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THE STRUCTURE OF XYLEM VESSELS IN GRAPEVINE (VITACEAE) AND A POSSIBLE PASSIVE MECHANISM FOR THE SYSTEMIC SPREAD OF BACTERIAL DISEASE[1]

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Xylem-dwelling pathogens become systemic, suggesting that microorganisms move efficiently in the xylem. To better understand xylem pathways and how bacteria move within the xylem, vessel connectivity between stems and leaves of *Vitis vinifera* cv. Chardonnay and *Muscadinia rotundifolia* cv. Cowart was studied. Three methods were used: (1) the light-producing bacterium, *Yersinia enterocolitica, (Ye)* strain GY5232 was loaded into petioles and followed using X-ray film, (2) fluorescent beads were loaded and followed by microscopy, and (3) low-pressure air was pumped into leaves and extruded bubbles from cuts in submerged leaves were followed. Bacteria, beads, and air moved through long and branched xylem vessels from the petiole into the veins in leaves of both varieties. From the stem, bacteria and air traveled into primary and secondary veins of leaves one, two, and three nodes above the loading point of the bacteria or air. Particles and air could move unimpeded through single xylem vessels or multiple vessels (conduits) connected possibly through broken pit membranes from within the stem axis into leaf blades. Bacteria were also able to move long distances within minutes from stem to leaf passively without having to cross pit membranes. Such complex, open xylem conduits have not been well documented before; these findings will help elucidate mechanisms involved in the systemic spread of pathogens.

Key words: grapevine; *Muscadinia rotundifolia*; particle movement; pit membrane; *Vitis vinifera*; xylem vessel connectivity.

During the formation of vascular bundles in leaves, xylem development is discontinuous, with an auxin-regulated bidirectional differentiation such that xylem maturation occurs both basipetally and acropetally until neighboring longitudinal segments connect to form the xylem network (Esau, 1965a, b; Sachs, 1969, 1981; Aloni, 2005). As xylem vessel members mature, secondary cell walls thicken according to some pattern. The vessel members making up a vessel have perforated end walls, but the vessel members at the ends of a vessel have an imperforate end wall (Esau, 1965a). Thus, xylem vessels have a finite length and form discrete units hydraulically linked to adjacent vessels by pit membranes through which water and low molecular mass solutes can pass, but microorganisms (except for very small viruses) and gas bubbles cannot (Tarbah and Goodman, 1987; Fosket, 1994; Bové and Garnier, 2002; Tyree and Zimmermann, 2002; Zwieniecki et al., 2002; Choat et al., 2005). Perforation plates can also form on the unreinforced sections of lateral walls when vessels are in physical contact (Fosket, 1994). Accordingly, a certain number of neighboring xylem vessel members may be connected by perforation plates to form branched xylem vessels.

Many pathogenic bacteria use the xylem to move throughout the plant to become systemic (Bové and Garnier, 2002). Researchers examining the vascular interconnectedness of organs (e.g., stem to leaf) have described the hydraulic connections within a plant (Howard, 1974; Ewers and Fisher, 1989a, b) and focused nearly exclusively on the movement of water (Rouschal, 1940; Zimmermann, 1983; Tyree and Zimmermann, 2002; Zwieniecki et al., 2002; Choat et al., 2005). However, the presence of pit membranes creates a distinct difference between where water flows and where particles or microorganisms are able to reach via the transpiration stream. In the few investigations into potential xylem pathways available to particles or bacteria between stems and leaves, only insignificant movement from one organ to another was observed (Canny, 1997; Suhayda and Goodman, 1981; Wiebe et al., 1984). Within leaves, the majority of xylem vessels ended near junctions, specifically the petiole–lamina (Wiebe et al., 1984; Canny, 1997) and the petiole–stem junction (Wiebe et al., 1984; Tyree and Zimmermann, 2002). In this organization, microorganisms would have to pass across pit membranes to leave the stem and move into a leaf blade.

To gain a better understanding of how xylem-dwelling pathogens travel throughout the plant, our primary objective was to determine the xylem pathways through which such pathogens might move unimpeded. The movement of the bacterium *Yersinia enterocolitica* (strain GY5232, engineered to emit light), fluorescent beads, and low-pressure air was followed to determine the xylem pathways. We will show long, branched xylem conduits that facilitate the rapid and passive

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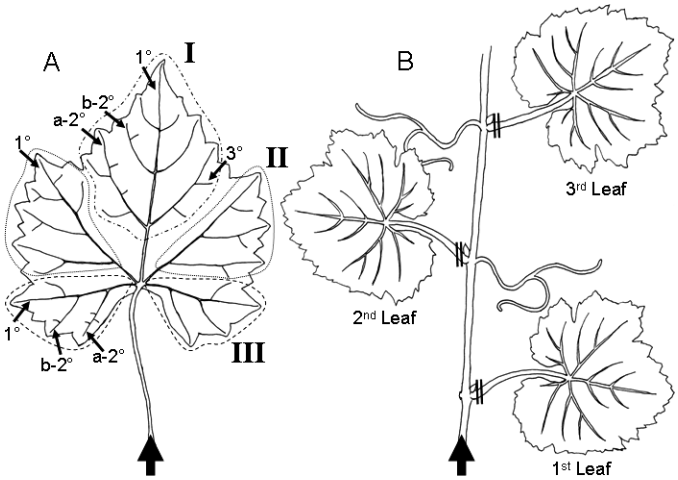

