Research Note
Passive Pathogen Movement via Open Xylem Conduits in Grapevine Graft Unions

Gregory A. Gambetta,[1] Thomas L. Rost,[2] and Mark A. Matthews[3]*

Abstract: In grapevines, scion-rootstock grafting is a common practice to impart pathogen resistance and to manipulate aspects of grapevine physiology, including vigor, yield, and fruit composition. Successful grafting requires the integration of the scion and rootstock vascular networks. The nature and extent of this integration was evaluated to determine the impacts of the graft union on the movement of vascular pathogens between scion and rootstock. Using both xylem-mobile dyes and the xylem-limited bacterial pathogen *Xylella fastidiosa*, we demonstrate that the graft union contains open xylem conduits providing for passive pathogen movement. These open conduits may facilitate bacteria overwintering in below-ground vine tissues of grafted vines and systemic infection in subsequent growing seasons.

Key words: Pierce's disease, *Xylella fastidiosa*, vessel length, pathogen movement

In viticulture, interspecific scion-rootstock grafting has been used for over 100 years, primarily to avoid the devastation of phylloxera. By exploiting phylloxera-resistant rootstocks of particular species, the fruit qualities of prized winegrape scions in the susceptible *Vitis vinifera* can be maintained. Rootstocks also affect vine vigor, yield, and fruit composition. The success of interspecific grafting requires the integration of the scion and rootstock vascular systems, and this integration has the potential to facilitate pathogen transmission. Indeed, scion-rootstock grafting has led to the inadvertent transmission of pathogenic viruses. Similarly, natural root grafts provide a potential mechanism for vine-to-vine transmission of pathogens (Epstein 1978). However, surprisingly little is known about the anatomy at the graft union in grapevine, and more specifically, the integrated vascular anatomy across the graft.

In general, the establishment of graft unions first involves the formation of callus tissue, then continuous cambial tissue, and finally the differentiation of this cambial tissue into specific tissues such as xylem and phloem (Coombe 1999). In tree crops, the integration of the xylem network across the graft appears to occur in two parts: first, the formation of a relatively disorganized conglomeration of tracheids and, second, the differentiation of more organized xylem vessels (Copes 1969, Weatherhead and Barnett 1986). There are few studies assessing the extent to which vasculature is integrated across the graft, but in light of the observation that plasmodesmata form between cells of different taxa in graft unions, the integration is evidently extensive (Kollmann and Glockmann 1985, Kollmann et al. 1985). Nevertheless, in *Vitis* there is some evidence that grafting leads to decreased hydraulic conductance (Bavaresco and Lovisolo 2000), suggesting that the vascular integration across the graft does not match that of the intact plant. If vascular integration across the graft union is limited, pathogen transmission from shoot to root or vice versa may be restricted or slowed.

Xylella fastidiosa (Xf) is a xylem-limited, gram-negative bacteria and the causal agent of numerous economically important plant diseases including Pierce's disease (PD) of grapevine. Pierce's disease may be limited to environments that allow successful overwintering which may occur in roots (Feil and Purcell 2001, Purcell and Saunders 1999). The flow of air, xylem-mobile dyes, and xylem-specific bacteria was used to show that the unwounded grapevine stem forms long open conduits that offer no barrier to pathogen movement (Chatelet et al. 2006). However, shoot-root transitions and graft unions were not investigated. The formation of a graft union originates with the joining of two wounded (excised) stem sections. This wounding leads to extensive vascular occlusions in the phloem (Evert et al. 2006) and xylem (Sun et al. 2006) that may limit vascular integration.

In the present study, we used various methodologies previously described (Chatelet et al. 2006) to determine to what extent the vascular anatomy of the graft union limits movement across the graft in *Vitis vinifera*. In addition, we

investigated the possibility of passive movement of Xf across the graft union. This work builds upon the understanding that Xf can rapidly move long distances through stems and leaves, suggesting that in *Vitis* there is the possibility of passive systemic movement of vascular pathogens.

