



## Research paper

# Surface tension phenomena in the xylem sap of three diffuse porous temperate tree species

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In plant physiology models involving bubble nucleation, expansion or elimination, it is typically assumed that the surface tension of xylem sap is equal to that of pure water, though this has never been tested. In this study we collected xylem sap from branches of the tree species *Populus tremuloides*, *Betula papyrifera* and *Sorbus aucuparia* over 3 months. We measured the instantaneous surface tension and followed changes over a period of 0.5–5 h using the pendant drop technique. In all three species the instantaneous surface tension was equal to or within a few percent of that of pure water. Further, in *B. papyrifera* and *S. aucuparia* the change over time following drop establishment, although significant, was very small. In *P. tremuloides*, however, there was a steep decline in surface tension over time that leveled off towards values 21–27% lower than that of pure water. This indicated the presence of surfactants. The values were lower for thinner distal branch segments than for proximal ones closer to the trunk. In some species it appears valid to assume that the surface tension of xylem sap is equal to that of water. However, in branch segments of *P. tremuloides* close to the terminal bud and hence potentially in other species as well, it may be necessary to take into account the presence of surfactants that reduce the surface tension over time.

**Keywords:** pendant drop, surface tension, surfactants, temperate trees, xylem sap.

## Introduction

The surface tension of xylem sap is an important factor influencing a range of physiological events in plants. These include air seeding or winter embolism by which the vessels may become air filled and cease to function (Lewis 1988, Sperry and Tyree 1988, Pittermann and Sperry 2006) as well as potentially the refilling of the vessels. The mechanism of the latter is still poorly understood (Zwieniecki and Holbrook 2009). Numerous theoretical models exist predicting how these events occur, which factors may be of influence and how they affect the performance of the plant. These include models of air seeding (Sperry and Hacke 2004), bubble growth and embolism (Holttä et al. 2002, 2007), frost-induced embolism (Pittermann and Sperry 2006) and embolism repair (Konrad and Roth-Nebelsick 2003). In these, it is assumed that the

surface tension of xylem sap is equal to that of pure water. The validity of this assumption has, to our knowledge, never been tested even though it is well known that xylem sap is a complex solution. It contains numerous chemical species, including sugar species, amino acids, organic acids, polyamines, phenyl propanoids, flavonoids, alcohols, small inorganic ions and minerals (e.g. Andersen et al. 1995a, 1995b, Bialczyk and Lechowski 1995, Schill et al. 1996, Alvarez et al. 2008).

Solutes may influence the surface tension of the solution in two ways. Non-surface active molecules within the solution can increase the surface tension as in the case of inorganic salts and, to a lesser extent, sugars, or decrease the surface tension as in the case of alcohols. Their effect will be to instantly lower or increase the surface tension to a new constant value, and the magnitude of the effect will typically be

proportional to the concentration. Surface active molecules, on the other hand, decrease the surface tension over time after the establishment of the air–water interface until a minimum is reached. At this point the surface tension levels off towards a constant value. The amphiphilic surfactants reduce the surface tension of water by adsorbing at the liquid–gas interface. A stable state is approached when surfactant molecules move away from the surface at a rate similar to that at which molecules are adsorbed, and so the surface tension levels off towards a constant value.

In xylem sap, a number of non-surface active components known to change the surface tension of solutions have been shown to be present, including sugars and alcohols (Andersen et al. 1995b, Schill et al. 1996). These are typically present in concentrations so low that they are unlikely to influence the surface tension significantly or even measurably. Alcohols, for instance, are among the more potent non-surface active chemical species and yet 10% ethanol must be added to pure water to reduce the surface tension by ~33%. In plants alcohols are typically present in concentrations of only up to a few mM, and sugars influence surface tension much less. We thus hypothesized that any difference in the surface tension of xylem sap compared with that of water would most likely be caused by surfactants instead. A number of different surfactants have been found in plants, most notably sapogenins, which are found in a wide range of different plant species and used throughout the world as a soap replacement (Karrer et al. 1924, Zkova 1966). The presence of surfactants within the xylem sap itself has, to our knowledge, not been described for healthy trees.

