

# A Requirement for Sucrose in Xylem Sap Flow from Dormant Maple Trees<sup>1</sup>

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## ABSTRACT

The response of excised stem segments of several tree species to freezing and thawing cycles was studied. All species studied (*Thuja occidentalis*, *Fagus grandifolia*, and *Betula papyrifera*) except maple (*Acer* spp.) exuded sap while freezing and absorbed on thawing. Maple stems absorbed sap while freezing and exuded sap during the thaw only when sucrose was present in the vessel solution. Increased concentration of sucrose in the vessel sap led to increased exudation. In the absence of sucrose, maple stems absorbed sap on thawing. The presence of sucrose enhanced sap absorption during freezing cycles in maples. In general, large sugars, disaccharides and larger, could substitute for sucrose in the maple exudation response while sugar hexoses could not. The results are discussed in relation to the O'Malley-Milburn model (1983 Can J Bot 61: 3100-3106) of sap flow in maples.

The physiological basis for xylem sap exudation in dormant maple trees has not yet been adequately characterized. Various hypotheses involving both vitalistic (5, 19) and physical (7, 17) mechanisms have been proposed but none as yet satisfies all observations. It is known that exudation in maples results from positive pressures which develop within the stem itself (8, 17). Root pressure is not considered a significant driving force since exudation may occur in excised stem segments whereas little exudation is found from root stocks after removal of shoots. It has been established that sap flow is temperature dependent with maximum yields occurring during the period when the wood temperature fluctuates above and below 0°C (9, 10, 19). Uptake of soil solution occurs during the cooling period and has been termed a "conditioning" phase by Marvin (8). This conditioning is required for large sustained volume flows during the warm period.

Recently Milburn and O'Malley (13) proposed a new hypothesis to describe sap flow in dormant maple trees. Absorption of sap is described as a biphasic process and is entirely apoplasmic moving from the vessels through wall material into gas-filled fibers (Fig. 1). Initial absorption of sap can be entirely explained as a consequence of gas dissolution and contraction within the stem, presumably in embolized fibers surrounding vessels. Absorption associated with freezing occurs as a direct result of ice crystal formation (on the inner wall of fibers) and proceeds as long as ice crystal growth occurs. Growth of ice crystals does not progress into cell wall capillaries because the freezing point is

depressed due to surface absorption effects. Continuous flow of liquid water occurs up to the site of crystal formation. Flow by vapor distillation is considered small by the authors. Gas entrapped in the fibers becomes compressed as ice crystal growth continues and contributes to the positive pressure driving sap out of the stem during the thaw. This system is similar to the process used to describe water flow in frozen soils (1, 3, 4, 20).

The O'Malley-Milburn model is a physical one which requires neither living cells nor sucrose. Experimental evidence in support of these negative requirements was obtained in both field and laboratory freezing experiments on *Acer pseudoplatanus* L. In these experiments O'Malley (15) observed absorption of sap on freezing and exudation during thawing in the absence of living cells. Stems collected in summer which presumably contained no sucrose in the vessel sap performed similarly. Most important, O'Malley (15) clearly demonstrated the simultaneous occurrence and rapid absorption and a freezing exotherm.

Much earlier Marvin (8, 10) had demonstrated sap flow without freezing in *Acer saccharum* Marsh. Stems cooled from +15 to +5°C showed absorption; stems exuded slightly when rewarmed to +15°C. The O'Malley-Milburn hypothesis provides a satisfactory explanation for flow without freezing as observed by Marvin. Volume flow was small and could be the result of gas contraction and dissolution. However, Marvin also showed a requirement for osmotically active substances in the vessel solution. This requirement is in direct conflict with O'Malley's (15) observations. The conflict needs to be resolved before the O'Malley-Milburn hypothesis can be accepted as a general explanation for sap flow. Should sucrose be shown conclusively to be required for sap flow then it must be incorporated into any model of sap flow. Because these conflicting results concerning the role of sucrose have not been addressed we have reinvestigated freezing responses in both species.

