DYNAMICS OF FREEZE–THAW EMBOLISM IN
SMILAX ROTUNDIFOLIA (SMILACACEAE)¹

ALEXANDER R. COBB,²,⁴ BRENDAN CHOAT,³ AND N. MICHELE HOLBROOK²

²Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138 USA; and
³Department of Viticulture & Enology, University of California, One Shields Avenue, Davis, California 95616 USA

Freeze–thaw cycles pose a major physiological challenge for all temperate perennial plants, but monocotyledonous vines face a still greater risk because their few large vessels are especially susceptible to embolism and are not replaced by secondary growth. The genus Smilax is particularly remarkable because it is widespread in the tropics but includes species that survive the hard frosts of New England winters. Smilax rotundifolia was monitored for a year for evidence of stem xylem freeze–thaw cavitation and refilling. Embolism of metaxylem was complete by late November and was completely reversed by late April, when root pressures rose as high as 100 kPa. Protoxylem remained full of sap throughout the year in cryogenic scanning electron micrographs. Three methods were used to quantify embolism: percent loss conductivity (PLC), gravimetric air fraction (GAF: mass of water in stem xylem relative to capacity), and cryogenic scanning electron microscopy (cryo-SEM). The three methods corroborated one another well and gave quantitatively similar results. Osmolality of xylem sap extracted from exuding stems was 64 mol/kg (±7.0, N = 8), consistent with the root pressures observed. Strong root pressure can account for Smilax’s survival in temperate regions with severe frosts, where few monocots with persistent aboveground organs are found.

Key words: Bedford, Massachusetts; cryo-SEM; embolism reversal; gravimetric air fraction; percent loss conductivity; root pressure; Smilacaceae; vine.

Vascular plants cannot survive freezing unless they avoid or overcome the xylem-obstructing bubble formation, or embolism, caused by freezing of xylem sap. Freeze–thaw embolism is a significant enough physiological challenge to constrain the distributions of some plants (Tyree and Cochard, 1996; Langan et al., 1997; Pockman and Sperry, 1997), lianas in particular (Ewers, 1985; Sperry and Sullivan, 1992). Monocotyledonous vines are doubly challenged by freezing because they often have large xylem vessels in the stem, which are highly susceptible to freeze–thaw embolism (Sperry and Sullivan, 1992; Davis et al., 1999; Feild and Brodribb, 2001; Pittermann and Sperry, 2003), and they lack a vascular cambium to replace these vessels. Vines from the genus Smilax, for example, often have vessels 100 μm or more in diameter, far above the 30–45 μm transition from low to high susceptibility to freeze–thaw cavitation in a range of North American plants (Davis et al., 1999; Pittermann and Sperry, 2003). The distributional range of Smilax nonetheless extends far into frost zones around the world (Sakai and Larcher, 1987; Heywood, 1993). We examined seasonal changes in the extent of embolism in the xylem of Smilax rotundifolia L. (Smilacaceae), a rhizomatous, winter-deciduous tendrill-climbing vine, to ascertain its strategy for coping with freeze–thaw embolism. We predicted that S. rotundifolia’s large (40–90 μm diameter) vessels would suffer extensive embolism during the many freezes and thaws of the New England winter but would be repaired in the spring by a local or systemic pressurization of the xylem.

Freeze–thaw cavitation occurs when dissolved gas expelled from sap during freezing expands to fill a xylem conduit when the sap thaws while under hydrostatic tension (Zimmermann, 1983). Liquid xylem sap generally contains dissolved gases in equilibrium with their concentrations in the atmosphere. The solubility of gases in ice is very low, however, and when sap freezes naturally, dissolved gas is by and large excluded from the crystal structure of the ice and accumulates in bubbles (Davis et al., 1999; Sperry and Robson, 2001; Pittermann and Sperry, 2006). In smaller vessels, gas bubbles are smaller and may collapse due to surface tension, but bubbles in larger vessels tend to persist and obstruct the xylem (Davis et al., 1999 and references therein; Feild and Brodribb, 2001; Pittermann and Sperry, 2003). Vines generally have larger vessels than free-standing plants, and the greater risk of freeze–thaw embolism associated with large vessels may help to explain the paucity of woody vines at higher latitudes (Ewers et al., 1990; Sperry and Sullivan, 1992).

In some ring-porous trees, conductance decreases drastically in the winter from freeze–thaw cavitation, but growth of a highly conductive new ring of xylem in the spring can support all or most of the transpirational needs of the plant with little need for a repair mechanism (Cochard and Tyree, 1990; Cochard et al., 1997; Davis et al., 1999; Cochard et al., 2001). The temperate vine Celastrus orbiculatus thrives without significant root pressure, overcoming loss of conductance with secondary growth (Tibbetts and Ewers, 2000). In contrast, Smilax has no vascular cambium and large vessels and must have some way of clearing gas from the xylem during the spring in order to support transpiration by the new year’s foliage. This might occur by root pressure, in which the xylem is pressurized from the roots by what appears to be reverse osmosis (Kramer, 1983; Kramer and Boyer, 1995) as occurs in the vine Vitis vinifera (Sperry et al., 1987), some monocotyledons (e.g., sugar cane, the bamboo Rhizocladium racem-
florum, and rice; Meinzer et al., 1992; Cochard et al., 1994; Stiller et al., 2003) and several temperate trees such as Betula cordifolia and Acer saccharum (Sperry et al., 1988b; Sperry, 1993), or by some other mechanism, probably involving a more local generation of xylem pressure (reviewed in Clearwater and Goldstein, 2005).

