three regions of the stem: basal (10–20 cm from the base branch), middle (60–80 cm from the base branch), or apical (20–40 cm from the shoot end) (Fig. 1). A 4-cm-long sample was collected from the apical side of each cut immediately after pruning and fixed in formalin-acetic acid-alcohol (FAA) (Ruzin, 1999) for later analysis of xylem anatomy and tylose development as samples for day 0. Similar samples for analysis of tylosis were then collected from the basal side of pruning cuts each day for six consecutive days using a different shoot for each date and treatment. Each treatment was repeated on six shoots in June 2004 on each of three replicate vines.

Structural investigation of secondary xylem—Each day 0 sample from above the cut was washed in 50% ethanol twice for 5 min each and trimmed into four 1-cm segments. One segment was used for examination with SEM. The other three segments were hydrated through an ethanol series to water in 30-min steps, then sectioned with a sliding microtome (AO-860, American Optical, Buffalo, New York, USA) in transverse, tangential, and radial planes with a section thickness of 20 or 25 μ m. Samples of basal and middle regions were softened in boiling water for 1–2 h before sectioning. To insure that this

¹ Manuscript received 16 January 2006; revision accepted 12 September 2006.

This work was supported by USDA grant 2003-34442-13148 and California Department of Food and Agriculture Agreement 01-0712. The authors thank E. Weber, Napa County Farm Advisor, Napa, California, USA, for first bringing the localized wounding response to our attention; J. F. Stevenson for the preliminary investigation related to this work; and N.-M. Nguyen for assistance in data collection.

⁴ Author for correspondence (e-mail: qiasun@ucdavis.edu)

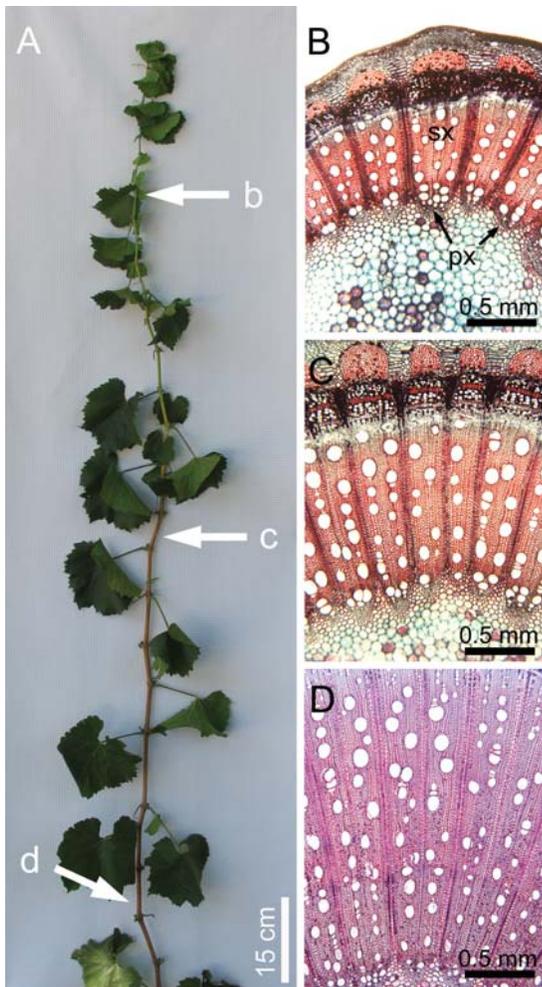


Fig. 1. Pruning sites on current-year shoots of (A) *Vitis vinifera* and (B–D) stem anatomy at each site. (A) A current-year shoot. Pruning was done at the apical (b), middle (c) or basal (d) region of the stem. (B) Transverse section of a stem at the apical region, showing smaller xylem width and diffuse-porous vessels. px = primary xylem, sx = secondary xylem. (C) Transverse section of a stem in the middle region, showing medium xylem width and diffuse-porous vessels. (D) Transverse section of a stem in the basal region, showing relatively large xylem width and diffuse-porous vessels.

treatment did not create artifacts, vessel diameters were measured in the same samples before and after boiling. In 10 stem pieces tested in this way, the treatment did not cause any measurable shrinkage or swelling (data not shown). The sections were dehydrated via an ethanol series (Ruzin, 1999). The 50% ethanol step contained 1% safranin O, and sections were stained for 4 h. The 95% ethanol step included 0.5% fast green FCF, and samples were stained for 1 min. Sections were further dehydrated and cleared in an ethanol–xylene series (2:1, 1:1, 1:2) followed by two xylene rinses of 10 min each. Sections were mounted with cover slips in Permount (Fisher Scientific, Fair Lawn, New Jersey, USA), and then observed with a light microscope (Olympus Vanox-AHBT; Olympus Optical, Tokyo, Japan) equipped with a digital camera (Pixera Pro 600ES; Pixera, Los Gatos, California, USA).

In preparing SEM samples, several smaller blocks were cut from the 1-cm segments with a razor blade; including some 2–3-mm thick stem discs exposing the transverse surface and some 2–3-mm thick longitudinal sections exposing the radial and tangential surfaces. The trimmed samples were dehydrated via the ethanol series mentioned but with 20 min at each step and without staining, and finally, specimens were critical-point dried (Samdri-780A; Tousimis Research, Rockville, Maryland, USA). The dry samples were coated with gold

(Denton Vacuum Desk II Cold Sputter-Etch Unit; Denton Vacuum, Moorestown, New Jersey, USA) and observed with an SEM (Hitachi S-3500N; Hitachi Co., Hitachi, Japan) at an accelerating voltage of 5 kV.

Two day-0 samples for each region were selected for maceration and hydrated to water as mentioned. Eight to 10-mm long, toothpick-thick rods were trimmed out of secondary xylem and incubated at 60°C for 36 h in a 1:1 mixture of glacial acetic acid and 6% hydrogen peroxide. Macerated tissues were washed with distilled water three times for 10 min each and then stained with 1% aqueous safranin O solution. Macerated xylem cells were mounted with a cover slip in 10% glycerin solution for observations and measurements.

The terminology for structural descriptions of secondary xylem in this study followed the definitions of the International Association of Wood Anatomists Committee (1989). Vessel density was determined for each sample from 10 fields of 1 mm² (0.5 mm² for samples of the apical stem region), which were selected randomly from the transverse sections. Tangential vessel diameter was an average of measurements for 30 randomly selected vessels in transverse section for each sample. Vessel element length and fiber length, diameter, and wall thickness were based on measurements of macerated cells; 30 vessel elements and fibers for each sample were randomly selected.

Quantitative analyses of tylose development in stems—Samples from days 0–6 were hydrated to water via the aforementioned ethanol series. Each sample was free-hand sectioned in transverse direction at 2, 4, 6, 8, and 10 mm below the pruning cut. Stem discs between these sections were taken at the same time, and some were prepared for SEM observations of tylose development in individual vessels following the protocol mentioned.

The sections were temporarily mounted with a cover slip in water for light microscopy. Five areas, each containing 40–50 vessels and including some consecutive xylem sectors bounded by rays, were chosen randomly for analysis. All vessels in each area were categorized as vessels without tyloses, vessels partially filled with tyloses, or vessel completely occluded with tyloses. Two parameters—percentage of vessels with tyloses (PVT) and percentage of vessels occluded completely by tyloses (PVO)—were calculated to quantify tylose development.