Materials and Methods

Plant material. One-year-old grafted grapevines (*Vitis vinifera* L. Chardonnay #37 scion grafted onto rootstock 110-R) were grown from dormancy in 4-L pots filled with 1/3 peat, 1/3 sand, 1/3 redwood compost, with 2.4 kg m^{-3} dolomite lime in a greenhouse (30/20°C ± 3°C; 40/70% ± 10% RH; and natural light with a daily maximum of 1200 μmol photons m^{-2} s^{-1} PAR). The vines were pruned to two shoots, and the shoots were vertically trained to ~1.5 m. Pots were drip-irrigated four times a day (at 0600, 0900, 1400, and 1800 hr) for 4 min at 7.57 L hr^{-1} (2 L d^{-1}) with dilute nutrient solution (90 mg/L calcium, 24 mg/L magnesium, 124 mg/L potassium, 6 mg/L nitrogen as NH_4, 96 mg/L nitrogen as NO_3, 26 mg/L phosphate, 16 mg/L sulfate, 1.6 mg/L iron, 0.27 mg/L 195 manganese, 0.16 mg/L copper, 0.12 mg/L zinc, 0.26 mg/L boron, and 0.016 mg/L molybdenum) at pH 5.5 to 6.0.

Movement of air. Plants were removed from soil, the entire plant was placed in water, and shoots were excised ~15 cm above the graft. A rubber tube was attached to the apical end of the graft union and household air was applied at 35 kPa, controlled by a pressure regulator. This pressure is well below (7%) that required for air seeding of vessels in grapevine (Sperry et al. 1987). After a few minutes under pressure, large roots (≥2 mm diam) were excised every 2 cm starting from the tip and moving toward the base (graft). The excisions were repeated until a stream of air bubbles appeared at the cut surface. The distance from where the bubbles first appeared to the air loading point was recorded. Experiments were replicated three times.

Movement of paint. Paint movement across the graft was carried out as reported previously (Chatelet et al. 2006). Briefly, red latex paint solution (1:300, paint:water) was prepared and large particles were allowed to settle for 24 hr. The solution was filtered through Whatman no.1 filter paper (Whatman International, Kent, UK) to remove particles greater than 11μm diam, thus allowing the paint solution to freely move within open xylem conduits, but not through pit membranes. The paint solution was loaded at 35 kPa for three days at the cut end of one shoot, while the other shoot was left intact.

Thin free-hand cross sections were cut every centimeter starting from the infused shoot, through the graft union, and into the root system. Stained xylem vessels were observed in cross section on a Zeiss dissecting microscope and photographed with a Pixera Pro 600ES digital camera. The number of stained xylem elements were counted and compared above and below grafts. Experiments were carried out in triplicate for a total of six plants.

Movement of Xf. Six plants were brought into the laboratory and the stems were cut at the soil line under water.

Three plants were placed in a 100-mL beaker with 25 mL Xf (at a concentration of ~1 x 10^8/mL) in PD3 (without agar) medium (Davis et al. 1981), and three control plants were placed in PD3 alone as negative controls. All plants were held in place by laboratory stands and fanned under ambient light conditions for 1 hr to promote transpiration by leaves and solution uptake at cut surface. Starting above the graft union, four 10-cm sections were excised, placed in a pressure chamber, and subjected to 0.25 MPa pressure. The exuded xylem sap was collected onto a nitrocellulose membrane. The membrane was then placed into a microcentrifuge tube containing 200 μL dd H_2O, spun at 14,000 rpm for 2 min, and the supernatant was collected and stored at -20°C. Polymerase chain reactions (PCR) were performed with the Xf-specific PCR primers RST33 and RST31 as established in a previous protocol (Minsavage et al. 1994). PCR products were assessed by agarose gel electrophoresis.

Results and Discussion

Air and paint movement across grafts. Both air and latex paint particles readily moved across graft unions from the shoot toward the roots. The movement of air through vessels across the graft was revealed in the appearance of air bubbles from the cut surface below the graft in every sample. The movement of paint across the graft was also observed in every sample (Figure 1, Figure 2). Many open xylem conduits, indicated by continuous staining of vessels, extended through the graft union, but several vessel endings were also clearly visible (Figure 2, small arrows). Accordingly, the number of paint-filled vessels in cross section diminished with distance from the infusion site (Figure 1). Comparisons of stained vessels in cross sections above and below the graft indicated that 10% of all vessels provided open conduits across the graft union. The maximum length of the open conduits across the graft, determined from air-loading experiments, was ~50% less in grafted vines compared to own-rooted vines, at 219 mm and 439 mm, respectively.