Numerous authors have argued that wide-vesseled temperate lianas could rely on root pressure to recover from winter embolism (Sperry et al., 1987, 1988b; Ewers et al., 1997; Fisher et al., 1997). Reports on root pressure in Smilax species have been equivocal, however. Ewers et al. (1997) found no root pressure in S. panameensis in a survey of vines in Panama, even in the wet season. Fisher et al. (1997) noted exudation in S. aristolochiaefolia and S. auriculata in Mexico and southern Florida, respectively, but measured pressures no greater than 2 kPa in S. auriculata, even though measurements were made in the rainy season. Studies of a range of other species outside the genus Smilax have sometimes found a root pressure inadequate to produce positive pressure in the upper canopy (Ewers et al., 1997) or have noted that the concentration of solutes in root sap gave an osmotic potential too small to generate positive pressure (Kramer and Boyer, 1995; McCully et al., 1998; McCully, 1999; Clearwater and Goldstein, 2005); the significance of these small root pressures is unclear.

We used three techniques to determine the extent of winter embolism and reversal in S. rotundifolia: percent loss conductivity (PLC), cryogenic scanning electron microscopy (cryo-SEM), and gravimetric air fraction (GAF). Percent loss conductivity (PLC) is the most established measure of embolism; it quantifies how much the occlusion of the xylem by air increases the resistance of an organ (Sperry et al., 1988b). Cryogenic SEM has generally been used to evaluate the extent of embolism qualitatively (Utsumi et al., 1996), or quantitatively by counting of empty conduits (Pate and Canny, 1999; Cochard et al., 2000; Melcher et al., 2001). Gravimetric air fraction (GAF) takes the relative difference between the mass of xylem sap in a stem at collection and its mass when all conduits are full. Pate and Canny (1999) employed a similar method to quantify the extent of embolism in roots, measuring the volume of sap extracted with a vacuum pump relative to an estimated xylem volume.

Though they measure slightly different things, PLC, cryo-SEM, and GAF all quantify the extent to which the xylem is filled with gas rather than sap and can be expected to provide similar results on average depending on how much vessel diameters vary and the extent to which freeze–thaw embolism is biased toward larger conduits. Melcher et al. (2001) found reasonable agreement between PLC and cryo-SEM, and Pate and Canny (1999) found a good correlation between PLC and cryo-SEM. Améglio et al. (2002) found a negative correlation between PLC and extracted sap volume per stem dry mass, a measure roughly proportional to 1 – GAF. In the Appendix we show, modeling the xylem as an array of pipes, that all three measures will give the same average result if vessels have the same diameter or if freeze–thaw embolism is unbiased with respect to conduit size.

We also monitored the pressure in the stem xylem of selected S. rotundifolia plants beginning in late February to determine whether positive pressures sufficient to reverse the observed embolism could be detected at the base of the stem. In addition, we sampled spring xylem sap and measured its solute concentration to determine the range of soil water potentials that would allow positive pressure in the xylem from root pressure.

**Materials and Methods**

Each month, samples of Smilax rotundifolia were collected from Concord Field Station (Bedford, Massachusetts, USA). Because leaves generally fall off during winter, we estimated the typical number of internodes produced in the 2003 growing season by the mean number of leaf-bearing nodes in October 2003, or about 15 internodes. The year’s growth, (i.e., the most apical 15 internodes) was used for cryo-SEM, GAF, and PLC sampling. In all cases, samples were collected before dawn to minimize the tension in any liquid xylem sap.

**PLC and GAF**—At each sampling time, the most apical 15 internodes of 10 stems were collected by cutting stems with pruning shears in air. Preliminary experiments showed that the longest vessels in S. rotundifolia stem segments almost always passed through exactly one node (B. Chvat and A. Cobb, unpublished data); therefore, we were confident that the 11 apical internodes used for GAF and PLC measurements were unaffected by cavitation induced by cutting. Collections were made shortly after dawn, even during winter, to ensure that stem tensions were low, and any remaining leaves were cut off before sampling to minimize tension in the xylem sap. Samples were immediately double-bagged with large plastic bags containing damp paper towels to prevent desiccation during transport.

For measurements of PLC, stem segments were cut underwater in the laboratory just apical to the fifth node, then cut again under water just basal of the sixth node and just apical to the seventh. The resulting sample from the internode between nodes six and seven was then cut back on both ends to a length of three to six centimeters. The ends of this segment were trimmed with a scalpel and the segment attached to the flow meter under water. Hydraulic conductance was then calculated by measuring the rate of flow of a 10 mmol KCl solution onto an electronic balance, while the pressure difference across the segment was measured with a differential pressure transducer (PX26-DV, Omega Engineering, Stamford, Connecticut, USA); the conductance was then calculated as flow rate divided by pressure difference. Pressure difference was measured with a transducer to avoid the errors that can arise from measuring the height of short water columns: hydraulic head for measurements needed to be kept below 15 cm to avoid displacement of air-water interfaces in the largest vessels (less than 150 μm, corresponding to a pressure difference of 1.9 kPa through Laplace’s law). After the initial measurement of hydraulic conductance (Kn), segments were flushed with solution using a syringe for 20–30 s at 100 kPa, measured using an analog pressure gage.