In this study, we test the instantaneous surface tension as well as changes over time in xylem sap extracted from three different temperate diffuse porous trees: *Populus tremuloides*, *Betula papyrifera* and *Sorbus aucuparia*. *Populus tremuloides* and *B. papyrifera* are both small- to medium-sized trees native to the colder parts of North America, including the area of Edmonton, Canada, in which the branches were harvested. *Sorbus aucuparia* is native to most of Europe as well as Northern Asia, but has been widely planted as an ornamental tree across the world, including the Edmonton area where it now grows wild.

## Material and methods

### Branch material

Branches of *P. tremuloides*, *B. papyrifera* and *S. aucuparia* were harvested around the north campus of the University of Alberta, Edmonton, Canada, 53°32'N, 113°33'W. Edmonton has a dry continental climate with extreme seasonal temperatures (Koppen climate classification *Dfb*, USDA Plant Hardiness Zone 3a). Branches were harvested throughout the growing season, a period spanning from the beginning of June until the end of

August 2008. In the case of poplar, harvesting was planned so that dry as well as wet periods were represented. One branch was harvested from each of seven different *P. tremuloides* trees, three different *B. papyrifera* trees and two *S. aucuparia* trees. The branches were brought back to the lab for further treatment. If the time span from the cutting of the branch until submersion in water was expected to exceed 10 min, the cut ends were kept wet by wrapping them in soaked tissue paper and sealing them with plastic foil taped tightly to the branch end. Branch lengths varied from 0.4 to 1.6 m.

In the lab, branches were re-cut under water. A minimum of 10 cm was removed from the cut end to avoid embolisms in the xylem, which could reduce sap extraction efficiency, and the branch was subdivided into lengths of 30–60 cm. This was done so that side branching within the individual segments was avoided and so the branching pattern determined the lengths of the branch segments. Although uptake of pure water during re-cutting may have decreased the concentration of solutes in the xylem sap at the exposed ends, the effect is not likely to have been substantial. Tension had already been released in the initial cutting events, and the diameters of the vessels were very small compared with the lengths.

In five of the seven branches of *P. tremuloides*, the distance from the middle of the branch segment to the terminal bud of the branch was recorded immediately prior to cutting.

In the case of *P. tremuloides*, four additional branch segments were further subdivided into 10- to 17-cm-long sections, the sap of which was combined, in order to increase the cut surface area relative to the extracted sap volume. This was done to test whether the wounding of the branch at the time of cutting might influence the results obtained. This could occur either due to leakages from the damaged living cells at the surface of the cut or due to chemical species excreted into the xylem vessels by surrounding living cells upon wounding. In these cases cutting was always done immediately before the sap extraction to have the wounded surface as fresh as possible, and in order to maximize any wounding effects the ends were not cleaned thoroughly as in the standard procedure but only rinsed briefly. Pairs of neighboring branches of similar length and distance from the terminal bud were compared, one prepared by the usual routine and one sub-sectioned in the above-described way. The pairs were compared directly, and further the ratio between total cut area and volume extracted was compared with the surface tension values obtained for these as well as all previously studied branches.

### Sap extraction

The branch segments were re-cut with a razor blade and rinsed in pure water. One end was then connected to a 0.1% acid fuchsin solution at a pressure head of  $5.3 \times 10^{-3}$  MPa. Acid fuchsin is a strong dye that does not bind to the cell walls and so is carried with the sap through the xylem.

Sap was collected from the exposed ends in three vials. The first drop was discarded to further minimize the possibility of contamination and to remove sap potentially diluted with water. From the second drop onward, sap was collected in two collection vials that were alternately emptied into a separate common storage vial so that only the first extracted of the two drops was stored. The collection was continued until the first trace of dye penetration was observed. The existing sap in both collection vials was then discarded, and only the sap in the storage vial was used for further experiments. In this way, both the drop that showed the first signs of coloring and the drop before this were removed, eliminating any possibility of contamination with traces of acid fuchsin too dilute to be observed by the eye.