This movement denotes continuously open xylem conduits; that is, the movement of air and paint across the graft did not require crossing intact intervessel pit membranes (Chatelet et al. 2006). These open paths were present in young (one-year-old) grafted vines. Although published data on xylem development in grafts are limited, analyses of cambium and xylem development in grafts of tomato (Turquois and Malone 1996) and coleus (Stoddard and McCully 1980) indicate that xylem connections are established within one week of grafting. Grapevines may behave similarly, and as young grafted grapevines such as the plants used in this study develop additional secondary xylem, larger vessels differentiate (Schultz and Matthews 1993), especially in the ventral and dorsal regions of the stem (Stevenson et al. 2004). Vessel length is correlated with vessel diameter (Ewers et al. 1990). Therefore, a young vine is the appropriate system to test for discontinuous conduits because more open and longer conduits are likely to de-

velop as grapevines age. In older grapevines, it has been shown that open conduits exist from the stem, through the petiole, and well into the leaf lamina of grapevine (Thorne et al. 2006), and these results have been confirmed (Chatelet et al. 2006). These open xylem conduits can be greater than 1 m in *V. vinifera* (Chatelet et al. 2006) and 7.75 m in *Vitis labrusca* (Zimmermann and Jeje 1975). Thus, several studies demonstrate that in *Vitis*, long open xylem conduits are commonplace and integrate the xylem network across grafts, through stems, and into leaves.

Our observations reveal a xylem network that is well integrated across the graft union despite the presence of shorter open-conduit lengths in grafted vines. Although there is limited evidence of a decrease in hydraulic conductance of some scion-stock combinations (Bavaresco and Lovisolo 2000), the integration shown here is consistent with several studies of graft unions in other woody crops that have found that the graft union has no effect on hydraulic conductance when compared to that of own-rooted plants. Tests for a hydraulic constriction in the graft union of peach, kiwi, apple, and olive have been negative (Basile et al. 2003, Clearwater et al. 2004, Solari and DeJong 2006, Cohen et al. 2007, Gasco et al. 2007).

Pathogen movement across grafts. PCR was used to determine the transpiration-driven, passive movement of Xf across grafts and into the shoot. Just one hour after introduction below the graft, Xf was readily detected in stem fragments as much as 66-cm distal from the graft (Figure 3). *Xylella fastidiosa* was never detected in negative control plants. Studies in our laboratory have demonstrated that the threshold for PCR detection of Xf in plant tissues is ~10^3 cells per gram of fresh weight tissue (Gambetta et al. 2007).

Comparison of some known parameters of xylem anatomy with the results of the experiments with air, paint, and Xf reveal clear information about the structure of the xylem network in the graft union of young grapevines. The dimensions of pit apertures averaged 2 x 50 μm for the scalariform intervessel pits in similarly grown Chardonnay grapevines (Sun et al. 2006). The rod-shaped bacterium is ~0.5 x 3 μm (Davis et al. 1978) and relatively large compared to the paint particles (>220 nm diam) (Chatelet et al. 2006). A pressure drop of 35 kPa (used in these experiments) would pull an air-water meniscus through a pore with a diameter larger than 8 μm (Zimmermann 1983). Thus, during the experiments air probably did not even enter a pit, although

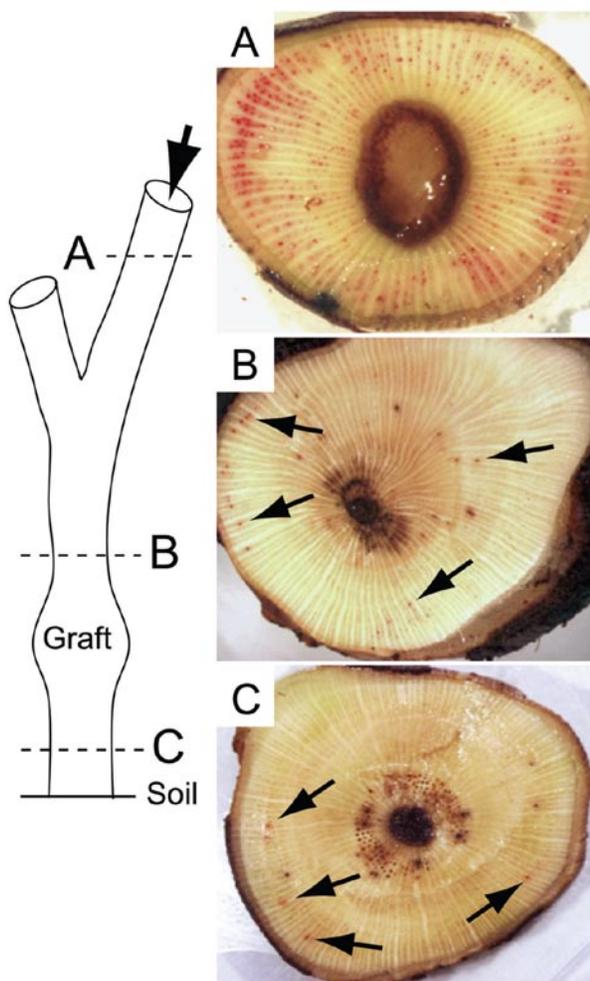**Figure 1** Canes loaded with latex paint (large black arrow) and sectioned at three locations: (**A**) ~10 cm from the graft union, (**B**) directly proximal to the graft union, and (**C**) directly distal the graft union (dotted lines). Red-stained vessels (small arrows) were observed in all sections, but never in the root system.