Anatomical study of S. rotundifolia showed that vessel endings occur at nodes (B. Chvat and A. Cobb, unpublished data). Therefore, the segments used for measuring PLC and the subsegments used for GAF contained few vessel endings. This made flushing the stems very easy, because gas was simply pushed out of the xylem (Sperry et al., 1987). PLC measurements therefore indicate the relative conductance of stem internodes, or of vessel lumens. Raw conductances of stem segments are not reported here because the conductances we measured did not include vessel ends and therefore would not be representative of whole stems.

Samples for GAF consisted of more basal portions of the same stems used for PLC. After the sample for PLC had been removed, the stem was cut again, in air, just basal to node 7 and just apical to node 11. The resulting segment was then cut into 5 cm internodal subsegments to facilitate flushing and weighing. These subsegments were quickly dried with a paper towel and then weighed (native mass). Each subsegment was then flushed with 2–5 mL of distilled water at 100 kPa until no more bubbles could be seen to emerge from the subsegment and dried with a paper towel for weighing (water flush mass). Finally, each subsegment was flushed with air at 200–275 kPa (laboratory’s compressed air supply, measured with analog pressure gage) for 5–10 s until no water could be seen to emerge, then weighed again (air-flush mass). GAF was then calculated as

$$GAF = \frac{M_n - M_a}{M_n - M_e}$$

where $M_n$ represents the native mass of the segment and $M_a$ and $M_e$ its masses after flushing with water and with air, respectively.

Agreement between GAF and PLC was evaluated by measuring the $t$...
statistic for the difference in means between the two values by month (Sokal and Rohlf, 1995). The correlation coefficient was not used because the two values can be expected to be different individually but the same on average when cationation is unbiased with respect to conduit diameter (see Appendix).

Cryo-SEM—Cryo-SEM samples were collected concurrently with samples for PLC and GAF measurements. Because some researchers have warned of artifactual cavitation in stems when they are frozen under tension (Cochard et al., 2000), care was taken to sample at dawn, when tension in any unfrozen xylem sap was minimal. Sections of internode between the sixth and seventh nodes were frozen with liquid nitrogen while still attached to the plant with either copper-plated pliers cooled in liquid nitrogen or by dunking into a cup of liquid nitrogen. The internode was held in the pliers or submerged in liquid nitrogen for 15 s before the segment was cut from the plant with pruning shears. Samples were stored in liquid nitrogen before preparation for cryo-SEM. Samples were mounted on aluminum stubs with TissueTek (Bayer, Pittsburgh, Pennsylvania, USA) and planed with a glass knife at ~140°C on an ultramicrotome with a cryogenic bath attachment (Leica, Wetzlar, Germany). Pate and Canny (1999) reported some difficulty in imaging whole cross-sections of Xanthorrhoea root steles due to their toughness; however, in S. rotundifolia stems vessels are all in the stem interior, and minor cracking at the stem periphery did not remove any functionally significant xylem.

After planing, mounted samples were transferred under liquid nitrogen to an SEM (FEI, Hillsboro, Oregon, USA) equipped with a cold stage (Gatan, Pleasanton, California, USA). Samples were etched at ~10°C for 5–10 min before being withdrawn to the prep chamber and sputter-coated with gold. Samples were observed at ~160°C in the SEM and digital images taken for analysis. A month was chosen to represent each of the four seasonal periods (October, February, April, and July), and three samples were examined for each month. Counts of full and empty metaxylem vessels were made on each image to assess embolism in the stem in each seasonal period. Counts of protoxylem vessels were made as the percentage of vascular bundles in the stem that possessed one or more gas-filled protoxylem vessels.

Bench-drying—In addition to testing agreement between GAF and PLC for freeze–thaw-induced embolism as described, we measured GAF and PLC in stems after bench-drying, because drought-induced embolism is a distinct process with a different bias with respect to conduit diameter (Sperry, 1995; Pittermann and Sperry, 2006). Following Sperry et al. (1988a), we constructed bench-drying vulnerability curves for Smilax rotundifolia by allowing 39 stems to dry out in the laboratory over the course of several days, periodically selecting stems to measure GAF and PLC as described earlier. Before each embolism measurement, the xylem sap tension was estimated by measuring covered-leaf water potential in a pressure bomb (PMS Instrument, Albany, Oregon, USA) using the balancing pressure method (Scholander et al., 1965; Boyer, 1967). The procedure for collecting stems was the same as that used for PLC and GAF experiments, i.e., the 15 most apical internodes were used (about 1 m). The protocol for GAF measurement was varied, in some cases flushing first with water and in other cases flushing first with air, to observe the effect of order of flushing on the measurement.