### Surface tension measurements

The surface tension was measured with the pendant drop apparatus described in Kang et al. (2008) and functionally similar to the setup described in detail by Andreas et al. (1938). Briefly, a drop of the solution was formed at the tip of a blunt end stainless-steel syringe needle held in place in an enclosed metal box. A computer-controlled syringe pump allowed for some control of the drop size and hence the surface area. Two windows in the box allowed for a movie to be taken of the drop over time as outlined against background illumination. The change in surface tension over time was calculated from drop shape analysis of the consecutive movie stills of the silhouettes of the hanging drop. The calculations were performed using FTA32 software (First Ten Angstroms) in which the gravity-distorted shape of the drop was fitted to the Young–Laplace equation and the interfacial tension was thus calculated. The volume and surface area of the drop were calculated simultaneously (Figure 1).

Unlike the experiment of Andreas et al. (1938), there was no control of the temperature inside the box during our experiment,

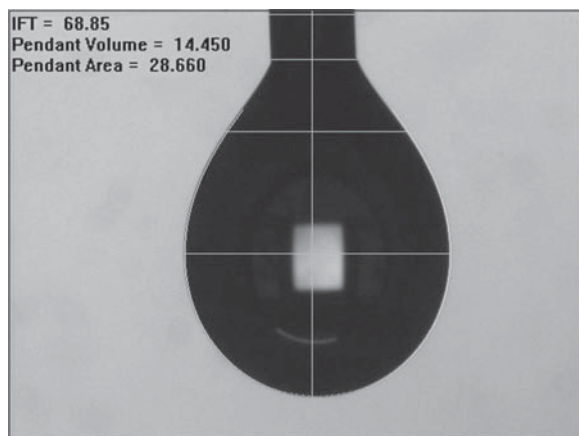


Figure 1. A pendant drop movie still showing the drop shape approximations, based on which the surface tension calculations were performed.

and so the air surrounding the drop was at room temperature. To compensate for fluctuations in temperature and other effects that could influence the calibration, measurements of pure water were performed several times a day and the data for the sap were compared with the values obtained for water.

Furthermore, because the box was not completely sealed, evaporation did take place. Water-soaked tissue placed in the bottom of the box saturating the air with moisture eliminated any measurable decline in drop size over the first half hour or so, but following that, the volume of the drop declined. This was a problem because reducing the surface area of an established drop through evaporation would have artificially increased the concentration of surfactants at the interface. Because of this, most experiments were kept to half an hour. When longer experiments were required, tests were performed to compensate for the change in surfactant concentration as the surface area changed. In these, drops of different sizes were allowed to stabilize for 1 h. Following this, they were rapidly inflated by variable amounts, and the effect of this on their surface tension was recorded. In this way a correlation between area and surface tension was obtained, and these equations were used to correct the data of long-term experiments for the change in surface area. Because of increasing rates of evaporation as the drop got smaller and the surface area to volume ratio increased, it was difficult to maintain any drop for longer than 3 h and the longest we ever managed to keep a drop was 5 h.

### Nitrogen experiments

To test whether the interfacial phenomena observed for *P. tremuloides* were influenced by oxidation of the surfactants at the surface, experiments were done in which the drops were suspended in nitrogen instead of ambient air. A capillary tube was attached to a high-pressure nitrogen bottle through a valve that allowed accurate adjustment of the flow of nitrogen. The capillary tube was fed into the metal box that enclosed the syringe tip and the suspended drop, and the box was sealed with waterproof medical tape. Since it was not possible to completely seal the box around the top where the tube was inserted, the open end of the capillary tube was placed towards the bottom of the box and a low-level flow of nitrogen of  $\sim 0.5 \text{ ml s}^{-1}$  was maintained. The flow rate was controlled by inserting the end of the capillary tube into an inverted vial filled with water and measuring the time it took for 5 ml of nitrogen to run into the vial. The flow rate was adjusted and the control repeated until the desired flow rate was obtained.