Fig. 1. Diagrams of grapevine leaves. (A) Introduction of bacteria, beads or air (large arrow at base of petiole or stem). Grapevine leaves have five primary veins (1°) and numerous secondary (2°) and tertiary veins (3°). Each 1° vein and associated 2° and 3° veins were grouped into sectors I, II, and III. There are no differences between left and right sectors of II and III. (B) Stem explants with three leaves and four internodes. Leaves are numbered in ascending order starting from the cut end of the stem where bacteria or air were introduced (large arrow). For bacterial experiments, leaves were removed at the base of the petiole (double lines) and exposed to photosensitive film to evaluate the distance bacteria moved from the stem.

movement of particles from stems into leaves in grapevine shoots.

MATERIALS AND METHODS

Well-watered 3-year-old *Vitis vinifera* cv. Chardonnay and *Muscadinia rotundifolia* cv. Cowart grapevines were grown in greenhouses in 7.9 liter plastic pots; the leaves and stems assayed in this study were from the current year's growth. Leaf and stem segments were generally taken between the 6th and 14th leaves basal to the shoot meristem and leaf blades ranged from 6 to 12 cm in width and height. Chardonnay leaves from 8-yr-old vineyard-grown vines in Napa, California were harvested in May 2004. Leaf and stem sampling occurred at the same time of day (1000 to 1100 hours). During this time, the relative humidity in the vineyard ranged from 40–70%, and photosynthetically active radiation (PAR) ranged from 1300 to 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Shoots were held under water, and three or four leaves were severed from the vines at the basal end of the petiole (Fig. 1A). Likewise, shoot segments having three attached leaves were severed underwater for bacterial and air movement experiments (Fig. 1B). In transferring leaves or stems from the water to bacterial or bead suspensions, a drop of water was left on the cut end. Cutting the leaves and stems underwater and retaining a water droplet on the cut surface minimized cavitation and the introduction of air emboli. In the bacteria and bead experiments, cut ends were not exposed to air at any point during the experiments. In the air-movement experiments, cut ends were exposed to air only as air was introduced into the stem or petiole.

Grapevine leaves have five primary veins (1°): one center vein and two symmetrical veins on both the left and right sides (Fig. 1A). Each primary vein has numerous secondary (2°) veins and tertiary veins (3°). To evaluate open vessel lengths, each primary vein and associated secondary and tertiary veins in leaves were grouped into sectors I, II, or III as shown in Fig. 1A. Secondary vessels in each sector were assigned a letter "a" or "b" beginning with the basal secondary veins and moving up the primary vein towards leaf margins (Fig. 1A).

Bacterial movement—The psycrotrophic bacterium *Yersinia enterocolitica* (*Ye*) strain GY5232 engineered to express the *lux* operon was introduced into leaves by submerging either the cut end of the petiole or the cut end of the stem in a test tube of *Ye* GY5232 bacterial suspension. Leaves or stems with leaves were allowed to take up bacterial suspension under transpiring conditions for 0.5, 1, 2, 3, or 5 h. The same results were obtained for all times, so 2 h was used for convenience.