Root pressure—In the spring of 2004 and 2005, the hydrostatic pressures at the cut ends of nine S. rotundifolia stems were measured with pressure transducers (PX26-005GV, Omega Engineering, Stamford, Connecticut, USA), while soil temperature was monitored with a thermocouple probe (TMX88-125U-12, Omega Engineering). To control for passive changes in pressure from freezing and other temperature effects, a 10th transducer was connected to a 10th transducer (PX26-005GV, Omega Engineering, Stamford, Connecticut, USA), while soil temperature was monitored with a thermocouple probe (TMX88-125U-12, Omega Engineering). To control for passive changes in pressure from freezing and other temperature effects, a 10th transducer was connected to a sealed, water-filled piece of silicon tubing of the same size and kind as the pieces used to connect the transducers to the stems. Pressures and temperature were logged at 15-min intervals with a data logger (CR10X, Campbell Scientific, Logan, Utah, USA). Every 3–7 d, transducers were moved to fresh stems to avoid problems with gradual clogging of xylem (see Améglio et al., 2001; Cochard et al., 2001).

Sap content—In May 2005, sap from 8–10 one-internode segments of eight stems was pushed into Eppendorf tubes with a 60 ml syringe and returned to the laboratory for analysis. Samples were collected early on a wet morning to ensure that the soil was water-saturated and the xylem sap near equilibrium with soil water. Osmolality of sap in each sample was then determined with a vapor pressure osmometer (Vapro 5520, Wescor, Logan, Utah, USA). The osmotic potential of the sap was calculated using the Van’t Hoff equation:

\[ \Psi_s = -cR T \rho, \]

where \( \Psi_s \) is the osmotic potential, \( R \) the gas constant, \( T \) the Kelvin temperature, \( c \) the osmolality of the xylem sap, and \( \rho \) the density of water (Nobel, 1999). The solutes’ depression of the sap’s freezing point was calculated as

\[ \Delta T_f = K_s m, \]

where \( \Delta T_f \) is the freezing point depression in kelvins, \( K_s \) the molal freezing point depression constant for water (1.86 K kg/mol), and \( m \) the osmolality of the sap.

RESULTS

PLC and GAF—Both PLC and GAF methods showed moderate embolism of about 35% in late October, after a few mild freezes. For the remainder of the winter, the stem xylem was nearly empty of water, according to both methods. In the first measurement after the last freeze (late April 2003; see Fig. 1), both methods showed that the xylem had more or less completely refilled. Very little embolism was apparent from this date through the time of the last measurement, in late October. PLC readings from mid-summer are not shown: stem xylem seemed to become occluded during flushing in these months, so that after the flush the conductance had dropped by several orders of magnitude. This did not occur during the fall or the winter. GAF measurements were affected too, as can be seen by the negative averages from early June through late July (Fig. 1), but the effect was not as severe.

Mean GAF and PLC results agreed well (Fig. 1). Both methods showed a fair amount of scatter in individual measurements, but the differences between their averages were insignificant (\( P = 0.20 \) to 0.78; \( N = 5–10 \) for both PLC and GAF; averages: PLC: 8.6, GAF: 8.9) for all sampling times except early March, when average PLC was significantly higher than average GAF (\( P = 0.008; \) PLC: \( N = 9 \), GAF: \( N = 8 \)).
root pressure as early as the end of March, when we first and protoxylem vessels were filled with sap, but again sap concentration (Figs. 6, 8). In the spring (April), all metaxylem vessels were gas-filled, but the protoxylem vessels contained sap. However, the sap contained a much greater concentration of solutes, apparent by the patterning of electron-emissive solutes excluded from the micrographs, while living cell sap is lighter because of the liquid during freezing. The dark ice in metaxylem and protoxylem of samples collected in summer (July) showed some rehydration of dry tissue would occur during the water flush). The complete embolism of metaxylem vessels was consistent with the extensive winter embolism observed in other temperate vines (Sperry et al., 1987; Tibbetts and Ewers, 2000) and ring-porous trees with earlywood vessels of similar size (Cochard and Tyree, 1990; Sperry and Sullivan, 1992; Hacke and Sauter, 1996; Utsumi et al., 1996). Cryo-SEM showed that the protoxylem, in contrast, suffered very little embolism over the winter (only about 4%). Similarly, Utsumi et al. (1996) found that smaller latewood vessels remained sap-filled in *Fraxinus mandshurika* while large earlywood vessels became completely embolized. In contrast, embolism may accumulate gradually over the winter in species with more uniformly small conduits, such as conifers and diffuse-porous angiosperms (Sperry et al., 1988b; Cochard and Tyree, 1990; Borghetti et al., 1993; Utsumi et al., 1996; Cochard et al., 1997; Utsumi et al., 1998, 2003).

More empirical work is needed in a broader range of species installed the pressure transducers. Pressures registered by transducers were low but measurable through April, peaking at about 15 kPa (Fig. 15). Early May measurements showed substantially larger pressures, about 50 kPa—sufficient to generate atmospheric pressure at 5 m above ground level. Leaf flush began soon after the first measurements (early April) and was nearly complete by the time of the last measurements shown (late April). By the time of measurements in early June, we measured no positive pressures, and we observed no further exudation of cut stems through our final measurements in October.