## MATERIALS AND METHODS

Some preliminary experiments were performed on excised shoots of the following species: Maple (*Acer saccharum* Marsh.), birch (*Betula papyrifera* Marsh.), beech (*Fagus grandifolia* Ehrh.), and cedar (*Thuja occidentalis* L.). Shoots 1.5 cm in diameter at the base and 2.0 to 2.7 m long were collected from Snake Island (courtesy of Harold McCue). All samples were collected between mid-December 1982 and mid-April 1983. The shoots were brought to the laboratory on Snake Island, and immediately placed inside a freezer. The basal end of the shoots protruded through a hole in one wall of the freezer and was connected to a flow rate transducer (see Tyree [18] for details of the apparatus used). The purpose of these experiments was to measure the rates of sap uptake and/or exudation during freezing and thawing cycles. For all subsequent experiments the materials

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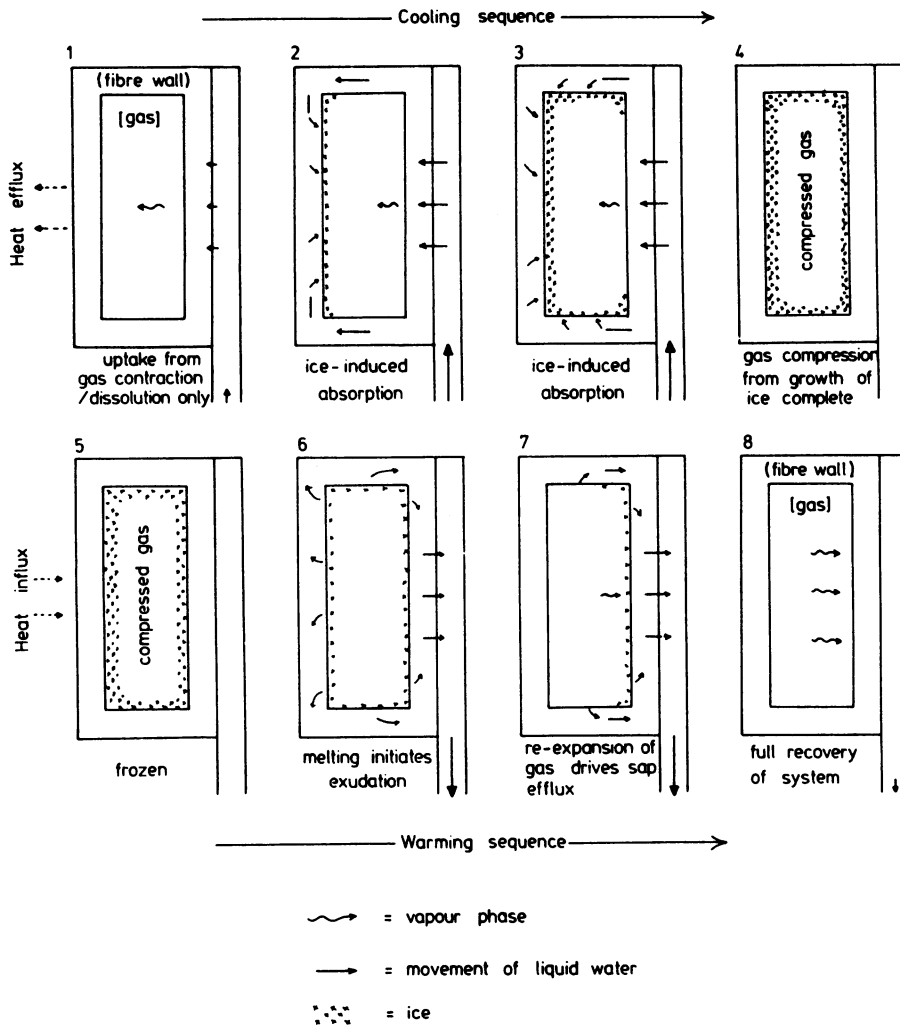


FIG. 1. Sap flow model from O'Malley (13, 15). Biphasic absorption of sap is shown in the cooling sequence, panels 1 to 4. Exudation as a result of warming is shown in the bottom sequence.

and methods were as described below.