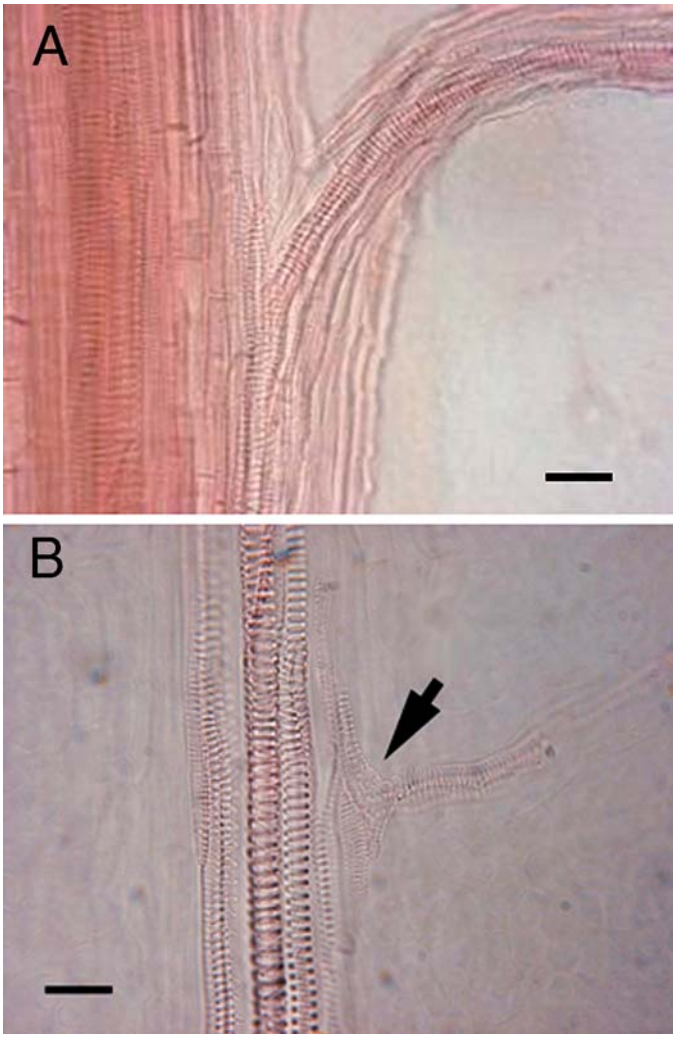

Fig. 2. Leaf clearings of xylem vessels branching from 2° to 3° veins in (A) *Muscadinia rotundifolia* cv. Cowart leaves. As indicated by the arrow, irregular tracheary elements branch off from tertiary veins to form quaternary veins in *Vitis vinifera* cv. Chardonnay (B). Scale bar = 50 μm (A); scale bar = 25 μm (B).

At the end of the time period, bacterial movement was evaluated in leaves containing *Ye* GY5232 by either removing leaves from the suspension or cutting the leaves from stems in the suspension at the base of the petioles (as indicated by the double lines in Fig. 1B), then exposing the leaves (petiole and leaf blade) to photosensitive x-ray film. Bacteria were detected by the light they produced due to expression of the *lux* operon. The *luxCDBAE* operon contains genes that encode proteins needed for the enzyme luciferase, synthesis of the luciferase substrate, and other relevant elements required for the bacterium to emit light de novo (Winson et al., 1998). The leaf tissue was sufficiently transparent that the light emitted by the bacteria was easily detected by the photosensitive x-ray film. This resulted in images of the locations of *Ye* GY5232 inside the xylem vessels of the leaves (Fig. 3). Varying exposure times (0.25–24 h) to photosensitive film of the same leaves showed that no bacterial movement occurred after removal of leaves from bacterial suspension; therefore, a 16-h exposure time was used for convenience and to obtain the most easily visualized bacterial pathways.

Construction of lux$^+$ strain GY5232 of Yersinia enterocolitica—*Ye* was engineered to emit light by introducing a plasmid encoded *flhB-lux* operon