**Sap content**—Sap extracted from stems had a mean osmolality of 6.44 \( \times 10^{-2} \) mol/kg (±7.0, 6). This osmolality gives an osmotic potential of 157 kPa by the Van’t Hoff equation (Eq. 2) and a freezing point depression of 0.12 K (Eq. 3).

**DISCUSSION**

All three methods used to assess the extent of embolism (PLC, GAF, and cryo-SEM) showed nearly complete embolism of *Smilax rotundifolia* xylem after the first few hard freezes and complete refilling after the appearance of root pressure in the spring. None of the methods suggested significant embolism in the summer, indicating that the studied plants completely avoided or reversed drought-induced embolism in the summer months. The three methods agreed quantitatively in most cases, although PLC measurements met with some difficulties in the summer, when flushes often led to dramatic increases in resistance, perhaps due to occlusion of the xylem by mucilage.

The complete embolism of metaxylem vessels was consistent with the extensive winter embolism observed in other temperate vines (Sperry et al., 1987; Tibbetts and Ewers, 2000) and ring-porous trees with earlywood vessels of similar size (Cochard and Tyree, 1990; Sperry and Sullivan, 1992; Hacke and Sauter, 1996; Utsumi et al., 1996). Cryo-SEM showed that the protoxylem, in contrast, suffered very little embolism over the winter (only about 4%). Similarly, Utsumi et al. (1996) found that smaller latewood vessels remained sap-filled in *Fraxinus mandshurika* while large earlywood vessels became completely embolized. In contrast, embolism may accumulate gradually over the winter in species with more uniformly small conduits, such as conifers and diffuse-porous angiosperms (Sperry et al., 1988b; Cochard and Tyree, 1990; Borghetti et al., 1993; Utsumi et al., 1996; Cochard et al., 1997; Utsumi et al., 1998, 2003).

More empirical work is needed in a broader range of species

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**Table 1. Percentage of gas-filled xylem conduits for four seasonal periods based on cryo-SEM observations in *Smilax rotundifolia* at Concord Field Station in Bedford, Massachusetts, USA.**

<table>
<thead>
<tr>
<th>Month</th>
<th>Metaxylem</th>
<th>Protoxylem</th>
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<tbody>
<tr>
<td>October</td>
<td>32.7 (±17.0)</td>
<td>0.0 (±0.0)</td>
</tr>
<tr>
<td>February</td>
<td>99.5 (±0.5)</td>
<td>4.4 (±3.0)</td>
</tr>
<tr>
<td>April</td>
<td>0.0 (±0.0)</td>
<td>0.0 (±0.0)</td>
</tr>
<tr>
<td>July</td>
<td>0.0 (±0.0)</td>
<td>0.0 (±0.0)</td>
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**Notes:** Values are means of three samples for each seasonal period with SE in parentheses.

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**Fig. 2. Vulnerability to xylem embolism by bench drying in *Smilax rotundifolia*.** Each point represents a measurement on a stem allowed to dry in the laboratory after pre-dawn collection in Bedford, Massachusetts, USA. Water potential is mean balancing pressure of two to three attached leaves with a pressure bomb. Up triangles: Gravimetric air fraction (GAF) with air flush after native weighing (expected to overestimate embolism when xylem was full of air, because complete removal of air was difficult); down triangles: GAF with water flush after native weighing (expected to underestimate embolism when xylem was full of air, because some rehydration of dry tissue would occur during the water flush). \( N = 38 \) for percent loss conductivity (PLC), \( N = 24 \) for GAF (air first), \( N = 15 \) for GAF (water first). Curves were fit using the Levenberg–Marquardt algorithm to adjust parameters \( a \) and \( b \) in the equation \( y = 1/2 + 1/2 \text{erf}(\Psi - b/a) \), where erf is the Gauss error function.

**Bench-drying**—PLC and GAF measurements from the bench-drying experiment showed similar correspondence to that observed in native freeze–thaw embolism measurements. The resulting vulnerability curves were similar for the different methods (Fig. 2). In GAF measurements, though flushing with water first gave better results in more hydrated stems, and flushing with air first gave better results in drier stems, either method generated a similar overall curve.