## Results and discussion

### *Populus tremuloides*

In *P. tremuloides*, the initial surface tension at time 0 was significantly lower than that of the control experiments with pure water (paired *t*-test,  $t = 4.82$  with 10 degrees of freedom,

$P < 0.001$ ). That said, the difference in magnitude was only 2.7%, as the mean for water was  $72.32 (\pm 0.15 \text{ SD}) \text{ mN m}^{-1}$  and the mean for xylem sap was  $70.39 (\pm 1.37 \text{ SD}) \text{ mN m}^{-1}$ . It is unlikely that a difference this small alone would have much of a physiological effect on the functioning of the tree.

Over time, however, we observed a strong decline in the surface tension of the suspended drop of sap (Figure 2). Within the first 5 min the values fell to an average of  $64.98 (\pm 4.19 \text{ SD}) \text{ mN m}^{-1}$  and within half an hour to  $59.88 (\pm 5.51 \text{ SD}) \text{ mN m}^{-1}$ . There were relatively large differences within and between the branches. Within-tree differences were as large as or larger than between-tree differences (Figure 2).

Since none of the studied drops had values that leveled off within the 0.5 h experimental period, long-term experiments were attempted as described in Materials and methods. Of the four drops we managed to hold stable for the longest period of

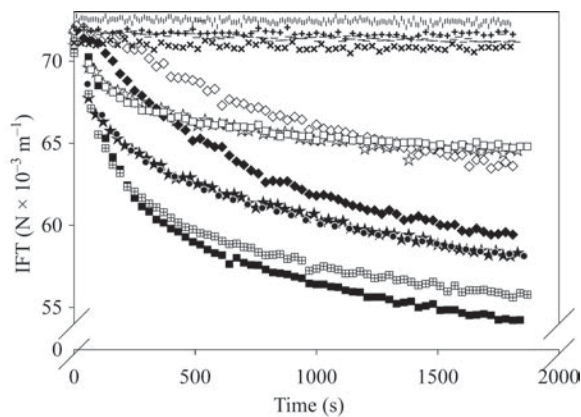


Figure 2. Change in surface tension (IFT) over a half-hour period. Squares, circles, diamonds and stars show data for branches from four trees of *P. tremuloides*, crosses the values for branches from two trees of *B. papyrifera*, and horizontal and vertical bars the values for branches from two trees of *S. aucuparia*. Identical shapes are used for different segments of the same branch. Proximal segments closer to the trunk are marked in white and distal segments closer to the terminal bud in black. Gray segments are in between the two. Only a representative subset of the studied branches is shown for clarity.

time, two leveled off within the study period. Leveling off was defined as the point in time beyond which the slope was extremely close to and not significantly different from 0. One leveled off towards the value of  $53.20 (\pm 0.17) \text{ mN m}^{-1}$  after 3.9 h and the other towards  $52.48 (\pm 0.09 \text{ SD}) \text{ mN m}^{-1}$  after 3.6 h (Figure 3). Both of these time spans were beyond the length of time that the third drop was maintained and close to the length of time that the fourth drop was maintained. Thus, it is likely that the remaining two drops would also have leveled off if it had been possible to maintain them for a longer period of time.

As described earlier, this decrease in surface tension over time leveling off towards a constant value is characteristic of solutions containing amphiphilic molecules that act as surfactants. As xylem sap contains hydrophobic as well as hydrophilic amino acids (Andersen et al. 1995a, 1995b, Bialczyk and Lechowski 1995, Schill et al. 1996, Alvarez et al. 2008), it is possible that these could react to form the surfactants responsible for the fall in surface tension observed here. Isolating the surface active component responsible for these interfacial phenomena in the sap of *P. tremuloides* is beyond the scope of this study.