RESULTS

Xylem structure in stems of current-year shoots—Three stem regions (basal, middle, and apical) of current-year shoots were investigated to determine the structural and quantitative characteristics of the secondary xylem at each region (Fig. 1A). Stem diameter was approximately 10, 6, and 3 mm in the basal, middle, and apical regions, respectively (Table 1). Secondary xylem of the three regions was diffuse-porous and similar in structure, except for the width of the xylem growth ring, (Fig. 1B–D). The width of the xylem increased more than the stem diameter with internode age, resulting in greater xylem width to stem diameter ratios in the basal region (0.29) than in the middle (0.24) or apical (0.20) regions (Table 1). Vessel density in secondary xylem was lower in the basal (38.7 vessels/mm², Fig. 1D) than in the apical region (53.5 vessels/mm², Fig. 1B), but tangential vessel diameter did not differ among the stem regions. Vessel element length was greater in the basal and middle regions (491 μm and 487 μm, respectively) than in the apical region (448 μm). There were no obvious differences in fiber diameter (20–21 μm) or wall thickness (3.8–3.9 μm) among the three regions, but fiber length was greater in the basal region (684 μm) than in the middle and apical regions (593 μm and 549 μm, respectively) (Table 1).

Vessels were round or oval in transverse section, with exclusively simple perforation plates, and generally solitary or in radial multiples of two to three (Fig. 2A, B). Radial chains of more than five vessels were uncommon in the middle and apical region, but radial chains of 4–10 smaller vessels were present in the basal region, especially in the outer xylem. Xylem fibers were septate (typically 2–4 septa) and abundant,

TABLE 1. Quantitative characteristics of secondary xylem structures in three regions of stems of current-year shoots of *Vitis vinifera*. Data are means \pm SD.

Structural characteristic	Basal stem	Middle stem	Apical stem
Stem diameter (mm) $N = 20$	9.73 \pm 0.61	5.85 \pm 0.45	3.46 \pm 0.27
Xylem width (mm) $N = 20$	2.54 \pm 0.32	1.38 \pm 0.17	0.64 \pm 0.15
Xylem width/stem diameter $N = 20$	0.29 \pm 0.03	0.24 \pm 0.02	0.20 \pm 0.03
Vessel density (No./mm ²) $N = 30$	38.7 \pm 5.6	36.3 \pm 4.7	53.5 \pm 8.6
Tangential vessel diameter (μ m) $N = 60$	60.5 \pm 24.5	67.6 \pm 22.9	66.7 \pm 15.5
Vessel element length (μ m) $N = 60$	491 \pm 85	487 \pm 79	448 \pm 96
Fiber length (μ m) $N = 60$	684 \pm 137	593 \pm 146	549 \pm 110
Fiber diameter (μ m) $N = 60$	20.0 \pm 4.0	20.8 \pm 2.9	20.9 \pm 3.2
Fiber wall thickness (μ m) $N = 60$	3.8 \pm 0.9	3.9 \pm 0.9	3.8 \pm 0.9

comprising the majority of secondary xylem. Axial parenchyma cells were scanty to vasicentric paratracheal (Fig. 2B). Scanty paratracheal parenchyma cells were often present around smaller vessels, especially those in a radial chain, while vasicentric paratracheal parenchyma cells in a single layer were more common around larger vessels. There were as many as eight axial parenchyma cells in a strand along a single vessel element and as many as 72–180 axial parenchyma cells

attached to large vessel elements. Rays were predominantly 4–10 seriate and rarely bi- or triseriate (Fig. 2C). They were heterocellular with rows of procumbent, upright, and square cells within the body of the ray, and of upright and/or square cells along the margin of the ray (Fig. 2D). Rays were more than 1 mm in height. There were usually less than four rays per mm width in the tangential section of stem.

Structure of pits on vessel walls—In secondary xylem of current-year shoot of grapevines, vessels have lateral contact with axial parenchyma cells, ray parenchyma cells, fibers, and other vessels. Pit pairs occurred between vessels and all other xylem cells except fibers (Fig. 3A). Pits between vessels (intervessel pits, Fig. 3C) were present in all the lateral walls contacting neighboring vessels. Intervessel pits occurred as bordered pit pairs in a scalariform arrangement and were much larger than other types of pits (Table 2, Fig. 4). The aperture of intervessel pits was as wide as the pits themselves (26.5–63.0 μ m, mean 50.8 μ m) and ranged in height from 1.4 μ m to 2.8 μ m (2.3 μ m mean) (Table 2, Fig. 4). Pits between vessels and axial parenchyma (vessel-parenchyma pits, Fig. 3B) were half-bordered pit pairs with a simple pit on the parenchyma cell side and a bordered pit on the vessel side. They were round, oval, or transversely elongated and were arranged in one or two more or less regular rows in the longitudinal direction of the stems. Vessel-parenchyma pits were the smallest among the different types of pits on the vessel wall (Table 2, Fig. 4). Width and height of the pit aperture of the bordered pit on the vessel side were usually 5.5–13.5 μ m (10.5 μ m mean) and 2.8–4.7 μ m (3.5 μ m mean), respectively. Pits between vessels and ray parenchyma cells (vessel-ray pits, Fig. 3D) were also half-bordered pit pairs with a simple pit on the ray parenchyma cell side and a bordered pit on the vessel side. They were oval or transversely elongated in shape and were usually arranged in regular rows in the radial direction of the stems. Vessel-ray pits were generally larger than vessel-parenchyma pits but much smaller than intervessel pits (Table 2, Fig. 4). Pit apertures of the bordered pit on the vessel side were 10.5–25.0 μ m (17.5 μ m mean) in width and 3.6–4.8 μ m (4.3 μ m mean) in height.

Tylose development in individual vessels—Tyloses developed from both axial parenchyma cells and ray parenchyma cells in response to pruning. Because less than 10% of vessels had contact with ray parenchyma cells (Fig. 2A), tyloses derived from them contributed little to tylose development in secondary xylem compared to those from axial parenchyma cells.

Typically, there were no tyloses in vessels of the stems used

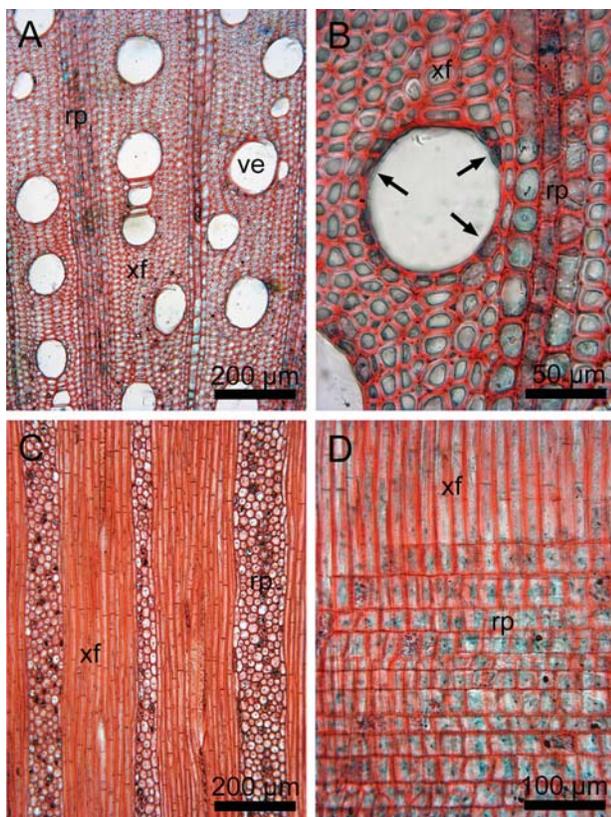


Fig. 2. Secondary xylem structure in basal stems of current-year shoots. (A) Vessels in transverse section are solitary or in radial multiples and rarely have contact with xylem rays. (B) Axial parenchyma cells (arrows) are scanty paratracheal, and libriform fibers comprise the majority of secondary xylem. (C) Tangential section of a stem, showing multiseriate xylem rays and septate libriform fibers. (D) Radial section of a stem, showing a heterocellular xylem ray, consisting of a marginal row of upright cells and body rows of upright, square, and procumbent cells. rp = ray parenchyma, ve = vessel, and xf = xylem fibers.