**Cryo-SEM**—Cryo-SEM sections showed seasonal patterns of embolism matching the results of PLC and GAF (Table 1, Figs. 3, 4, 9, 10). Dilute sap appears dark in cryo-electron micrographs, while living cell sap is lighter because of the patterning of electron-emissive solutes excluded from the liquid during freezing. The dark ice in metaxylem and protoxylem of samples collected in summer (July) showed both to be full of water with a low solute concentration (Figs. 12, 14). Preliminary examinations of samples from June and September showed that all vessels were full in these months also. In the fall (October), most metaxylem vessels and all protoxylem vessels contained sap. However, the sap contained a much greater concentration of solutes, apparent by the patterns of solute lines in the xylem sap of October samples compared with samples from July (Figs. 5, 7). In the winter (February), all metaxylem vessels were gas-filled, but the smaller protoxylem vessels were full of sap with a high solute concentration (Figs. 6, 8). In the spring (April), all metaxylem and protoxylem vessels were filled with sap, but again sap solute concentration was high (Figs. 11, 13).
Figs. 3–8. Representative cryogenic scanning electron micrographs of *Smilax rotundifolia* stems in fall and winter at three levels of detail. The variation in contrast between vessels and the surrounding tissue in different images results from differences in the level of etching; embolized vessels are empty and appear black. 3, 5, 7. Fall (October): most metaxylem (mx) vessels contain sap with high solute concentrations, indicated by electron-emissive lines of solutes. 4, 6, 8. Winter (February): metaxylem is fully embolized (gas-filled), while protoxylem (px) is full of sap with a high concentration of solutes.
Figs. 9–14. Cryogenic scanning electron micrographs of *Smilax rotundifolia* stems in spring and summer at three levels of detail. 9, 11, 13. Spring (May): metaxylem (mx) has been completely refilled; metaxylem and protoxylem (px) alike contain solute-rich sap. 10, 12, 14. Summer (July): metaxylem and protoxylem are still full of sap, but now its dark color and smooth texture indicate a low solute concentration.
to assess the value of GAF as a companion or alternative technique to PLC. However, in this study, GAF, PLC, and cryo-SEM corroborated one another. Agreement between GAF and PLC was much better than the worst-case agreement that would result from a deterministic bias toward larger conduits in freeze–thaw embolism, that is, if wider vessels always cavitated before narrower ones. Stems with larger average conduit diameters are consistently more susceptible to freeze–thaw cavitation, but because ice nucleation is intrinsically probabilistic (Sakai and Larcher, 1987; Debenedetti, 1996), chance may play a major role in determining where embolism first occurs within a particular plant stem, how water is redistributed within the stem during freezing, and where bubbles are sufficiently large that they persist after a thaw. For example, the gradually sloping histogram of cavitated vessels in Betula occidentalis as measured by Sperry and Sullivan (1992) suggests that occasionally, though improbably, a smaller conduit will become embolized while larger ones remain sap-filled. In general the stochastic element of freeze–thaw cavitation should favor agreement between GAF and PLC (see Appendix). Differences between average GAF and average PLC may be still smaller under drought-induced cavitation because it has been difficult to detect much conductivity of the segment, if cavitation of vessels is random there will be no difference between average GAF and PLC (see Appendix). In addition, GAF seems to be less susceptible to some of the sources of error associated with PLC. Small bubbles introduced during the cutting of segments for PLC can occlude pit membranes, whereas such bubbles have little effect on GAF measurements. In summer measurements, the xylem became obstructed during flushing, presumably by mucilage, often giving spurious PLC values. In winter months, great care needed to be taken with PLC measurements to avoid expelling air from the stem segment when connecting the flow apparatus.

However, PLC is the more physiologically interesting quantity, so GAF is valuable only to the extent that it can be shown to bear a consistent relationship to PLC; more study is certainly needed in a broader range of species before its use can be widely recommended.

Average GAF in the early March measurement may have been lower than average PLC (Fig. 1) because the stem portion used for GAF measurements was basal to the stem portion used for PLC measurements, and at that time the stem xylem was beginning to be refilled from the base of the plant by root pressure. Alternatively, it may be that smaller vessels refill before larger ones, causing GAF to initially increase faster than PLC.

Root pressures had reversed essentially all winter embolism by the time of leaf flush in late April. Work on temperate vines and trees with strong root pressure, such as Vitis, Betula, Alnus, Salix, and Juglans, has also shown full recovery of xylem conductance by root pressure before spring cambial growth and leaf expansion (Sperry et al., 1987, 1994; Utsumi et al., 1998; Améglio et al., 2002). However, Tibbetts and Ewers (2000) observed more limited spring recovery from complete winter embolism in the vines Vitis riparia (recovered to 30–35%) and Celastrus orbiculatus (recovered to 50%).

Root pressures were great enough to account for the spring reversal of embolism, often reaching 50 kPa during the day and occasionally increasing to 100 kPa during rains. These values are of the same magnitude as root pressures measured in other studies, both tropical and temperate, where typical pressures have generally been in the range of 15 to 50 kPa and highest recorded pressures around 150 kPa (Sperry et al., 1988b, 1994; Sperry, 1993; Steudle and Meshcheryakov, 1996; Ewers et al., 1997; Fisher et al., 1997; Tibbetts and Ewers, 2000; Améglio et al., 2001). The only considerably higher measurement of which we are aware is 500 kPa in Vitis reported by Scholander et al. (1955). Some workers have seen maximum root pressures during the day (Sperry et al., 1994; Kramer and Boyer, 1995), but in our experiments, as in some other studies (Ewers et al., 1997; Fisher et al., 1997), pressures increased overnight, peaking shortly after dawn (Fig. 15). The “tension” apparent thereafter (not a true tension, because shown values are gage pressures) was presumably because of transpiration from other stoma-covered stems connected to the measured stems by rhizomes.