Stem segments from sugar maple seedlings, 1.5 to 2.0 cm diameter, were collected monthly throughout the winter from Century Farms, Toronto (courtesy of Amos Baker) as well as from Snake Island. Red (*A. rubrum* L.) and Manitoba (*A. negundo* L.) maple were also collected in late winter from these sites. Seedlings were cut in the early morning, wrapped in damp paper toweling and aluminum foil, and returned to the laboratory while still frozen. Samples collected in nonfreezing conditions were maintained at 4°C in an insulated box. Stems not used immediately were stored at -15°C for no longer than 3 weeks.

Sycamore maple (*A. pseudoplatanus* L.) seedlings were purchased from Keith Sommers Tree Farm, Tilsonburg, Ontario. Excised branches were also collected as needed from mature trees growing on the University of Toronto campus.

Stem segments were chosen to be free of any lateral branches. Fifty centimeter lengths were used in the freezing experiments; approximately half of the stem was completely inside an insulated freezing box. At least 6 cm on each end was needed to connect the stem to brass fittings and copper tubing which supplied external sap to the stem (Fig. 2). The insulated box consisted of a double layer of styrofoam insulation; total thickness was 5 cm. The inside dimensions were 26 cm wide, 45 cm tall, and 55 cm long. This design permitted perfusion of the stem segments with various solutions. Since the stem segments were of approximately uniform diameter within the freezer, the rate of freezing was more uniform than in Tyree's (18) apparatus where the smaller

twigs were completely frozen long before the main stems.

Pressure seals and differential pressure transducer design and use are as described by Tyree (18). Copper/constantan thermocouples were used to measure stem and air temperatures and an Omega Ice Point Cell served as reference. Thermocouples were placed near the cambium by slicing into the bark with a razor blade. The output of the differential thermocouple pair was amplified by a LM 725 op amp (Dixon 1982). Data collection was automated using a Systemaster S-100 Z-80 microcomputer (Teletek Enterprises Inc, Sacramento, CA) and a TM-AD212 S-100 analog to digital converter (Tecmar, Inc. Cleveland, OH). A FORTRAN program was used to call Assembly language routines to collect data from the A/D converter and print real time graphics on a Gemini 10X printer.

In the interest of uniformity and reproducibility, freezing cycles were carefully controlled. Once a stem was placed in the insulated box and connected to the plumbing system, all tubing was vacuum infiltrated with a 10 mM NaCl solution. The stem was allowed to equilibrate overnight. On the following morning the stem was supercooled to -3°C until both temperature and solution flow equilibration was reached. Freezing of the sap was induced by spraying the stem with liquid freon. The exotherm and absorption period lasted 3 to 4 h. After thermal and flow equilibration the stem was warmed to +3°C. Three to 4 h were required for equilibration. The stem was then perfused with 100 to 200 ml of some other solution. Pressurized nitrogen at 0.05 to 0.075 MPa was introduced to the supply flask and used to