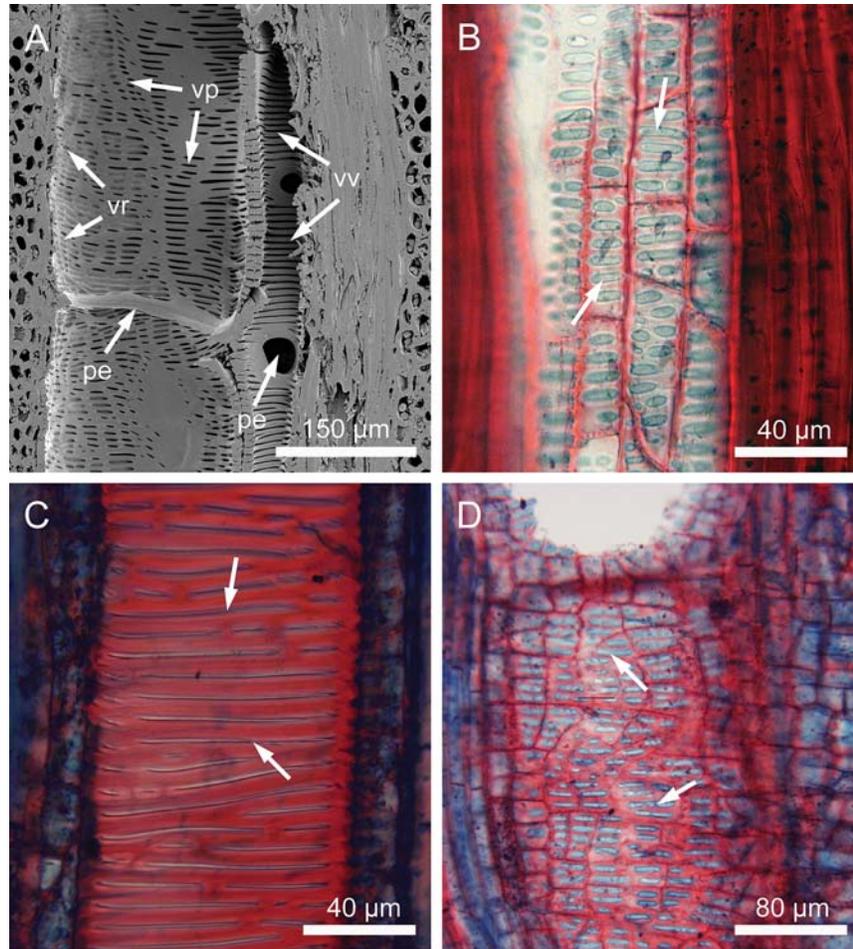


Fig. 3. Structure of pits on vessel walls in stems. (A) SEM micrograph of secondary xylem in tangential section showing morphology and distribution of three types of pits in a single vessel lateral wall: vessel-parenchyma pits (vp), vessel-ray pits (vr), and intervessel pits (vv). pe = perforation plate. (B–D) Light micrographs of secondary xylem. (B) Tangential section of a stem showing half-bordered vessel-parenchyma pits (arrows) that are oval or transversely elongated and are arranged regularly in the axial direction of stem. (C) Tangential section of a stem showing scalariform, bordered intervessel pits (arrows). (D) Radial section of a stem showing half-bordered vessel-ray pits (arrows) that are oval or transversely elongated and are arranged regularly in the radial direction of the stem.

in these experiments unless the stems were wounded by pruning (Figs. 5A and 6A, B). Tylose initiation started as early as 1 day after pruning as indicated by one or two small spherical outgrowths into the vessel lumen (Fig. 5B). Initiated tyloses were generally similar in size and grew uniformly (Fig. 5C). In a single vessel, multiple parenchyma cells from a single or from different axial parenchyma strands were involved in tylose development. Multiple tyloses from a single parenchyma cell were sometimes observed. Tylose initiation into a single

vessel lumen generally occurred simultaneously from adjoining parenchyma cells. When the enlargement of tyloses finally caused them to come in contact with each other, they became oval, polygonal, or irregular in shape. Nuclei were observed in many tyloses as they grew or after they became stable in size (Fig. 5G), showing that the nucleus must have passed through the pit into the outgrowth. Fully developed tyloses were compactly arranged in the vessel lumen.

In transverse view of the vessel there were usually more than

TABLE 2. Range of dimensions for pits and pit apertures on vessel walls in stem secondary xylem of current-year shoots of grapevines. See Fig. 4 for location of measurements. Values in parentheses are means \pm SD.

Pit type	Pit		Pit aperture	
	Width (μm)	Height (μm)	Width (μm)	Height (μm)
Intervessel $N = 60$	28.5–64.5 (53.6 \pm 15.2)	5.0–6.3 (5.8 \pm 0.3)	26.5–63.0 (50.8 \pm 13.7)	1.4–2.8 (2.3 \pm 0.3)
Vessel-parenchyma $N = 60$	6.5–16.5 (13.5 \pm 2.6)	3.3–5.5 (4.6 \pm 0.4)	5.5–13.5 (10.5 \pm 2.2)	2.8–4.7 (3.5 \pm 0.4)
Vessel-ray $N = 60$	13.0–27.5 (19.5 \pm 3.3)	4.0–5.0 (4.6 \pm 0.2)	10.5–25.0 (17.5 \pm 3.5)	3.6–4.8 (4.3 \pm 0.2)

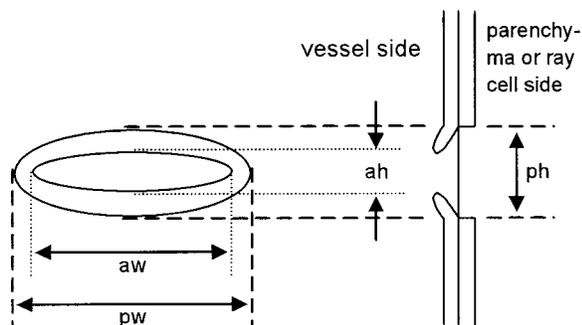


Fig. 4. Schematic drawing of a surface view of a bordered pit (left) and a sectional view of a vessel-parenchyma or vessel-ray pit pair (right) in stems of current-year shoots in grapevines. The pw and ph represent the width and height of a pit, respectively, and aw and ah represent the width and length of pit aperture of the bordered pit on the vessel side.

three but less than 10 tyloses blocking the lumen in larger vessels (Fig. 5D). In the longitudinal direction of the vessel, tyloses developed along the axis and were observed in up to eight consecutive vessel elements in a row (Fig. 5E). Tylose development in these vessel elements was also approximately simultaneous and finally occluded the vessel. There was usually an obvious boundary along the vessel beyond which no tyloses formed (Fig. 5F). There was no evidence of wound gels in any section of current-year shoots collected in summer.