The mean osmotic potential of extracted xylem sap, 157 kPa, sufficed to account for even the highest hydrostatic pressures measured with transducers. The appearance of xylem sap in cryo-SEM indicated that the highest solute concentrations occurred earlier in the spring before sap was collected, so the peak sap osmolarity may have been still higher. The xylem sap’s hydrostatic pressure \( p \) and osmotic potential \( \Psi_s \) at ground level are related to the soil water potential \( \Psi_r \) through the following equation when there is no transpiration:

\[
\Psi_s = p + \Psi_r.
\]
Because soil water is held in small capillary spaces, the soil water potential $\Psi_s$ is usually less than zero, like the sap osmotic potential $\Psi_o$. For the hydrostatic pressure $p$ to be positive, the soil water potential must be less negative than the sap’s osmotic potential. This may help to explain why New England’s *Smilax* species favor wet habitats (Gleason and Cronquist, 1991): a dry spring could prevent refilling and leaf flush in *Smilax* plants, regardless of their tolerance of dryness in the summer and fall. *Smilax rotundifolia* is usually found in wet and low-lying sites, even though its vulnerability curve suggests a relatively low susceptibility to drought-induced cavitation for a mesophyte (50% PLC at −3.0 MPa; Fig. 2) and it appears to entirely avoid embolism in summer (Fig. 1). The vines *Vitis riparia* and *Celastrus orbiculatus*, also in northeastern North America but with secondary growth, are considerably more vulnerable (Tibbetts and Ewers, 2000). Some other monocots with root pressure, such as rice and maize, also appear to be more vulnerable to drought-induced cavitation (Tyree et al., 1986; Stiller et al., 2003). Of other monocots studied to date, the bamboo *Rhipidocladum racemiflorum* is perhaps most similar in having root pressure but also tolerating quite large xylem tensions (50% conductivity loss only at −4.2 MPa; Cochard et al., 1994), though in that species root pressure repairs drought-rather than freeze–thaw-induced embolism.

Cryo-SEM images made in the fall (late November) show high concentrations of solutes in the protoxylem tracheids and the few full metaxylem vessels, though we never observed sap exudation at that time of year. It would be interesting to explore whether and how *Smilax* might benefit from loading solutes into xylem sap during fall. Though the presence of solutes in xylem sap probably does not reduce the probability sap will freeze, it may reduce the likelihood of freeze–thaw cavitation by elevating the hydrostatic pressure in the xylem sap. The measured sap osmolality would result in a trivial freezing point depression of 0.12 K, but the higher hydrostatic pressures caused by solutes in the xylem could help to ensure collapse of bubbles during thaws in vessels that otherwise would experience cavitation. Indeed, smaller hydrostatic tensions appear to reduce freeze–thaw cavitation in stems, whether at the time of freezing (Yang and Tyree, 1990; Tyree et al., 1994; Pittermann and Sperry, 2006) or thawing (Sperry and Sullivan, 1992; Davis et al., 1999).

As a temperate monocotyledonous vine, *Smilax rotundifolia* faces exceptional demands on its ability to cope with freeze–thaw embolism. Clearly, spring root pressure is the key to its survival. Interestingly, in contrast with some annual monocots such as *Zea mays* or *Oryza sativa*, *S. rotundifolia* followed the root pressure schedule of temperate trees and vines such as *Vitis riparia*, producing root pressure only in the spring, rather than daily (Tyree et al., 1986; Tibbetts and Ewers, 2000; Stiller et al., 2003). *Smilax rotundifolia* may not need root pressure during the growing season because of the low vulnerability of its xylem to drought-induced embolism. This contrasts with the aforementioned grasses, which seem to cavitate and repair on an almost daily, rather than seasonal basis (Tyree et al., 1986; Stiller et al., 2003). Future work might explore why certain species appear to have a perennially inductive root pressure mechanism, while in others such as *S. rotundifolia* root pressure seems to occur only seasonally.

**LITERATURE CITED**


McCully, M. E. 1999. Root xylem embolisms and refilling: relation to...


APPENDIX. Agreement among measures of embolism.

The extent of embolism in a stem can be quantified in several ways: by counting the proportion of vessels that are gas-filled (embolized vessel fraction, EVF), measuring the relative deficit in xylem sap mass (GAF), or measuring the relative deficit in xylem conductance (PLC). Here, we show that although the three measures must differ at some level of embolism in any particular stem with vessels of nonuniform radii, the averages of all three measures will be equal for any distribution of vessel diameters if embolism is random with respect to vessel radius.

The number of vessels $N$ in a particular stem segment from a *Smilax rotundifolia* internode can be modeled as an array of $N$ pipes with radii $R$. Embolized vessel fraction is the number of embolized vessels $n$ relative to the total $N$:

$$
EVF = n/N.
$$

Gravimetric air fraction is the deficit in xylem sap mass (Eq. 1 in Materials and Methods under PLC and GAF) and can be calculated by dividing the volume of empty vessels by the total xylem volume. After simplification, this gives:

$$
EVF = n/N.
$$


Wilcox, D. C. 1997. Basic fluid mechanics. DCW Industries, La Cañada, California, USA.


The expressions for the three measures of embolism in the above equations (5–7) all have a similar form: they consist of the sum of some function evaluated for every embolized vessel divided by the sum of that function evaluated for all vessels. Therefore, embolism fraction and conductivity for a stem segment with \( n \) embolized vessels is

\[
\text{GAF} = \frac{\sum_{i=1}^{n} R_i^4}{\sum_{i=1}^{n} R_i^4}
\]

(6)

where the radii \( R_1, R_2, \ldots, R_n \) correspond to the radii of the vessels that are embolized.