When looking at the surface tension as a function of the square root of time ( $t^{1/2}$ ), there was some evidence that the process might have been more complicated than the simple adsorption of a single surfactant (Figure 3b). In the case of a simple adsorption mechanism of a surfactant species from a bulk phase to a spherical drop surface, the change in surface tension over time is directly proportional to  $t^{1/2}$  (Fainerman et al. 1995). When the surface tension of the four poplar branches subjected to long-term studies was plotted as a function of  $t^{1/2}$ , we did not find the expected linear relationship (Figure 3b).

Initially, there appeared to be a linear phase from 0 to between 17 and 20  $\text{s}^{1/2}$ , with slopes in the range  $-0.24$  to  $-0.31$  (mean  $-0.028 \pm 0.03 \text{ SD}$ ). It should be noted that this phase was characterized by relatively few data points, making

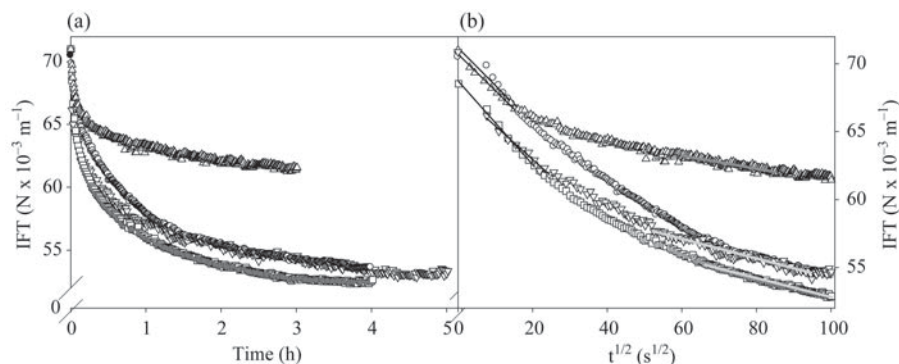


Figure 3. (a) Changes in the surface tension of xylem sap from four branches of *P. tremuloides* over a 3- to 5-h period. Two of the four leveled off towards constant values within the experimental period. (b) Dynamic surface tension as a function of  $t^{1/2}$  for the same four branches as shown in (a). At least two distinct linear phases could be identified, an initial one marked in black and a later one marked in gray.

an exact interpretation of the curve difficult. Following this there was a non-linear transition phase returning to a second linear phase starting at between 550 and 760 s<sup>1/2</sup> and lasting until between 840 and 1000 s<sup>1/2</sup> with slopes in the range −0.048 to −0.070 (mean −0.065 ± 0.011 SD). In most cases, the last portion of the curve again deviated from linearity, probably due to reduced surface accessibility (saturation of the available free surface area), which most likely resulted in the plateau observed for two of the four branches.

The presence of two distinct linear phases could indicate that the surface activity measured in this study might have been the result of the action of two separate surfactant species, one with a higher and one with a lower diffusion rate. The initial linear phase could be ascribed to a regime dominated by adsorption of the rapidly adsorbing surfactant, and the intermediate phase by the two surfactants adsorbing simultaneously. During the second linear phase, the first surfactant could have approached equilibrium so that any further changes would be due to the activity of the second slower surfactant.

At present, however, we cannot rule out the possibility that other chemical reactions could have influenced the observed surface phenomena or been responsible for the deviation from linearity. For example, surfactants with appropriate chemical structures could undergo gelation or condensation. Moreover, protein surfactants potentially present in the sap sample may have denatured and modified their conformation when exposed to the gaseous phase.

Patterns in time and space

The exact composition of the xylem sap of a given species has been found to vary with a number of environmental conditions, including state of hydration (Andersen et al. 1995b, Alvarez et al. 2008), time of year (Schill et al. 1996) and time of day (Andersen et al. 1995a). Our study was conducted over a 3-month period and under conditions that varied greatly in terms of rainfall. In spite of this, no correlation was found between either the time of year or the recent rainfall and the surface tension of the sap harvested (Table 1). It appeared that the concentration of the surfactant in question did not change over time.