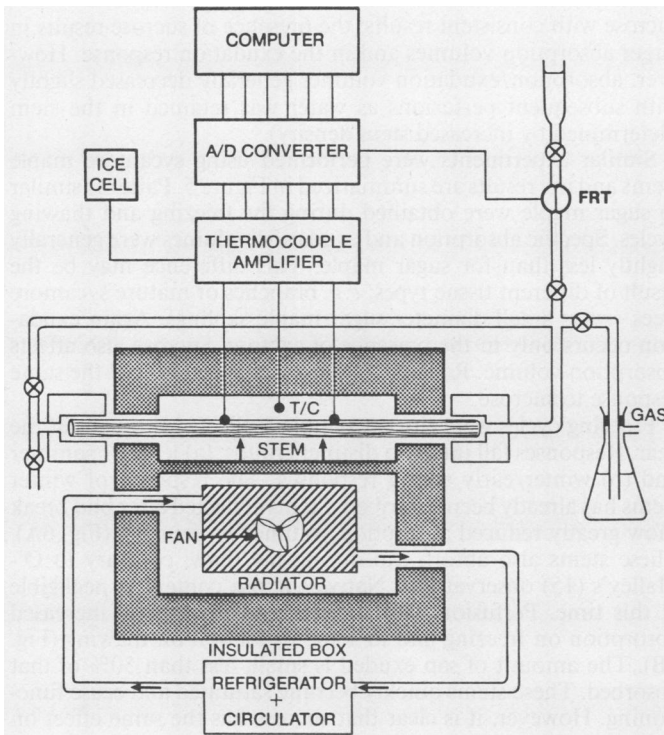


FIG. 2. Schematic of equipment layout for freezing experiments. Stem segments extend through the insulated box and are connected to copper tubing. A flow rate transducer (FRT) measures instantaneous flow rate into and out of the stem segment. The flask allowed pressurized gas to perfuse solutions through the stem. Data collection was automated with computer equipment.

Table 1. Perfusion Sequences for all Experiments

Perfusion solutions: NaCl = 10 mM NaCl; % = percent sucrose plus 10 mM NaCl; None = unperfused stem segment.

	1	2	3	4	5	6
Figures 4 and 5	None	NaCl	2%	NaCl <sup>a</sup>	0.2% <sup>a</sup>	
Figure 6	None	2%	NaCl	2% <sup>a</sup>	NaCl <sup>a</sup>	
Figure 8						
Sugar maple						
A	0.5%	3%	5%	1%	2%	
B	5%	2%	3%	0.5%	1%	
C	2%	0.5%	5%	0.0%	3%	1%
D	3%	2%	1%	5%	0.5%	
Sycamore maple						
A	0.5%	5%	2%	3%	1%	
B	5%	1%	3%	0.5%	2%	0.0%

<sup>a</sup> Results not shown.

perfuse the stems. Perfusion lasted 1 to 3 h depending on conductivity of the stem. Repeated perfusions tended to reduce conductivity slightly; however, this did not significantly affect absorption/exudation volumes. Typically, after five cycles the stem was removed from the box and measurements made of the stem weight and volume. Measurements were made on both that portion of stem completely inside the box and the total stem. Dry weights were obtained after 7 to 10 d at 65°C. The sequence of solution changes for the various experiments after each freeze/thaw cycle is as shown in Table I.

The perfused solution was analyzed for sugar content by HPLC using 75/25 acetonitrile/water at 2 ml/min in a Waters NH<sub>2</sub>Z module column. Sugars were detected in an R401 refractive index detector and quantified by triangulation.

RESULTS

In Figure 3 we show the rate of sap flow versus time for freezing and thawing cycles performed on excised shoots of sugar maple, birch, cedar, and beech. All shoots were in their 'native' state, i.e. the stems were not perfused with any solution before the freeze-thaw cycle. Positive flows indicate an exudation and negative flows indicate sap uptake. The duration of the freezing exotherm is indicated by X and the thawing endotherm by E in Figure 3. The response for sugar maple shoots is typical of that reported by Tyree (18) for the same rate of freezing. It should be noted that only sugar maples absorb sap during the freezing cycle and exude sap during the thaw. All other species behave in the other way, i.e. birch, cedar, and beech exude sap during the freeze and absorb sap during the thaw.

In Figure 4A we characterize the response of sugar maple stem segments in the native state collected during the winter. Temperature measurements are included in this and the remaining figures. At 0 h the stem began supercooling to -3°C at approximately 0.1°C/min (Fig. 4A). At this rate no cellular freezing occurs (12). As cooling progressed approximately 0.1 ml was absorbed. Approximately 30 min after thermal and solution flow equilibrium was reached, freezing was induced as indicated by the distinct exotherm. Rapid absorption began at this time and peaked at approximately 3.4 ml/h (Fig. 4A). The extremely high peak absorption rates measured by Tyree (18) and shown in Figure 3 were generally not seen in these experiments. The reduced freezing surface area to volume ratio, the smaller mass