Lost conductivity is normally expressed as a percentage, but for simplicity of calculation and consistency with the other measures, here we use fraction lost conductivity \( \text{FLC} = \text{PLC}/100 \). Conductivity of a straight-walled pipe is proportional to the fourth power of its radius \( (\text{Wilcox, 1997}) \), and the conductivity of an array of such pipes is equal to the sum of their conductivities. Therefore, fraction lost conductivity for a stem segment with \( n \) embolized vessels is

\[
\text{FLC} = \frac{\sum_{i=1}^{n} R_i^4}{\sum_{i=1}^{n} R_i^4}
\]

(7)

where again the \( n \) lowest indices \( 1, 2, \ldots, n \) iterate over the embolized vessels.

The expressions for the three measures of embolism in the above equations (5–7) all have a similar form: they consist of the sum of some function \( f \) evaluated for every embolized vessel divided by the sum of that function evaluated for all vessels. Therefore, EVF, GAF, and FLC can all be considered as generic measures of embolism \( E \) varying only in the function \( f \) that is evaluated for each vessel:

\[
E = \frac{\sum_{i=1}^{N} f(R_i)}{\sum_{i=1}^{N} f(R_i)}
\]

(8)

where \( f(R) = R^4 \) for EVF, \( f(R) = R^2 \) for GAF, and \( f(R) = R^4 \) for PLC.

First we consider embolism in a particular stem and show that the three measures must differ at some intermediate level of embolism (i.e., xylem neither completely full of sap nor full of air) if vessel radii are not uniform. When \( \mu \) is defined as the mean of the function \( f \) applied to the radii of all vessels \( R_1, R_2, \ldots, R_N \), a measure of embolism \( E \) can also be expressed as

\[
E = \frac{\bar{E}}{N} = \frac{1}{N} \sum_{i=1}^{N} \delta_i
\]

(9)

where \( \delta_i \) is the normalized deviation of \( f(R_i) \) from the mean \( \mu \):

\[
\delta_i = \frac{1}{\mu} [f(R_i) - \mu]
\]

(10)

Note that \( \delta \) sums to 0 for all three measures, and that for EVF, \( \mu = 1 \) and \( \delta = 0 \) for all \( i \).

A fully conductive stem segment has no embolized vessels \( (n = 0) \); after multiple freeze–thaw cycles, the number of embolized vessels \( n \) increases toward the total number of vessels \( N \). Using Eq. 9, the difference between a measure of embolism \( E \) (GAF or FLC) and EVF is

\[
E - \text{EVF} = \frac{1}{N} \sum_{i=1}^{n} \delta_i
\]

(11)

where the standardized deviations \( \delta \) vanish for EVF because they are always zero.

Equation (11) allows several useful conclusions. First, GAF or PLC can only be equal to EVF if the sum of its normalized deviations is zero. This is necessarily true when \( n = 0 \) (no embolism) or \( n = N \) (complete embolism); it is possible for it to be true for intermediate values as well. However, if the sum of the deviations is 0 at some intermediate value \( n \), it can only be true at \( n + 1 \) if \( \delta_{n+1} = 0 \), that is, if the next embolized vessel has a radius \( r \) such that \( f(r) \) gives the mean value \( \mu \). It follows that all three measures can only be equal at all intermediate levels of embolism if \( \delta_n = 0 \) for all \( n = 1, 2, \ldots, N \), that is, if all vessels have the same radius.

Equation (11) also shows that generally the difference between EVF and GAF or EVF and FLC evolves differently in a particular stem segment as more and more vessels are embolized (increasing \( n \)) depending on the order in which the vessels embolize. If the sequence is random, on average the difference between the two is zero (because the deviations \( \delta \) have mean zero). The maximum mean discrepancy between the two occurs if \( R \) is consistently sorted, that is, if, for example, the progression of embolism always proceeds from the largest to the smallest vessel. In this case, given the first freeze–thaw cavitation event will add the largest positive deviation, i.e., \( \delta_1 \) will be the largest positive deviation, and the deviations contributing to the discrepancy \( E - \text{EVF} \) (11) will be added in order from the most positive deviation to the most negative deviation \( \delta_N \).

Given embolism that is not biased by vessel radius, EVF and GAF in fact are unbiased estimators of FLC—meaning that the average difference among these measures over multiple identical experiments converges to zero when the three measures are evaluated on the same set of stem segments, regardless of the distribution of levels of embolism among those stem segments. The argument is somewhat more complicated to present in detail and is not included here, but can be developed by considering that the moments of an unbiased sample are unbiased estimators of the corresponding population moments.

In reality, freeze–thaw embolism is known to be more likely in larger-diameter vessels. This will have the effect of encouraging “sorting” of the sequence of vessel embolism so that more positive deviations are likely to be added first. However, the results of the present study and others indicate that this bias in freeze–thaw embolism is not deterministic, meaning that there will still be a degree of randomness in the sequence of embolism, encouraging agreement among embolized vessel fraction, gravimetric air fraction, and fraction lost conductivity. Agreement might be poorer in stems with vessel diameter distributions spanning the 30–45 \( \mu \)m transition below which freeze–thaw embolism seems to be extremely rare.