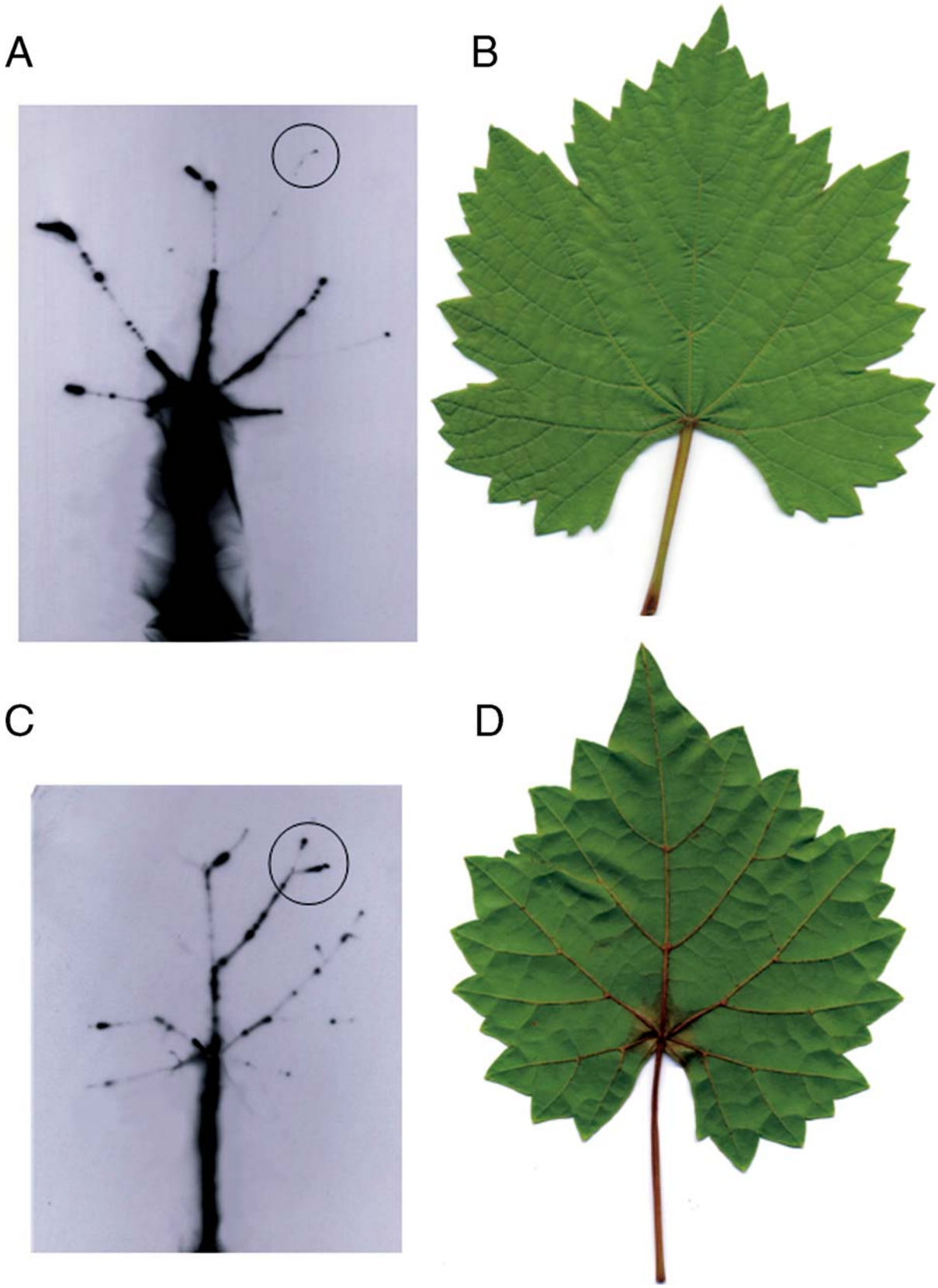

Fig. 3. Photosensitive x-ray film and leaves showing the movement of *Yersinia enterocolitica* strain GY5232 from petiole to leaf blade in *Vitis vinifera* cv. Chardonnay (A, B) and *Muscadinia rotundifolia* cv. Cowart (C, D) leaves. Circles show 3° veins into which bacteria have moved.

fusion (pGY377). The *flhB* gene was amplified by PCR using primers that annealed approximately 500 bp upstream of the initiation codon and 80 bp downstream of the initiation codon. The primers used were flhB1 (5′ GCT CTA GAG CAT AAC AAG GGT ATG AGC 3′) and flhB2 (5′ TCC CCC GGG GGA TAT CTG GCC TTT CTC 3′). (A *Xba*I linker was included in the flhB1 primer and a *Sma*I linker was included in the flhB2 primer for another project that is not detailed here.) After amplification, the 580-bp fragment was cloned into the pTOPO vector (Invitrogen) to obtain pGY330. This plasmid was then digested with *Eco*RI to release a DNA fragment containing *flhB* and was subcloned into the EcoRI site of pSB401 (Winson et al., 1998). This formed a transciptional fusion of *flhB* with the *luxCDBAE* operon. The resulting plasmid, designated pGY377, was transferred by electroporation (BioRad Gene Pulser) into *Yersinia enterocolitica*. This strain was designated GY5232. *Ye* was routinely cultivated in tryptone (1%) yeast extract (0.5%) (TYE) at 26°C. Prior to the start of each experiment, *Ye* strain GY5232 was grown overnight in TYE at 26°C and then subcultured at a 1 : 30 dilution and incubated another 2 h at 26°C to ensure high expression of *flhB-lux CDBAE*.

Bead movement—Leaves were removed from stems as stated, then transferred to vials of fluorescent polystyrene beads suspended in TYE at a concentration of 1.0×10^8 microspheres/mL (FluoSpheres polystyrene microspheres, 1.0 μm, blue-green fluorescent [430/465 nm], Molecular Probes, Eugene, Oregon, USA). Leaves were allowed to transpire under lighted conditions from several hours to overnight. Petioles and leaf veins were then freehand sectioned in transverse view and observed under violet light wavelengths with an Olympus Vanox-AHBT compound microscope (Olympus America, Melville, New York, USA), and digital images were captured with a Pixera (Los Gatos, California, USA) 600ES digital camera.

Air movement—Leaves or stems with three leaves were removed as stated previously. Filtered (with glass wool), pressurized air at a maximum of 80 kPa, which was low enough to avoid inducing cavitation in xylem vessels (Skene and Balodis, 1968; Cohen et al., 2003), was applied to the cut ends of petioles or stems. Leaves were placed under water and, starting at the leaf margins, incisions were made at 1 to 2 mm intervals in the leaf blade. With each incision, newly cut vein endings were examined with a dissecting microscope for signs of bubbles emerging from the veins. The first appearance of a stream of bubbles was evidence of an open xylem vessel, starting at the cut end of a petiole or stem and ending at the location of the cut. When low-pressure air is forced through xylem vessels of fresh tissue, air will only pass through open vessels because wet pit membranes will block air flow (Skene and Balodis, 1968; Tyree and Zimmermann, 2002; Cohen et al., 2003). If vessels are already embolized, air will also be able to flow through those vessels, which could compromise the accuracy of continuous vessel length measurements. However, bacteria and beads cannot flow through embolized vessels. Because results from the three methods were uniform and all plant parts were detached under water, embolisms in vessels were highly unlikely to contribute or detract from the lengths of open, continuous vessels determined in this study.

Leaf clearing—Leaves were cleared using a NaOH and chloral hydrate method (Ruzin, 1999). Dissected leaf tissue was soaked in 70% ethanol to remove pigment, then placed in water, treated with 5% NaOH for several days to remove all cell contents, and finally treated with a saturated chloral hydrate solution overnight. Leaves were then rinsed several times in water, stained with 0.1% safranin O, and dehydrated and permanently mounted on microscope slides with Permount and a cover glass. All material was examined with an Olympus Vanox-AHBT compound light microscope (Olympus America, Melville, New York, USA) with bright-field optics, and photographed with a Pixera (Los Gatos, USA) 600ES digital camera.