Spatial and temporal tylose development in three regions of a current-year shoot—Unlike the relatively simultaneous development of tyloses in a single vessel, some vessels started to form tyloses ahead of others (Fig. 6C, D). Vessels with tyloses were not random but were often clustered in some sectors of xylem. Subsequently, increasing numbers of vessels were involved in tylose development, and many vessels became completely occluded by tyloses (Fig. 6E, F), but some

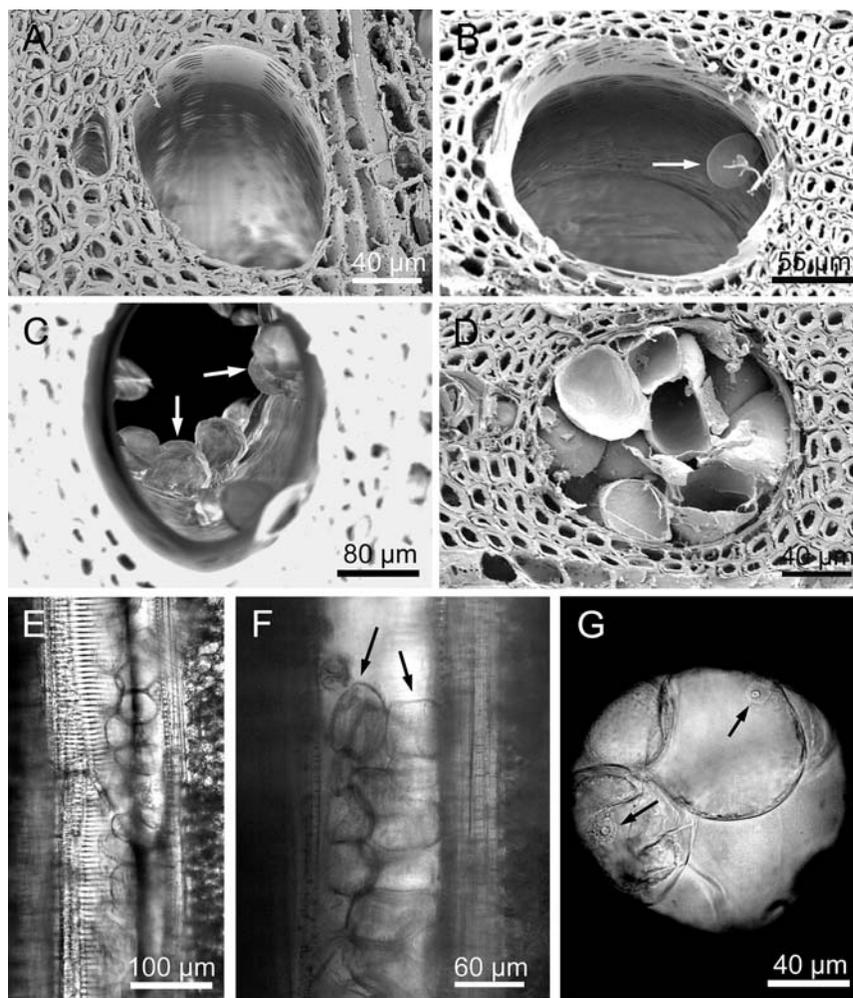


Fig. 5. Tylose development in individual vessels following pruning. (A–D) SEM micrographs of basal stems. (A) No tyloses in a vessel lumen of an unwounded control. (B) A small tylose initial (arrow) formed in a vessel lumen. (C) Several small tyloses (arrows) developed in a vessel lumen. (D) A vessel lumen is completely occluded by tyloses arranged compactly. (E–F) Tangential section of middle stems. (E) Tyloses occlude the vessel lumen over more than one vessel element. (F) There is an obvious boundary in the vessel lumen with and without tylose development (arrows). (G) Transverse section of a middle stem. Each tylose contains one nucleus after it enlarges (arrows).

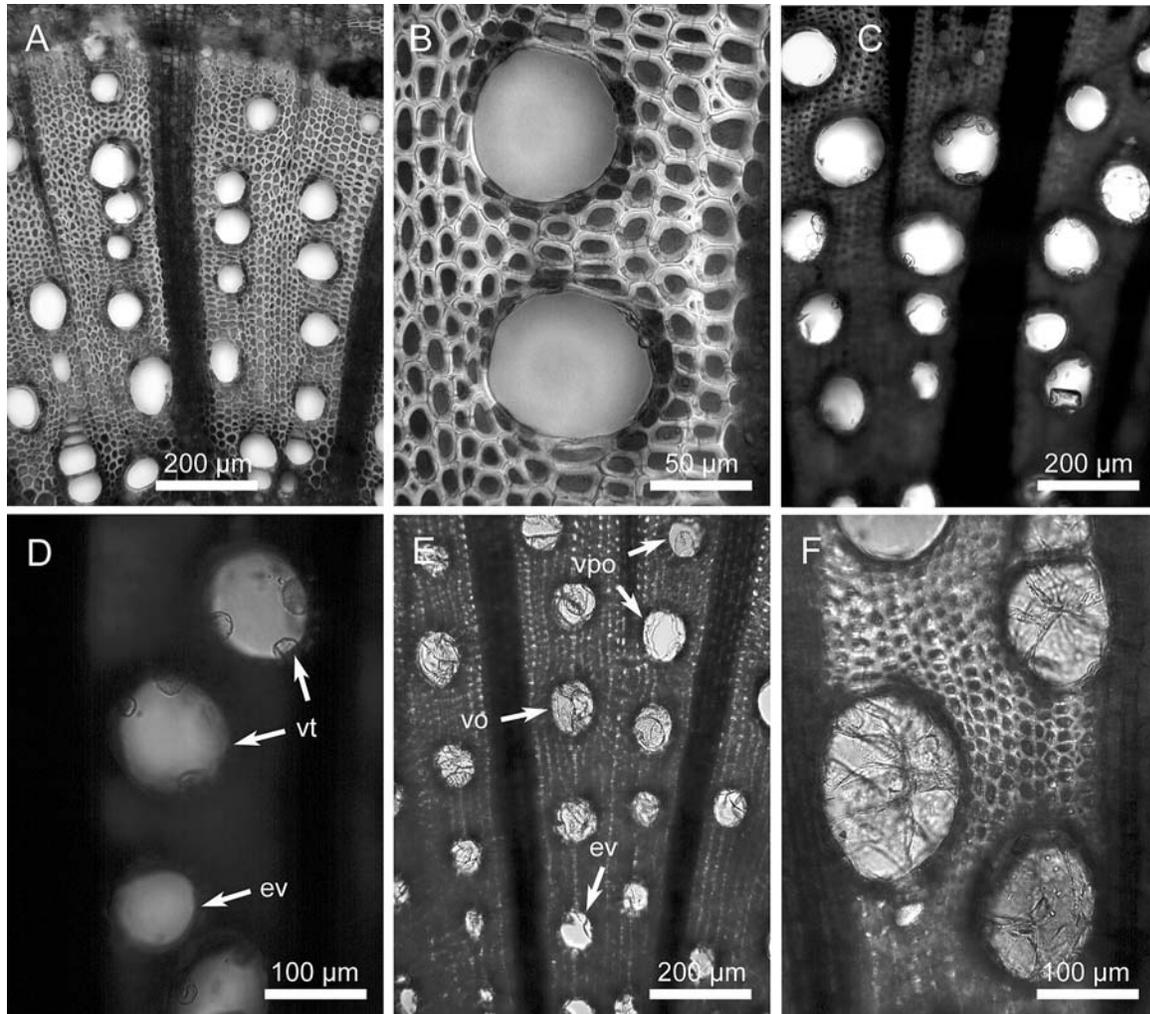


Fig. 6. Tylose development in xylem tissue of middle stems at the depth of 2 mm from the cut surface. (A–B) No tyloses in vessels at day 0. (C–D) Small round or oval tyloses in some vessels on day 3. (E–F) Vessels occluded by tyloses at day 6.

vessels remained either without tyloses or partially occluded with tyloses.