Table 1. Coefficients obtained from a linear regression of the surface tension after 30 min (in mN m<sup>−1</sup>) plotted against rainfall immediately prior to harvest of the branches, day of the year and wounded surface area compared with volume extracted.

|                                                                | Slope                     | r <sup>2</sup>          | P    |
|----------------------------------------------------------------|---------------------------|-------------------------|------|
| 24 h rain (mm)                                                 | 0.207                     | 0.01                    | 0.71 |
| 72 h rain (mm)                                                 | 0.114                     | 0.01                    | 0.70 |
| Day of the year (day number)                                   | − 1.56 × 10 <sup>−3</sup> | 8.18 × 10 <sup>−5</sup> | 0.98 |
| Wound area/extraction volume (m <sup>2</sup> m <sup>−3</sup> ) | − 0.807                   | 0.14                    | 0.23 |

There was a significant linear relationship between the distance from the terminal bud of the branch at which the sap was sampled and the fall in surface tension over time (Figure 4; slope: 0.11; r<sup>2</sup> = 0.67; P = 0.001). Thin distal parts of the branches closer to the terminal bud typically had a lower surface tension at any given time from the start of the surface tension measurements than did the thicker parts of the branches more proximal to the trunk (shown at 30 min in Figure 4). The surface tension of sap from distal branches fell in the range 52.1–58.19 mN m<sup>−1</sup> after half an hour, >20% lower than that of pure water. In contrast, it appeared that the surface tension of sap might have approached that of water in branches at a distance of ~1.8 m from the terminal bud if the trend in Figure 4 could be shown to continue. It is possible that the concentration of surfactants in the thinner distal branches might have been higher because they accumulate there due to sap flow. Further experiments on trees at different transpiration rates are required to determine whether this might be the case.

Zimmermann (1983), in his segmentation hypothesis, proposed that fine branches and petioles might be designed so that they embolize prior to the rest of the system, hence protecting the main parts of the plant against further drought damage. If true, the stronger decline in surface tension over time seen here in distal as compared with more proximal branches could help to achieve this. In this way it could potentially help enhance rather than reduce the fitness of the tree.

Potential artifacts of the experimental procedure

Since the environment of the sap was changed upon extraction, there was the possibility that the interface phenomena observed in the hanging drops of sap could differ from those

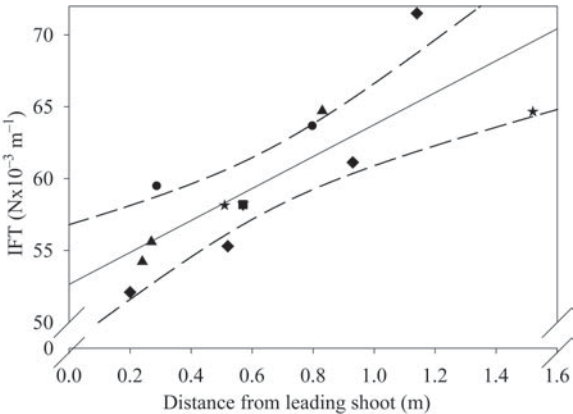


Figure 4. Data for the surface tension of xylem sap drops after half an hour of air exposure as a function of distance of the branch segment from the terminal bud. The sap was extracted from segments at various distances from the terminal bud cut from five different branches of *P. tremuloides*. Different segments from the same branch have identical symbols. Linear regression showed a positive slope between the two: slope 0.11; r<sup>2</sup> = 0.67; P = 0.001.

taking place inside living trees. For instance, there is evidence that oxygen levels in the woody tissue of trees are lower than that of ambient air (Spicer and Holbrook 2005, Sorz and Hietz 2008). Thus, it is possible that oxidation could affect the surface activity of the molecule in question. In our experiments, no differences were found between drops from the same branch segment suspended in nitrogen as opposed to ambient air (Figure 5). This, however, only means that oxidation of molecules at the sap–water interface did not play any substantial role in defining the shape or magnitude of the surface tension decline. We did not have the equipment needed to extract, transport and transfer drops under anoxic conditions, and so the sap could have been oxidized prior to the surface tension measurements.