Statistical analysis—Analysis of variance (ANOVA) was used to test the significance of the main effects and corresponding interactions (SAS, SAS Institute, Cary, North Carolina, USA). Three replications of 15 plants were used for each treatment. In all cases, there was no statistical difference between methods or between cultivars. Where appropriate, means were compared by Fisher's LSD (least significant difference) at the $P = 0.05$ level.

RESULTS

Analysis of leaf clearings of Chardonnay and Cowart showed vascular bundle branches between lower order veins (1°, 2°, 3°; Roth-Nebelsick et al., 2001) consisting of simple divergence of vessels (Fig. 2). Junctions between higher order veins (3° and quaternary) appeared to be mediated by irregular tracheary elements, possibly tracheids (Fig. 2B) (Esau, 1977). Chardonnay and Cowart showed equivalent branching patterns and vascular structure. The branching from lower to higher order veins suggested the potential for continuous vessels to exist within in the leaf blade. However, simple anatomical examination of leaf vasculature did not show which veins had open vessels that could allow particles, such as bacteria, to move freely from lower to higher order veins or from organ to organ. Therefore, potential xylem pathways for passive particle movement were identified using bead, bacterial, and air flow movement experiments.

Petiole to leaf blade—Light-producing *Ye* GY5232 traveled unimpeded through the transpiration stream from the cut, basal end of petioles, through the petiole–leaf blade junction, into primary (1°), secondary (2°) and tertiary (3°) veins as shown by exposing leaves to photosensitive film (Fig. 3). Film was blank if no light-producing *Ye* GY5232 were present. Bacteria consistently went into all five 1° veins and were quite uniformly distributed. Movement into 2° veins appeared to be random and *Ye* GY5232 also entered into 3° veins, often in sector I (Fig. 3A and C; refer to Fig. 1A). Particularly dark spots along vein traces on the photosensitive film may indicate possible aggregation of bacteria at vessel endings, producing a stronger (darker) collective light signal (Fig. 3A and C).

Bacteria moved rapidly throughout the leaf blade, reaching a maximum distance in 0.5 h, the shortest time interval evaluated. After 5 h, more bacteria had moved into the leaf (judging by a stronger signal on photosensitive film exposed for the same length of time), but the distances the bacteria traveled within the leaf from the 0.5 h time to the 5 h time did not differ. Identical results were obtained in these tests with light-producing *Escherichia coli* (*E.coli*) (data not shown). Further, there were no overall differences between Chardonnay and Cowart leaves in the distribution of bacteria or in the distances the bacteria moved.

Low-pressure air moved through open xylem vessels from the base of the petiole up to 3° veins in both Chardonnay and Cowart leaves as determined by the first appearance of a stream of bubbles. Air moved into all five 1° leaf blade veins, most 2° veins, and generally followed the same pattern as the *Ye* GY5232 bacteria. In addition, leaves of Chardonnay grown in the field were equivalent to Chardonnay leaves of greenhouse vines (Fig. 4 and Table 1).

Fluorescently tagged 1-μm beads were loaded into cut petiole ends. Similar to *Ye* GY5232 and air movement, fluorescent beads moved from the petiole, across the petiole–leaf blade junction, into the 1° and 2° veins of the leaf blade (Fig. 4). Beads were not found in 3° veins, but this was most likely due to the difficulty in freehand sectioning such thin leaf pieces and to the low signal strength from just a few beads in small veins.

Continuous, open vessels existed from the base of the petiole to all five primary veins based on air, bacterial and bead movement. The average distances that bacteria, beads, and air traveled through open, continuous vessels were similar, and ranged from 60 to 80% of the total length of the potential vascular path from petiole base to individual leaf blade vein endings (Table 1). The maximum distances reached by *Ye*