Figure 2 Paint staining (red traces) of xylem vessels across the graft union. The number of paint-stained vessels near the loading site (large arrow) diminishes as they extend through the graft. Small arrows indicate vessel endings. Scale bar equals 2.5 cm.

both the paint and bacteria particles would have, provided there was some orientation of the rod-shaped bacteria. Although the pore size in pit membranes of some species may be large enough to allow Xf movement (Siau 1984, McElrone et al. 2008), the size-exclusion limit in grapevine is ~5 nm (Labavitch et al. 2004). Rapid movement of air, paint, and Xf across the graft union should not be possible if vessel endings and pit membranes were in the pathway. Therefore, open conduits without vessel endings and pit membranes in the flow path were present through the graft union in young, grafted grapevines.

Freedom of Xf movement across the graft mirrors previous findings for particle movement in other species (e.g., McElrone et al. 2008). In one study, an Xf-sized animal bacterium that is unable to digest intervessel pit membranes, *Yersinia enterocolitica*, was used to demonstrate passive movement from the stem into leaves several nodes distal to the loading point (Thorne et al. 2006). Identical results were shown with a strain of Xf that had been modified to constitutively express green fluorescent protein (Chatelet et al. 2006). *Xylella fastidiosa* has also been shown to move across grafts in other species (He et al. 2000, Sanderlin and Melanson 2006), but a recent attempt to study Xf transmission across naturally occurring root grafts in *Vitis* was inconclusive due to a complete absence of the formation of natural root grafts (Krell et al. 2007). Interestingly, neither air nor paint was ever found to move into the root system, demonstrating the absence of open conduits extending through the union and into differentiated (branched) roots. This observation suggests that although continuous open conduits differentiate in stems even in the presence of complete severing of the stem, continuous open conduits may not develop from stem to roots. However, there is a study in which bacteria readily moved from root to shoot in *Vitis vinifera* (Compant et al. 2005). It is important to note that these experiments were conducted with vines that were in their second growing season after grafting. Older vines may better support movement into roots which become a reservoir for overwintering.

We hypothesize that, in *Vitis*, there are relatively widespread pathways that support the passive, systemic movement of vascular pathogens. However, we do not know how much movement occurs without active degeneration of pit membranes. Although it is generally assumed that the breaching of pit membranes is required for systemic infection and Pierce's disease, the kinetics of that process have not been studied and compared to passive movement.

Conclusion

There was rapid and extensive integration of vascular networks across the graft union resulting in open xylem conduits that supported passive movement of *Xylella fastidiosa* across the graft union of grafted grapevines in their second growing season. These conduits may be comprised of more than one vessel, but the nature of the open connection between vessels is unclear. The open conduits did not extend into the roots, indicating that Xf would have to pass at least one pit membrane in order to reach differentiated roots.

Literature Cited

Basile, B., J. Marsal, L.I. Solar, M.T. Tyree, D.R. Bryla, and T.M. DeJong. 2003. Hydraulic conductance of peach trees grafted on rootstocks with differing size-controlling potentials. J. Hortic. Sci. Biotech. 78:768-774.

Bavaresco, L., and C. Lovisolo. 2000. Effect of grafting on grapevine chlorosis and hydraulic conductivity. Vitis 39:89-92.