Despite a similar overall pattern, the quantitative characteristics of tylose development in response to pruning were dependent upon stem region, distance from the cut, and time from pruning. Almost all tyloses developed within the first 10 mm from the cut surface, although a few were observed deeper. At day five, the percentage of vessels with tyloses (PVT) was generally greatest in the basal region and decreased with depth from the cut surface for all stem regions (Fig. 7A). The PVT was about two times as great in the basal region than in the middle and apical regions at all depths except 2 mm. Tylose frequency was greatest in the middle and apical regions at that depth, but in the basal region the greatest tylose frequency was 4 mm from the cut surface. The maximum PVT was considerably greater in the basal and middle regions (about 70%) than in the apical region (about 45%).

The percentage of vessels completely occluded by tyloses (PVO) at the different depths had patterns similar to those of PVT in the three stem regions (Fig. 7B). Accordingly, the PVO was greatest in basal regions and generally decreased away

from the cut surface. Generally, no more than 40% of vessels at any depth were completely occluded.

Tylose development in the first 10 mm from the cut surface continued for 1 week after pruning. When the progression of tylosis was evaluated for the depth at which maximum tylose formation was observed (i.e., at 4 mm for basal and 2 mm for middle and apical regions), tylose development was clearly slower in the apical regions (Fig. 8A). The PVT increased rapidly from day 2 but more so in the basal and middle regions than in apical regions. The PVT reached about 85% in the basal region, 75% in the middle regions, and 50% in the apical region 6 days after pruning. The PVO had a temporal pattern similar to the PVT, although the rapid increase in PVO occurred after day 4 (Fig. 8B). The PVO was less than 5% in all three stem regions for the first 2 days after pruning. Thereafter, the PVO increased to about 50% in the basal region and about 40% in the middle and apical regions. The temporal progress of tylose development at depths over 8 mm from the cut surface was much slower. At 10 mm, the PVT on day 6 was about 20% for the basal region and less than 15% for the middle and apical regions, while PVO was less than 10% in the

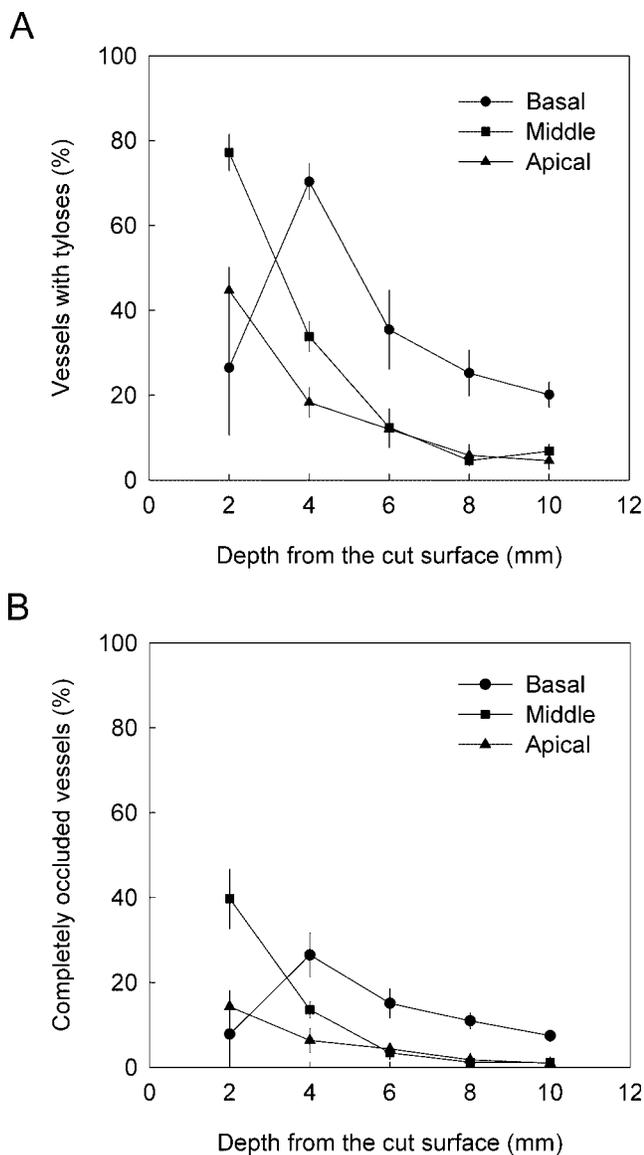


Fig. 7. Spatial differences in tylose development in basal, middle, and apical stems at day 5 after pruning. (A) Percentage of vessels with tyloses at different depths from the cut surface. (B) Percentage of vessels completely occluded by tyloses at the different depths from the cut surface. Data are means \pm SD, $N = 3$.

basal region and almost nil in the middle and apical regions (data not shown).

DISCUSSION

Plants are continuously subjected to environmental stresses, wounding, and attacks from pathogens. One reaction to these trauma-inducing agents is the formation of tyloses in secondary xylem to close wounds and compartmentalize pathogens that enter the plant body. Although there is some literature on these processes, we still need to know when, where, and how tyloses develop in response to wounding and pathogen infection. We don't know if there are differences in the capacity of xylem of

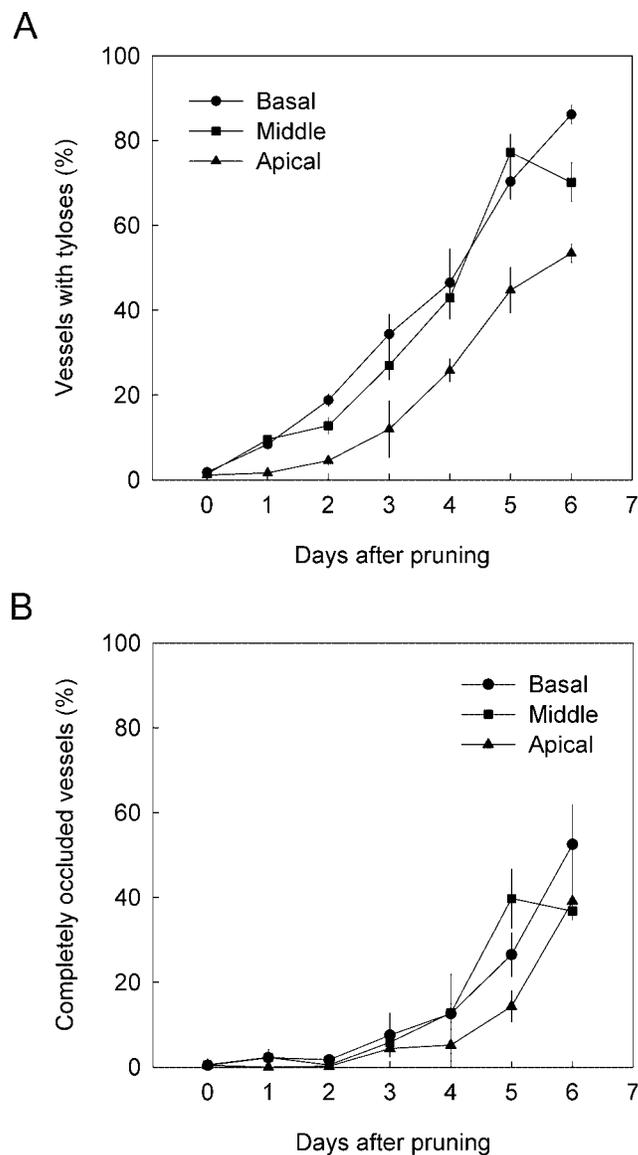


Fig. 8. Temporal progress of tylose development in basal, middle, and apical stems. (A) Percentage of vessels with tyloses. (B) Percentage of vessels completely occluded by tyloses. Data are for 2 mm from the cut surface in middle and apical stems, and 4 mm in basal stems; data are presented as means with SD, $N = 2$ or 3.

different ages to produce tyloses, and there is little information on the whole dynamic process of tylose development in single vessels and in the xylem as a whole. The object of our study was to show, in grapevine stems, the dynamic process of tylose development from xylem parenchyma and the differences in wound repair reaction in different ages of wood in a single cane in its first year of growth. We have shown that in secondary xylem of the three stem regions (base, middle, and apex) tyloses are induced by wounding, that tylose development has a similar pattern among different vessels but separates in time, and that the three regions show differences in spatial distribution and temporal progress of pruning-induced tyloses. In the following discussion, we will describe the literature related to tylose development in response to wounding and

pathogen infection and how tylose development is a function of secondary xylem structure.