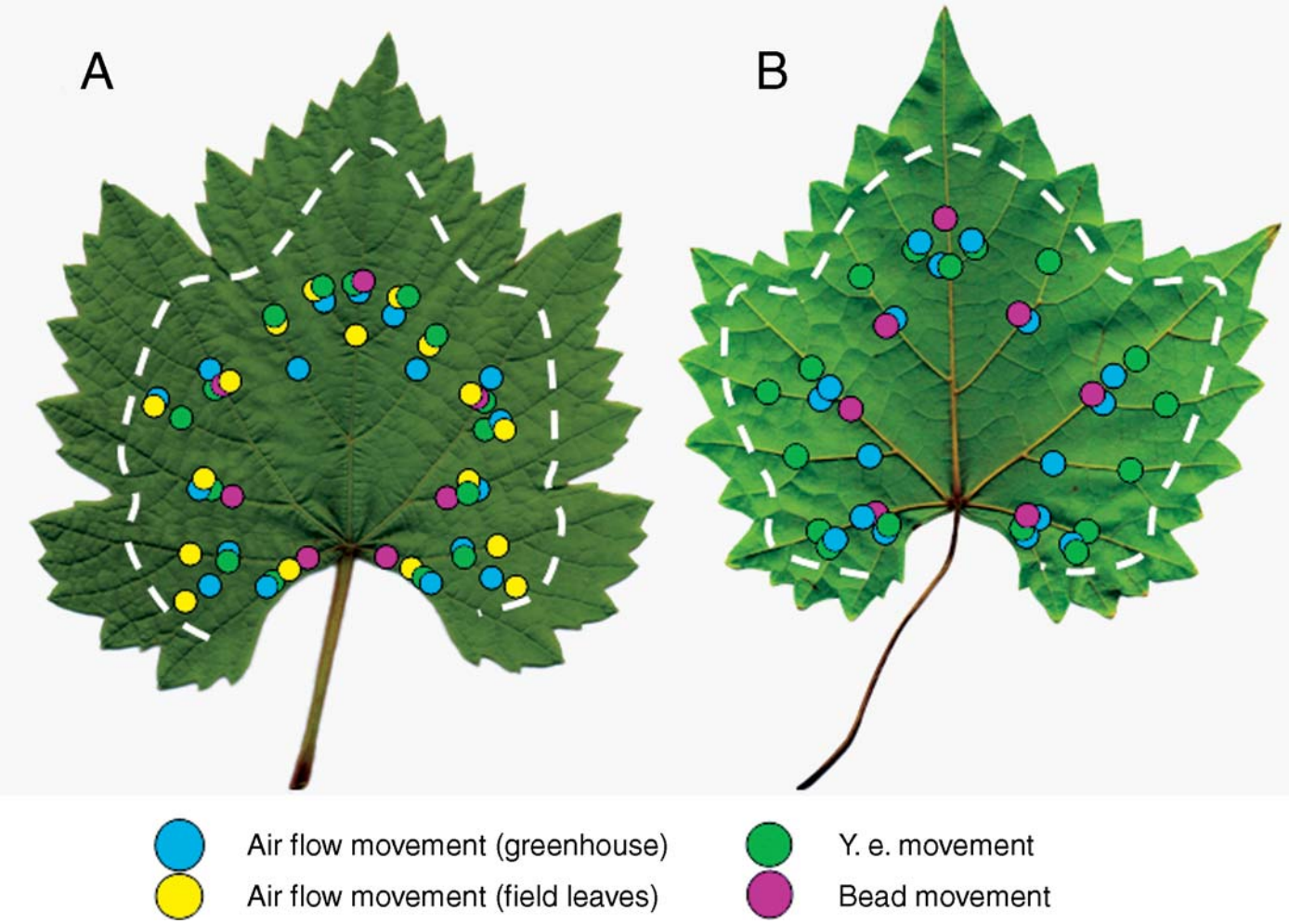

Fig. 4. A schematic representation of the lengths of open, continuous vessels as a percentage of the total length of the vascular path from petiole base to leaf blade vein endings in *Vitis vinifera* cv. Chardonnay (A) and *Muscadinia rotundifolia* cv. Cowart (B). Green dots represent the average distances traveled by the bacteria, *Yersinia enterocolitica* GY5232; blue dots represent the distances of air flow through greenhouse-grown vines, yellow dots, the distances of air flow in vineyard-grown Chardonnay vines, and pink dots, 1-μm polystyrene beads. The dashed white line shows the maximum distances the bacteria, beads, and air were observed. Data are means ± SE, based on three replications of 15 plants per treatment.

GY5232, the fluorescent beads, and air were identical, as indicated by the white dashed line in Fig. 4.

Stem to leaf blade—Low pressure air and *Ye* GY5232 were loaded at the cut end of a stem segment with three leaves still attached. Similar to the petiole-to-leaf-blade loading experiments, there were continuous open vessels from the point of loading on stems, which resulted in rapid particle movement from the stem into all three leaves, apparently without crossing any pit membranes. Bacteria and air traveled from the cut stem into 1° and 2° leaf blade veins of at least three leaves above the bacteria or air source (refer to Fig. 1B), moving passively between multiple organs. Cowart differed somewhat in that no bacteria were observed in the 2° veins of the third leaves, and only one 2° vein in the third leaf had any air movement (Table 2); other than this, Chardonnay and Cowart were indistinguishable. No *Ye* GY5232 or air flow was observed in 3° veins of any of the leaves (first, second, or third) when air or bacteria were introduced through the stem. As one would expect, the length of open vessels running from the stem into 2° veins was longest in the third leaves above the stem cut. Although fewer bacteria reached the third leaf than the first or second, the movement within leaf blades was strikingly similar in all three leaves. The distribution patterns were comparable to those seen when air, bacteria, or beads were administered from the petiole base (Fig. 3).

The number of leaves per stem (which was 3) in segments having open vessels was largely dictated by plant size and did not represent the maximum distance the bacteria or air may be able to move from organ to organ through open, continuous xylem vessels. Indeed, in larger stem segments taken between the 6th and 14th leaves basal to the shoot meristem, air flow was observed in 1° and 2° veins of the fifth leaf above a cut stem, having average vessel lengths of 37.8 cm with a standard deviation of 1.5 cm for 1° veins; and 36.7 cm, with a standard deviation of ± 1.6 cm for 2° veins. Thus, the maximum length of open, continuous vessels is expected to be greater than the three-leaf stem segments used in this study.