Chatelet, D.S., M.A. Matthews, and T.L. Rost. 2006. Xylem structure and connectivity in grapevine (*Vitis vinifera*) shoots provides a passive mechanism for the spread of bacteria in grape plants. Ann. Bot. 98:483-494.

Clearwater, M.J., R.G. Lowe, B.J. Hofstee, C. Barclay, A.J. Mandemaker, and P. Blattmann. 2004. Hydraulic conductance and rootstock effects in grafted vines of kiwifruit. J. Exp. Bot. 55:1371-1382.

Cohen, S., A. Naor, J. Bennink, A. Grava, and M. Tyree. 2007. Hydraulic resistance components of mature apple trees on rootstocks of different vigours. J. Exp. Bot. 58:4213-4224.

Compant, S., B. Reiter, A. Sessitsch, J. Nowak, C. Clement, and E.A. Barka. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl. Environ. Microbiol. 71:1685-1693.

Coombe, B. 1999. Grafting. *In* The Oxford Companion to Wine. 2d ed. J. Robinson (ed.). Oxford University Press, New York.
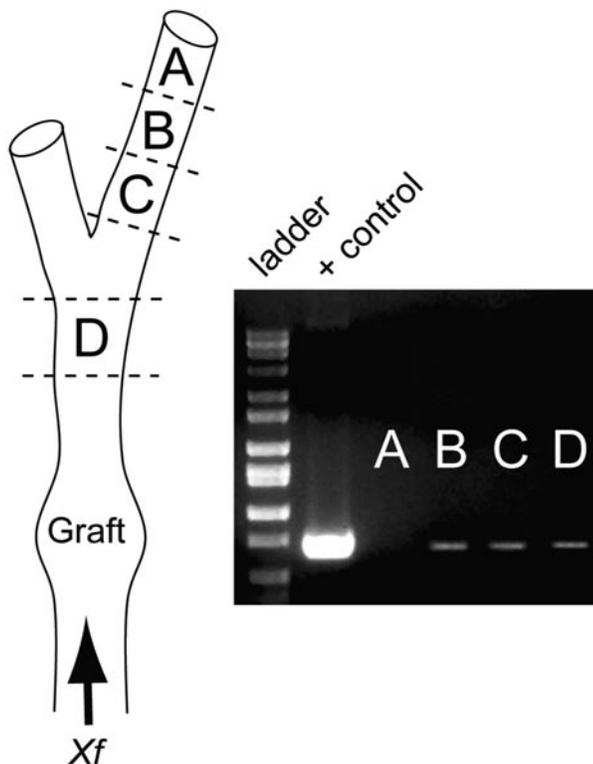
Figure 3 *Xylella fastidiosa* movement across the graft union. Xf was allowed to be taken up and across the graft via transpiration (black arrow). Xylem sap from 10-cm cane sections (A, B, C, and D) was assayed for Xf presence by PCR. Results of a single plant visualized via agarose gel electrophoresis are shown; lanes are labeled according to corresponding cane sections.

Copes, D. 1969. Graft union formation in Douglas-fir. Am. J. Bot. 56:285-289.

Davis, M.J., A.H. Purcell, and S.V. Thomson. 1978. Pierce's disease of grapevines: Isolation of the causal bacterium. Science 199:75-77.

Davis, M.J., R.F. Whitcomb, and A.G. Gillaspie Jr. 1981. Fastidious bacteria of plant vascular tissue and invertebrates (including so-called rickettsia-like bacteria). *In* The Prokaryotes: A Handbook on Habits, Isolation, and Identification of Bacteria. M.P. Starr et al. (eds.), pp. 2172-2188. Springer-Verlag, Heidelberg.

Epstein, A.H. 1978. Root graft transmission of tree pathogens. Ann. Rev. Phytopathol. 16:181-192.

Evert, R.F., K. Esau, and S.E. Eichhorn. 2006. Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. 3d ed. Wiley & Sons, Hoboken, N.J.

Ewers, F.W., J.B. Fisher, and S.T. Chiu. 1990. A survey of vessel dimensions in stems of tropical Lianas and other growth forms. Oecologia 84:544-552.

Feil, H., and A.H. Purcell. 2001. Temperature dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. Plant Dis. 85:1230-1234.

Gambetta, G.A., J. Fei, T.L. Rost, and M.A. Matthews. 2007. Leaf scorch symptoms are not correlated with bacterial populations during Pierce's disease. J. Exp. Bot. 58:4037-4046.