As an argument against the influence of oxygen on our results, there was no significant correlation between the time that passed from the extraction to the measurements (in hours, from 0.4 to 5 h) and the values obtained. In fact if anything, the slope was positive rather than negative (slope 1.86,  $r^2 = 0.19$ ,  $P = 0.33$ ). Thus, we conclude that it appears unlikely that oxidation significantly changed the interfacial behavior of the sap observed in this experiment.

Further, wounding appeared to have little or no effect on the observed results. When comparing whole branches prepared according to the standard procedure with those in which wounding was artificially increased by cutting the branch more times and rinsing the ends less thoroughly, no systematic differences were found ( $t$ -test of values at 15 min,  $P = 0.96$ ,  $t = 0.053$  with six degrees of freedom). At 15 min, the mean for the artificially wounded group was  $62.13 \pm 5.28 \text{ mN m}^{-1}$  (SD) and the mean for the group prepared by the typical protocol was  $61.96 \pm 3.42 \text{ mN m}^{-1}$  (SD). In accordance with this, the slight negative regression found between the cut surface area—sap volume extracted ratio and the surface tension was not significant (Table 1). It thus seems unlikely that the surfactants in

question were present in the xylem sap due to contaminants from wounded living cells or due to other wound responses.

### Physiological effects of the presence of surfactants

As discussed above, it appears that the values obtained here do represent the properties of xylem sap in living *P. tremuloides* branches rather than being an experimental artifact. The presence of surfactants may impact a number of physiological parameters in the fine branches of this species; however, the exact effect would depend on a number of factors, the study of which is beyond the scope of the present paper. The pressure difference required for air seeding, for instance, depends directly on the surface tension of the interface between sap-filled and embolized vessels (Sperry and Tyree 1988). Indeed, it has been found that the presence of surfactants strongly increases xylem vulnerability to cavitation in both angiosperms and gymnosperms (Cochard et al. 2009).

It is unclear, however, whether the decrease in surface tension at the air–sap interface in pits neighboring a newly cavitating vessel quantitatively would match that described in this paper. Because of the minute size of the pores, larger molecules might not be able to adsorb efficiently to the interface. The potential close proximity to negative charges in the plant cell wall (Dainty and Hope 1959) could influence the adsorption rate indirectly through ionic molecules or directly. Further, if the bubble expands very rapidly following an air-seeding event, the concentration of the surfactant at the bubble surface will only initially be close to the steady-state levels. As the bubble expands, the surfactant concentration will decrease and go towards ambient levels in the solution.

Cavitation experiments are normally carried out on branches flushed with water or KCl solution and so existing data are of little help in determining the exact effect of surfactants on cavitation events in the branches of *P. tremuloides*. Further studies similar to those of Cochard et al. (2009) are needed to elucidate this effect.

Analogously for winter embolism, the bubble radius above which an air bubble will expand rather than disappear upon thawing depends directly on the surface tension of the xylem sap (Pittermann and Sperry 2006). However, as the surfactant may freeze out of the solution alongside the air, the concentration and so the reduction in surface tension could be much greater than what one would expect from the numbers presented in this paper.