TABLE 1. Xylem vessel lengths were measured as the total possible distance (in mm) from the base of the petiole to the leaf margin divided by the distance moved by *Yersinia entericolita*, fluorescent beads, or low pressure air and expressed as a percentage for primary and secondary (2°) laminar veins, in *Vitis vinifera* cv. Chardonnay and *Muscadinia rotundifolia* cv. Cowart. Data are means ± SE. Values followed by an asterisk are significantly different from the other values in the column at $P = 0.05$. See Materials and Methods, statistical analysis section for further details.

Technique	Variety	Sector I primary veins	a-2° veins[a]	b-2° veins	Sector II primary veins	a-2° veins	b-2° veins	Sector III primary veins	a-2° veins	b-2° veins
Yersinia entercolitica	Chardonnay	67.6 ± 2.7	73.1 ± 2.2	78.2 ± 4.1	65.7 ± 2.3	66.9 ± 2.0	73.3 ± 2.1	63.6 ± 2.5	68.9 ± 3.0	—
	Cowart	67.5 ± 2.8	86.5 ± 1.7*	81.6 ± 2.0	75.3 ± 2.3	83.5 ± 3.4*	86.4 ± 3.8	81.9 ± 2.4	79.7 ± 3.7	86.5 ± 1.9
Low pressure air flow	Chardonnay	66.5 ± 2.3	65.1 ± 6.5	74.2 ± 1.1	68.7 ± 2.1	69.4 ± 2.7	78.9 ± 3.3	64.1 ± 2.9	69.7 ± 2.0	77.5 ± 2.7
	Cowart	68.4 ± 2.4	76.7 ± 3.0	82.9 ± 3.0	72.1 ± 2.0	70.7 ± 2.2	76.8 ± 0.3	73.6 ± 2.8	80.3 ± 10.6	85.3 ± 1.0
	Field Chardonnay	60.0 ± 3.2	72.0 ± 2.7	77.1 ± 4.5	65.2 ± 3.6	68.1 ± 2.4	78.6 ± 3.2	72.4 ± 3.4	64.8 ± 4.4	82.9 ± 3.4
Fluorescent bead	Chardonnay	68.2 ± 1.0	—	—	65.4 ± 6.0	63.6 ± 1.6	—	50.0 ± 1.0*	—	—
	Cowart	75.2 ± 0.9	76.4 ± 1.4	—	67.6 ± 5.6	—	—	72.0 ± 4.5	—	—

[a]To evaluate open vessel lengths, each primary vein and associated secondary and tertiary veins in leaves were grouped into sectors I, II, or III as shown in Fig. 1A. Secondary vessels in each sector were assigned a letter "a" or "b" beginning with the basal secondary veins and moving up the primary vein towards leaf margins (Fig. 1A).

DISCUSSION

In this study, we show for the first time, using three complementary methods, that xylem conduits are present that facilitate the movement of bacteria-sized particles long distances unimpeded and passively through xylem vessels from stems and petioles to higher order veins in the lamina, in some instances covering a distance of over 30 cm. The long pathways and their incorporation of stems, petioles, and lamina suggest that these conduits may be comprised of single branched vessels, but may also include more than one vessel joined via damaged pit membranes.

Long-distance bacterial movement within grapevine stems has been reported before, but no previous study investigating pathogen or particle movement within the xylem system has reported evidence of the open conduits shown in this study. In Jonathan apple trees (*Malus pumila*), *Erwinia amylovora* labeled with ^{32}P moved a total of 35 mm along the stem but not into leaves (Suhayda and Goodman, 1981). After a second treatment with ^{32}P-labeled *E. amylovora*, some bacteria were detected in the first petiole from the inoculation site (a total of 9 mm), but most of the labeled bacteria remained in the stem (Suhayda and Goodman, 1981). Tarbah and Goodman (1987) reported that the bacterium *Agrobacterium tumefaciens* moved up to 30 cm in 24 h in grapevine (*Vitis vinifera*) stem, but they did not follow the bacteria into leaves. Weibe et al. (1984) showed that India ink moved as much as 3 mm into the petiole from the stem in alfalfa (*Medicago sativa*), but not into the leaf lamina. Similarly, Canny (1997) introduced latex particles to petioles and found latex in only three of over 600 vessels in sunflower leaf lamina (*Helianthus annuus*), and no latex particles were found beyond a few millimeters into the leaf lamina. These particle movement studies have led to the conclusion that xylem vessels end in groupings at junctions, e.g., the petiole–lamina junction (Canny, 1997; Wiebe et al., 1984).