Gasco, A., A. Nardini, E. Raimondo, E. Gortan, A. Motisi, M.A. Gullo, and S. Salleo. 2007. Hydraulic kinetics of the graft union in different *Olea europaea* L. scion/rootstock combinations. Environ. Exp. Bot. 60:245-250.

He, C.X., W.B. Li, A.J. Ayres, J.S. Hartung, V.S. Miranda, and D.C. Teixeira. 2000. Distribution of *Xylella fastidiosa* in citrus rootstocks and transmission of citrus variegated chlorosis between sweet orange plants through natural root grafts. Plant Dis. 84:622-626.

Kollmann, R., and C. Glockmann. 1985. Studies on graft unions. 1. Plasmodesmata between cells of plants belonging to different unrelated taxa. Protoplasma 124:224-235.

Kollmann, R., S. Yang, and C. Glockmann. 1985. Studies on graft unions. 2. Continuous and half plasmodesmata in different regions of the graft interface. Protoplasma 126:19-29.

Krell, R.K., E.A. Boyd, J.E. Nay, Y.L. Park, and T.M. Perring. 2007. Mechanical and insect transmission of *Xylella fastidiosa* to *Vitis vinifera*. Am. J. Enol. Vitic. 58:211-216.

Labavitch, J.M., M.A. Matthews, and L.C. Greve. 2004. Linking the model of the development of Pierce's disease in grapevines to an understanding of the dynamics of glassy-winged sharpshooter transmission of *Xylella fastidiosa* to grapevines and grapevine gene expression markers of Pierce's disease. *In* Pierce's Disease Research Symposium Proceedings, pp. 15-18. California Department of Food and Agriculture, San Diego.

McElrone, A.J., S. Jackson, and P. Habdas. 2008. Hydraulic disruption and passive migration by a bacterial pathogen in oak tree xylem. J. Exp. Bot. 59:2649-2657.

Minsavage, G.V., C.M. Thompson, D.L. Hopkins, R.M.V.B.C. Leite, and R.E. Stall. 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84:456-461.

Purcell, A.H., and S.R. Saunders. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant Dis. 83:825-830.

Sanderlin, R.S., and R.A. Melanson. 2006. Transmission of *Xylella fastidiosa* through pecan rootstock. HortScience 41:1455-1456.

Schultz, H.R., and M.A. Matthews. 1993. Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): Evidence that low light uncouples water transport capacity from leaf area. Planta 190:393-406.

Siau, J.F. 1984. Transport Processes in Wood. Springer-Verlag, Berlin.

Solari, L.I., and T.M. DeJong. 2006. The effect of root pressurization on water relations, shoot growth, and leaf gas exchange of peach (*Prunus persica*) trees on rootstocks with differing growth potential and hydraulic conductance. J. Exp. Bot. 57:1981-1989.

Sperry, J.S., N.M. Holbrook, M.H. Zimmermann, and M.T. Tyree. 1987. Spring filling of xylem vessels in wild grapevine. Plant Physiol. 83:414-417.

Stevenson, J.F., M.A. Matthews, and T.L. Rost. 2004. Grapevine susceptibility to Pierce's disease I: Relevance of hydraulic architecture. Am. J. Enol. Vitic. 55:228-237.

Stoddard, F.L., and M.E. McCully. 1980. Effects of excision of stock and scion organs on the formation of the graft union in coleus: A histological study. Bot. Gaz. 141:401-412.

Sun, Q., T.L. Rost, and M.A. Matthews. 2006. Pruning-induced tylose development in stems of current-year shoots of *Vitis vinifera* (Vitaceae). Am. J. Bot. 93:1567-1576.

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. Am. J. Bot. 93:497-504.

Turquois, N., and M. Malone. 1996. Non-destructive assessment of developing hydraulic connections in the graft union of tomato. J. Exp. Bot. 47:701-707.

Weatherhead, I., and J.R. Barnett. 1986. Development and structure of unusual xylem elements during graft union formation in *Picea sitchensis* L. Ann. Bot. 57:593-598.

Zimmermann, M.H. 1983. Xylem Structure and the Ascent of Sap. Springer, Berlin.

Zimmermann, M.H., and A.A. Jeje. 1981. Vessel-length distribution in stems of some American woody plants. Can. J. Bot. 59:1882-1892.