These potential effects of surfactants provide an interesting dimension to the mathematical modeling of surface tension-related phenomena in xylem sap; they add a surface tension time scale dependency to the equations. A simple difference in surface tension with respect to that of water would just have resulted in a proportional change in most surface tension-dependent phenomena (Holttä et al. 2002, 2007, Pittermann and Sperry 2006). On the other hand, the observed

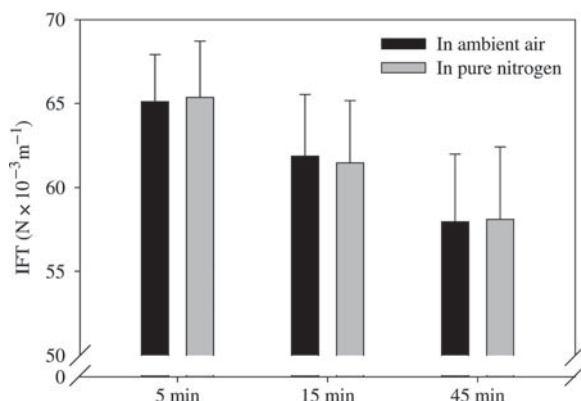


Figure 5. Mean surface tension of different drops from the same samples suspended in nitrogen as compared with ambient air. Data are shown for the surface tension after 5, 15 and 45 min. Error bars indicate 1 SD. No differences were found at any time.

decrease over time implies that the time an interface has existed combined with the rate at which the bubble is expanding or contracting will influence the surface tension of the bubble at a given point in time, adding a new level of complexity to the equations.

### *Betula papyrifera* and *S. aucuparia*

The initial surface tension values of *B. papyrifera* and *S. aucuparia* at time zero were very close to those obtained for the control drops of pure water. In *S. aucuparia*, the initial surface tension of xylem sap was only 0.50% lower than that of pure water, and the difference was not significant (paired *t*-test,  $t = 2.670$  with three degrees of freedom;  $P = 0.076$ ). The surface tension of the sap of *B. papyrifera* was significantly different from that of water (paired *t*-test,  $t = 3.34$  with six degrees of freedom,  $P = 0.016$ ), though only 0.52% lower.

Of the six *B. papyrifera* branches studied, five showed a significant decrease in surface tension over a 1-h period and one did not (linear regressions, slopes significantly different from 0, *P* values obtained:  $P = 0.148$ ,  $P = 0.026$ ,  $P = 0.029$  and three branches of  $P < 0.001$ ). The average slope, however, was only  $1.68 \times 10^{-4} \text{ mN m}^{-1} \text{ s}^{-1}$ , and so the total change over the 1-h period amounted to, on average, 0.88% ( $\pm 0.49$  SD). Two long-term experiments (3 h) were performed, and in these there was no difference between the values obtained at 1 and 3 h. All the four *S. aucuparia* branches showed a significant decline in surface tension over the 45-min experimental period (linear regressions, slopes significantly different from 0, *P* values  $< 0.001$ ). The average slope was  $1.56 \times 10^{-4} \text{ mN m}^{-1} \text{ s}^{-1}$  ( $\pm 1.05 \times 10^{-4}$  SD), resulting in a change of, on average, only 0.59% ( $\pm 0.39$  SD) over the 45-min period.

As in *P. tremuloides*, the change in surface tension over time in these two species implies that one or more surfactants were present in the sap, though either at very low concentrations or with very low surface activity. Whether or not these surfactants might be the same as those found in the sap of *P. tremuloides* remains at present purely speculative.

Although there were significant differences between surface tension phenomena in *B. papyrifera* and *S. aucuparia* sap and those of pure water, the magnitudes were small. We conclude that they are unlikely to have any substantial effect on the physiological functioning of the tree. It thus appears that, within an acceptable level of error, the assumption that the surface tension of xylem sap is equal to that of water is valid for these two species.

## Conclusion

Of the three species studied, *B. papyrifera* and *S. aucuparia* had xylem sap with a surface tension that was almost identical to that of water and changed little over time. In *P. tremuloides* however, the surface tension of xylem sap was only initially

close to that of water and dropped over time to level off at values that were up to 27% lower than those of pure water. This was true in all trees regardless of when they were harvested or the presence or absence of drought, although thin distal branches had lower surface tension values than branches closer to the trunk. Thus, it appears that although the surface tension of xylem sap may frequently be close to or identical to that of water, this cannot necessarily be assumed to be the case throughout the tree in all species.

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