Compant et al (2005) inoculated agar with *Burkholderia* spp. strain PsJN and then planted small tissue-cultured grape seedlings (~6 cm tall) on the agar. Using GFP- or GUS-tagged bacteria, they followed the movement of the bacteria onto the root surface, into the root tissues via small cracks in the root surface, and then into the xylem vessels. They observed that *Burkholderia* spp. was able to secrete cell-wall-digesting enzymes, suggesting that this may assist the bacteria in its

TABLE 2. Average lengths of open conduits from stem to primary and secondary veins in *Vitis vinifera* cv. Chardonnay and *Muscadinia rotundifolia* cv. Cowart measured as the distance traveled by *Yersinia entericolitica* or the movement of low pressure air from the loading point in the stem to the leaf blade of leaves 1, 2 or 3 apical to the loading point. Stem and petiole lengths are included in measurements of primary and secondary veins. Data are means (in mm) ± SE (except for * for which there was only one value). See Statistical analysis section for details.

Location		*Yersinia entericolitica* movement		Low pressure air flow	
		Chardonnay	Cowart	Chardonnay	Cowart
1st leaf	Stem length	58.3 ± 11.6	71.8 ± 1.5	51.8 ± 5.5	63.3 ± 10.8
	Petiole	60.0 ± 8.2	92.3 ± 11.0	80.3 ± 5.1	72.3 ± 5.5
	Primary veins	141.4 ± 9.0	201.0 ± 10.7	196.4 ± 8.6	164.2 ± 14.4
	Secondary veins	125.1 ± 4.3	226.0 ± 14.2	196.1 ± 5.6	187.1 ± 8.8
2nd leaf	Stem length	87.8 ± 11.3	144.3 ± 5.5	121.9 ± 15.5	96.7 ± 30.3
	Petiole	39.8 ± 3.9	55.7 ± 13.0	78.0 ± 6.2	61.7 ± 0.9
	Primary veins	153.9 ± 7.1	231.0 ± 10.5	261.8 ± 14.5	197.4 ± 19.1
	Secondary veins	167.4 ± 9.2	243.8 ± 16.3	267.8 ± 9.3	173.7 ± 22.7
3rd leaf	Stem length	109.7 ± 15.0	207.5 ± 7.5	204.6 ± 49.2	181.5 ± 40.4
	Petiole	34.0 ± 1.5	67.0 ± 6.0	66.6 ± 9.7	65.5 ± 4.5
	Primary veins	171.4 ± 9.5	311.7 ± 4.8	326.0 ± 33.8	254.1 ± 18.0
	Secondary veins	189.7 ± 2.5		336.7 ± 20.9	244.0*

passage into the root xylem. Compant et al. (2005) then observed that the bacteria were present inside the xylem vessels of the fifth leaf within 96 h of inoculation. This observation is consistent with that of the current study by showing the rapid movement of a bacterium through the xylem vessels into a leaf blade. Compant et al. (2005) used small plantlets instead of fully grown grape plants, so we would expect the xylem network in a plantlet to be considerably smaller, the xylem to be mostly primary xylem, and obviously the vessels appeared to be interconnected.

The open continuous xylem conduits observed in the current study are unlikely to be an artifact. Rupture of pit membranes due to air flow can be discounted, because we used very low pressures, and Choat et al. (2004) showed that in *Fraxinus americana* L., pit membranes stretched but did not tear at pressures as high as 6 MPa. Enzymatic degradation of pit membranes can also be discounted. Of the three methods used in this study (bacteria, beads, and air flow), only *Ye* GY5232 had the potential to synthesize wall-degrading enzymes (there is no evidence that it does), yet the distances traveled from stem to petiole to leaf blade were equivalent for all three methods. Scanning electron micrographs suggest that naturally occurring, degraded pit membranes are present in several species (Carlquist and Schneider, 2004). Ruptured pit membranes have been observed in grapevine xylem vessels (J. Stevenson, T. Rost, M. Matthews, and Q. Sun, unpublished data). However, more work needs to be done to document that the observed ruptured pit membranes existed before sample preparation.

Bacteria, beads, and air moved the same distance from the petiole into the leaf blade, delineated by the white line in Fig. 4, suggesting that beyond this point anatomical limitations of the xylem prevented further movement. One explanation for this is that increasingly narrow vessels with proximity to leaf margins prevented bead and *Ye* GY5232 movement. However, air movement would not have been inhibited because air would have been able to move through narrower vessels than bacteria or beads unless pit membranes were present to prevent air passage by imposing greater pressure requirements. Thus, the movement of *Ye* GY5232, beads, and air was most likely stopped by vessel ends with intact pit membranes.

The facility of travel within grapevine that open, continuous conduits allow has implications for the systemic spread of xylem-dwelling bacteria. For example, the systemic spread of *Xylella fastidiosa* increases Pierce's disease development and eventually results in plant death (Goodwin and Purcell, 1992; Purcell and Hopkins, 1996). The insect vector of *Xylella fastidiosa (Xf)*, the sharpshooter, feeds on petioles, leaf blades, and stems of grapevine (Purcell, 1975; Feil et al., 2003). If sharpshooters feed on open, continuous vessels and infest the vine with *X f*, the bacteria can be rapidly and passively moved into nearby leaves. The extent of the rapid, passive movement of *Ye* GY5232 through xylem vessels and conduits seen in this study suggests that passive movement of bacteria could be a contributing mechanism for systemic movement of xylem-borne bacteria, such as *Xf*in grapevine.

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