Xylem structure and tylose development—Grapevines have diffuse porous wood with vessel elements having simple perforation plates, vessels of various lengths up to 1 m (Thorne et al., 2006), and paratracheal axial parenchyma. We found three types of pits in vessel walls—intervessel pits, vessel-parenchyma pits, and vessel-ray pits. Intervessel bordered pit pairs were arranged in a scalariform pattern and were the largest pits. Vessel-parenchyma pits and vessel-ray pits were oval to transversely elongated half-bordered pit pairs with a simple pit on the axial parenchyma cell or the ray parenchyma cell side and a bordered pit on the vessel side, but the vessel-parenchyma pits were smaller than the vessel-ray pits. Tyloses developed from both parenchyma pit types, but tyloses from ray parenchyma cells contributed little to the total tylose population because few vessels in secondary xylem have contact with rays. Minimal pit aperture dimensions for tylose formation are suggested to be 8–10 μm in width (Chattaway, 1949) and 3 μm in height (Bonsen and Kučera, 1990). It is unclear whether tylosis was restricted to the vessel-parenchyma pits with larger pit apertures in grape shoots, but the dimensions of vessel-ray pits were greater than the suggested minimal values.

Revelation of the developmental process and characteristics of tyloses in a single vessel and in the secondary xylem generally is essential to understanding the effects of tyloses on wound healing and disease defense. Our results indicated that within a single vessel, the appearance of tyloses developing from different parenchyma cells was uniform and coordinated, extending up to eight consecutive vessel elements; but among vessels in xylem, tyloses developed separately in time. Thus, in a transverse section of xylem taken after wounding, there were vessels in which tyloses were present at different developmental stages, causing some vessels to be completely occluded and other vessels partially or not occluded. Also, tyloses developed earlier in some xylem sectors than in others. To our knowledge, these characteristics of tylosis in grape shoots have not been reported in other species.

Tylose development in response to wounding—Our results show that tyloses develop quickly and close to the wound in the xylem of current-year shoots of grapevine. Tyloses were first observed within 1 or 2 days after pruning, and up to 85% of vessels developed tyloses after 6 days. Tylose development was greatest at 2 mm (middle and apical stem regions) or 4 mm (basal stem region) from the cut and decreased to nil beyond 10 mm deep from the cut. Tylose-forming capacity (mean percentage of vessels with tyloses within 10 mm of the cut) was greatest in the basal region and decreased acropetally in the middle and apical regions. The basal and middle regions were also much faster in tylose development than the apical region.

There is little other information in the literature about the temporal progress of tylose development, particularly with respect to wounding. Tylose development induced by piercing stems of *Roystonea regia* was observed 7 days after wounding (Weiner and Liese, 1995). Tyloses were observed as early as 1–3 days after pathogen inoculation in some plant–pathogen combinations, such as *Gossypium barbadense*–*Verticillium dahliae* (Mace, 1978), *Musa acuminata*–*Fusarium oxysporum* f. sp. *cubense* (VanderMolen et al., 1987) and *Eucalyptus*

nitens–*Ganoderma adspersum* (Barry et al., 2001); not until one to several weeks after inoculation in other plants, e.g., *Vitis vinifera* inoculated with *Pseudopezizica tracheiphila* (Reiss et al., 1997) or with *Botryodiplodia theobromae* (Atia et al., 2003); and rarely or not at all in still other systems, e.g., *Chrysanthemum morifolium* inoculated with *F. oxysporum* f. sp. *chrysanthemi* (Stuehling and Nelson, 1981) and *Dianthus caryophyllus* inoculated with *F. oxysporum* f. sp. *dianthi* (Ouellette et al., 1999). Tylose development observed in *Quercus* species growing naturally was very slow. In earlywood, tyloses were initiated in winter and developed in summer; but in latewood, tylosis started much later and took several years for many vessels to become occluded completely (Cochard and Tyree, 1990). Thus, compared with tylose development in other species and under other conditions, the initiation and developmental progress of pruning-induced tyloses in grapevine stems was very rapid.

Few studies have reported on the spatial distribution of tyloses. In *Robinia pseudoacacia*, a few tyloses developed within the first 5 mm from the wounding surface, but tyloses were abundant at 20 mm from a wound made by partial drilling into a stem (Schmitt and Liese, 1994). Pruning-induced tylose development in current-year shoots of grapevines in summer was restricted to 10 mm from the wound and was therefore relatively localized.

There are also few investigations that quantify tyloses in stems of different ages. In perennial *Roystonea regia*, tyloses developed only in vessels of apical stems and not in those of basal stems (Weiner and Liese, 1995). Similarly in *Quercus petraea*, tylose development decreased from the apical trunk stems downwards in both healthy and diseased plants (Babos, 1993). In contrast, the tylose-forming capacity of a current-year shoot in the present study was greatest in basal stems and least in apical stems. The apparent contrast deserves further attention.

Formation of callus and/or secretion of resin and gels are common responses to pruning wounds (Brown, 1995). In grapevines, pruning of current-year shoots in the summer did not lead to formation of callus or secretion of resin and gels. Instead, tyloses developed close to the cut. The intensive and relatively rapid tylose development in grape may serve as an alternate means to seal wounds and reduce the risk of pathogen invasion, at least during the growing season. Thus, the distribution of tylose-forming capacity along the stem indicates that in viticultural practice summer pruning near the shoot apex (called hedging) may expose the vine to airborne pathogens for a longer period than does pruning at more basipetal stem locations used for cane and spur pruning.

Tyloses and pathogen movement—The kinetics, extent, and spatial distribution of wound tylose development reported here have implications for tylose function in general and in understanding responses to plant disease in particular. Tyloses could restrict movement of sap, pathogens, both, or neither depending upon the timing and condition of the vessel when tyloses develop.

In most research, tyloses have been studied in conjunction with pathogens, but the impact and roles of tyloses in disease are decidedly not clear. One viewpoint is that tylose development compartmentalizes the pathogens and prevents or helps prevent, in association with gels, their movement in xylem (Pearce, 1991; Clerivet et al., 2000) as a means of defense against diseases (Elgersma, 1973; Beckman and

Talboys, 1981). The main evidence for this opinion is the development of tyloses around the region of inoculation (Reiss et al., 1997; Barry et al., 2000) and the development of more tyloses in resistant genotypes than in susceptible genotypes (Bishop and Cooper, 1984; Grimault et al., 1994). It should be noted that many of the studies with pathogens involved a wound at the inoculation site near which tyloses formed. Yet, the effect of the wound alone has seldom been tested. Thus, some tylose formation associated with infections may have been due to wounding used to inoculate plants. Another opinion is that tylose development can exacerbate the symptoms of disease (Aleemullah and Walsh, 1996). This opinion is based mainly on reports of more tyloses in susceptible genotypes than in resistant genotypes (Dimond, 1955), a positive correlation of the number of tyloses and the severity of disease (e.g., *Fusarium* wilt disease in *Lagenaria siceraria*, Sen and Palodhi, 1984) and reduction of hydraulic conductivity of plant tissues with tyloses (Dimond, 1955). To complete the possibilities, the third opinion is that tylose development has no relation either to defense against disease or to symptom development (Palodhi and Sen, 1979; Jacobi and MacDonald, 1980) because some studies showed no differences in tylose formation between susceptible and resistant genotypes (Prior, 1979). Similarly, the frequency of tyloses in Pierce's disease-infected grapevines has been reported as greater in resistant genotypes (Hopkins and Mollenhauer, 1975), greater in susceptible genotypes (Krivanek et al., 2005), and unrelated to susceptibility of the grape genotype (Fry and Milholland, 1990). All this literature tacitly assumes tyloses form in functioning vessels. If they do not, the second hypothesis is rendered unlikely if not untenable, and the first is possible only to the extent that pathogen movement occurs in gas-filled vessels. In this study, the fastest tylose initiation occurred 1 day after pruning, and complete blockage of a vessel required several days. This is too slow to restrict spread of mobile pathogens such as bacteria moving upward in the mass flow of the transpiration stream (Thorne et al., 2006), although spread of low-mobility pathogens, such as fungal hyphae might be restricted. In all studies of Pierce's disease, the development of tyloses has been slow in that observations were made weeks after inoculation. Tyloses in the stem appear after causal *Xylella* bacteria have infected subtended leaves (Stevenson et al., 2004). This also indicates that tylosis is too slow to prevent upward movement of bacteria; however, the slower basipetal movement of bacteria that is necessary for the infection to become systemic (Stevenson et al., 2004) might be affected by tyloses. Especially if the bacteria move against a transpiration flux in sap-filled vessels (Meng et al., 2005), tyloses could present a significant obstacle to systemic infection.

Both wounding and infection may cause embolisms. If, upon infection, tyloses form only in functioning vessels containing the pathogen, effective control of migration with minimal loss of water transport might be affected. Alternatively, extensive tylose occlusions may seal a wound to prevent pathogen entry from outside or water loss from the exposed vessel lumen. Tylosis is a common component of abscission (Webster, 1973). Thus, if tylose development (and other sealing phenomena) was sufficiently extensive, plants could respond to wounding and to infection by sealing off affected parts, leaves, or shoots effectively, a natural pruning mechanism of the affected segments. The results of this study, however, suggest that that is not the case with grapevine. In this study, all vessels in a

cross section were cut and exposed to air, but not all vessels became occluded with tyloses. Over 50% of vessels remained at least partially open when the shoot was completely severed. A similarly low fraction of vessels was occluded in studies of Pierce's disease (Fry and Milholland, 1990; Krivanek et al., 2005) and other diseases (e.g., Pegg and Cronshaw, 1976). Bornman (1967) argued that tyloses and abscission are not causally related. Thus, there are two important questions that need resolution in order to understand whether tyloses are important in exacerbating disease by limiting xylem sap flow: do tyloses form in functioning vessels, and are enough vessels occluded by tyloses rather than embolisms to limit water and solute transport?

Conclusion—In our investigation of tylose development in pruned grapevine stems, the responses to wounding are xylem age-dependent; tyloses form deeper in wounds made at the base of a cane (maximum at 4 mm deep) compared to the apical region (maximum at 2 mm deep), and faster in the base (85% occluded at 7d) vs (50% occluded at 7d) when wounded at the apex. These data will be of use to biologists who study roles of tylose development in wound repair and pathogenesis and are of practical importance to growers to help them better understand pruning practices.

LITERATURE CITED

- ALEEMULLAH, M., AND K. B. WALSH. 1996. Australian papaya dieback: evidence against the calcium deficiency hypothesis and observations on the significance of laticifer autofluorescence. *Australian Journal of Agricultural Research* 47: 371–385.
- ATIA, M. M. M., A. Z. ALY, M. R. A. TOHAMY, H. EL-SHIMY, AND M. A. KAMHAWY. 2003. Histopathological studies on grapevine die-back. *Zeitschrift Für Pflanzenkrankheiten und Pflanzenschutz* 110: 131–142.
- BABOS, K. 1993. Tyloses formation and the state of health of *Quercus petraea* trees in Hungary. *International Association of Wood Anatomists Journal* 14: 239–243.
- BARRY, K. M., R. B. PEARCE, S. D. EVANS, L. D. HALL, AND C. M. MOHAMMED. 2001. Initial defence responses in sapwood of *Eucalyptus nitens* (Maiden) following wounding and fungal inoculation. *Physiological and Molecular Plant Pathology* 58: 63–72.
- BARRY, K. M., R. B. PEARCE, AND C. M. MOHAMMED. 2000. Properties of reaction zones associated with decay from pruning wounds in plantation-grown *Eucalyptus nitens*. *Forest Pathology* 30: 233–245.
- BECKMAN, C. H., AND P. W. TALBOYS. 1981. Anatomy of resistance. In M. E. Mace, A. A. Bell, and C. H. Beckman [eds.], *Fungal wilt diseases of plants*, 487–521. Academic Press, New York, New York, USA.
- BIGGS, A. R. 1987. Occurrence and location of suberin in wound reaction zones in xylem of 17 tree species. *Phytopathology* 77: 718–725.
- BISHOP, C. D., AND R. M. COOPER. 1984. Ultrastructure of vascular colonization by fungal wilt pathogens. 2. Invasion of resistant cultivars. *Physiological Plant Pathology* 24: 277–290.
- BOEHM, J. 1867. Über Function und Genesis der Zellen in den Gefässen des Holzes. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften* 55: 851–866.
- BONSEN, K. J. M., AND L. J. KUČERA. 1990. Vessel occlusions in plants: morphological functional and evolutionary aspects. *International Association of Wood Anatomists Bulletin* 11: 393–399.
- BORNMAN, C. H. 1967. The relation between tylosis and abscission in cotton (*Gossypium hirsutum* L.) explants. *South African Journal of Agricultural Science* 10: 143–154.
- BROWN, G. E. 1995. The pruning of trees, shrubs and conifers. Timber Press, Portland, Oregon, USA.
- CANNY, M. J. 1997. Tyloses and the maintenance of transpiration. *Annals of Botany* 80: 565–570.
- CHAFFEY, N. J., AND J. A. PEARSON. 1985. Presence of tyloses at the blade-

- sheath junction in senescing leaves of *Lolium temulentum* L. *Annals of Botany* 56: 761–770.
- CHATTAWAY, M. M. 1949. The development of tyloses and secretion of gum in heartwood formation. *Australian Journal of Scientific Research, B* 2: 227–240.
- CLERIVET, A., V. DEON, I. ALAMI, F. LOPEZ, J.-P. GEIGER, AND M. NICOLE. 2000. Tyloses and gels associated with cellulose accumulation in vessels are responses of plane tree seedlings (*Platanus × acerifolia*) to the vascular fungus *Ceratocystis fimbriata* f. sp. *platani*. *Trees* 15: 25–31.
- COCHARD, H., AND M. T. TYREE. 1990. Xylem dysfunction in *Quercus* vessel sizes tyloses cavitation and seasonal changes in embolism. *Tree Physiology* 6: 393–408.
- DAVISON, E. M., AND F. C. S. TAY. 1985. The effect of waterlogging on seedlings of *Eucalyptus marginata*. *New Phytologist* 101: 743–754.
- DIMOND, A. E. 1955. Pathogenesis in the wilt diseases. *Annual Reviews of Plant Physiology* 6: 329–350.
- DUTE, R. R., K. M. DUNCAN, AND B. DUKE. 1999. Tyloses in abscission scars of loblolly pine. *International Association of Wood Anatomists Journal* 20: 67–74.
- ELGERSMA, D. M. 1973. Tylose formation in elms after inoculation with *Ceratocystis ulmi*: a possible resistance mechanism. *Netherlands Journal of Plant Pathology* 79: 218–220.
- ESAU, K. 1948. Anatomic effects of the viruses of Pierce's disease and phony peach. *Hilgardia* 18: 423–482.
- ESAU, K. 1977. Anatomy of seed plants, 2nd ed. Wiley, New York, New York, USA.
- FRY, S. M., AND R. D. MILHOLLAND. 1990. Response of resistant tolerant and susceptible grapevine tissues to invasion by the Pierce's disease bacterium *Xylella fastidiosa*. *Phytopathology* 80: 66–69.
- GALET, P. 2000. General viticulture. Oenoplurimédia sal Château de Chaintré, Chaintré, France.
- GRIMAULT, V., B. GÉLIE., M. LEMATTRE, P. PRIOR, AND J. SCHMIT. 1994. Comparative histology of resistant and susceptible tomato cultivars infected by *Pseudomonas solanacearum*. *Physiological and Molecular Plant Pathology* 44: 105–123.
- HOPKINS, D. L., AND H. H. MOLLENHAUER. 1975. Tylose and gum formation in the xylem of Pierce's disease infected grapevines. *Proceedings of the American Phytopathological Society* 2: 65 (abstract).
- INTERNATIONAL ASSOCIATION OF WOOD ANATOMISTS (IAWA) COMMITTEE. 1989. IAWA list of microscopic features for hardwood identification. *International Association of Wood Anatomists Bulletin* 10: 219–332.
- JACOBI, W. R., AND W. L. MACDONALD. 1980. Colonization of resistant and susceptible oaks by *Ceratocystis fagacearum*. *Phytopathology* 70: 618–623.
- KRIVANEK, A. F., J. F. STEVENSON, AND M. A. WALKER. 2005. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. *Phytopathology* 95: 36–43.
- MACE, M. E. 1978. Contributions of tyloses and terpenoid aldehyde phytoalexins to *Verticillium dahliae*. *Physiological Plant Pathology* 12: 1–12.
- MENG, Y. Z., Y. X. LI, C. D. GALVANI, G. X. HAO, J. N. TURNER, T. J. BURR, AND H. C. HOCH. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching mobility. *Journal of Bacteriology* 187: 5560–5567.
- MOLLENHAUER, H. H., AND D. L. HOPKINS. 1976. Xylem morphology of Pierce's disease infected grapevines with different levels of tolerance. *Physiological Plant Pathology* 9: 95–100.
- OUELLETTE, G. B., R. P. BAAYEN, M. SIMARD, AND D. RIOUX. 1999. Ultrastructural and cytochemical study of colonization of xylem vessel elements of susceptible and resistant *Dianthus caryophyllus* by *Fusarium oxysporum* f. sp. *dianthi*. *Canadian Journal of Botany* 77: 644–663.
- PALODHI, P. R., AND B. SEN. 1979. Role of tylose development in a muskmelon *Cucumis melo* disease caused by *Fusarium solani*. *Plant Disease Reporter* 63: 584–586.
- PARAMESWARAN, N., H. KNIGGE, AND W. LIESE. 1985. Electron microscopic demonstration of a suberized layer in the tylosis wall of beech *Fagus sylvatica* and oak *Quercus robur*. *International Association of Wood Anatomists Bulletin* 6: 269–271.
- PEARCE, R. B. 1991. Reaction zone relics and the dynamics of fungal spread in the xylem of woody angiosperms. *Physiological and Molecular Plant Pathology* 39: 41–56.
- PEGG, G. F., AND D. K. CRONSHAW. 1976. Ethylene production in tomato plants infected with *Verticillium albo-atrum*. *Physiological Plant Pathology* 8: 279–295.
- PRIOR, C. 1979. Resistance of cocoa to vascular-streak dieback disease. *Annals of Applied Biology* 92: 369–376.
- RANJANI, K., AND K. V. KRISHNAMURTHY. 1988. Tyloses of the root wood of *Cassia fistula* L. *Feddes Repertorium* 99: 147–149.
- REISS, K., M. GUTMANN, H. C. BARTSCHERER, AND V. ZINKERNAGEL. 1997. *Pseudopezizica tracheiphila* on *Vitis vinifera*: microscopical studies of the infection mechanism. *Zeitschrift Für Pflanzenkrankheiten und Pflanzenschutz* 104: 483–491.
- RUZIN, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press, New York, New York, USA.
- SAITOH, T., J. OHTANI, AND K. FUKAZAWA. 1993. The occurrence and morphology of tyloses and gums in the vessels of Japanese hardwoods. *International Association of Wood Anatomists Journal* 14: 359–371.
- SCHMITT, U., AND W. LIESE. 1993. Response of xylem parenchyma by suberization in some hardwoods after mechanical injury. *Trees* 8: 23–30.
- SCHMITT, U., AND W. LIESE. 1994. Wound tyloses *Robinia pseudoacacia* L. *International Association of Wood Anatomists Journal* 15: 157–160.
- SEN, B., AND P. R. PALODHI. 1984. Vascular aberrations and disease grades in *Fusarium* wilt of cucurbits. *Zeitschrift Für Pflanzenkrankheiten und Pflanzenschutz* 91: 472–475.
- STEVENSON, J. F., M. A. MATTHEWS, L. C. GREVE, J. M. LABAVITCH, AND T. L. ROST. 2004. Grapevine susceptibility to Pierce's disease. II: Progression of anatomical symptoms. *American Journal of Enology and Viticulture* 55: 238–245.
- STUEHLING, B. A., AND P. E. NELSON. 1981. Anatomy of a tolerant *Chrysanthemum* cultivar infected with *Fusarium oxysporum* f. sp. *chrysanthemi*. *Phytopathology* 71: 1162–1168.
- THORNE, E. T., B. M. YOUNG, G. M. YOUNG, J. F. STEVENSON, J. M. LABAVITCH, M. A. MATTHEWS, AND T. L. ROST. 2006. The structure of xylem vessels in grapevine and a possible passive mechanism for the systemic spread of bacterial disease. *American Journal of Botany* 93: 497–504.
- VANDERMOLEN, G. E., C. H. BECKMAN, AND E. RODEHORST. 1987. The ultrastructure of tylose formation in resistant banana following inoculation with *Fusarium oxysporum* f. sp. *cubense*. *Physiological and Molecular Plant Pathology* 31: 185–200.
- WALLIS, F. M., AND S. J. TRUTER. 1978. Histopathology of tomato plants infected with *Pseudomonas solanacearum* with emphasis on ultrastructure. *Physiological Plant Pathology* 13: 307–318.
- WEBSTER, B. D. 1973. Anatomical and histochemical changes in leaf abscission. In T. T. Kozłowski [ed.], *Shedding of plant parts*, 45–83. Academic Press, New York, New York, USA.
- WEINER, G., AND W. LIESE. 1995. Wound response in the stem of the royal palm. *International Association of Wood Anatomists Journal* 16: 433–442.
- ZIMMERMANN, M. H. 1979. The discovery of tylose formation by a Viennese lady in 1845. *International Association of Wood Anatomists Bulletin* 2–3: 51–56.