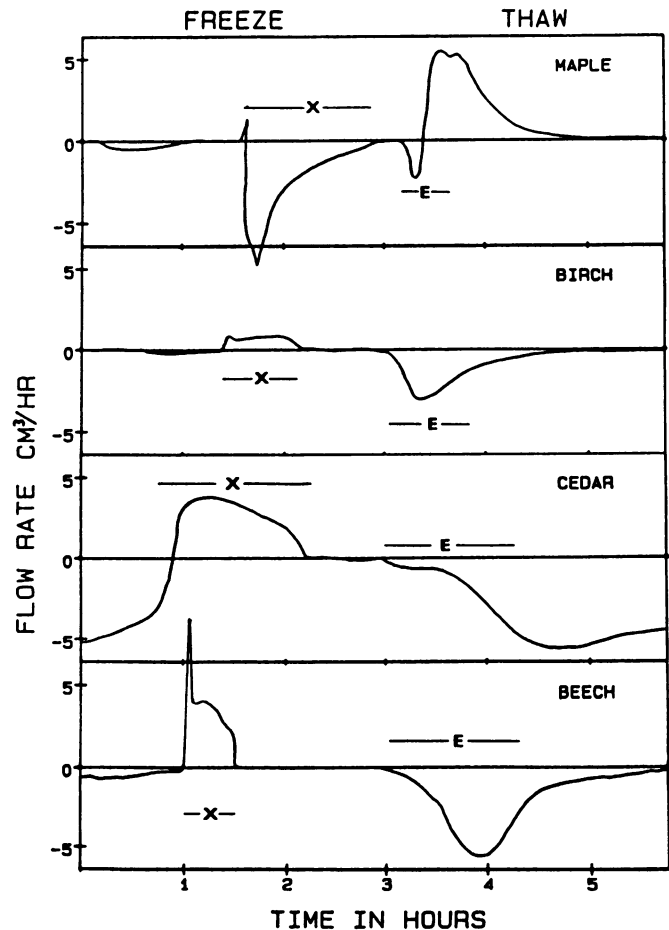


FIG. 3. Response of several tree species to freeze/thaw cycles. Positive flows indicate exudation and negative flows indicate sap absorption. Duration of the freezing exotherm is indicated by X and the thawing endotherm by E.

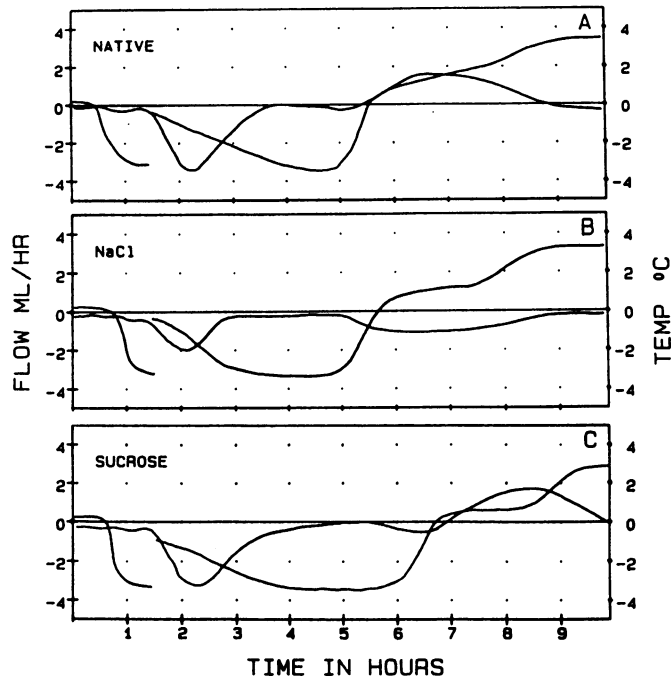


FIG. 4. Response of sugar maple to freezing cycles. Flow into and out of the stem is as indicated in Figure 3. Temperature measurements are included in this and the following figures. In A, the unperfused response is shown, in B response after perfusion with 125 ml of 10 mM NaCl, and in C the response of the same stem after reperfusion with 125 ml of 2% sucrose and 10 mM NaCl.

of tissue frozen, and the absence of small diameter stems which froze rapidly in the earlier experiments (18) probably account for the difference in peak flow rates. Total volume absorbed during this freeze was 2.0 ml. Temperature and solution equilibrium was reached at 3.8 h. After an additional 30 min the stem was warmed to +3.0°C at 0.2°C/min. An initial absorption, approximately 0.1 ml, occurred, possibly associated with ice contracting in the vessels as it melts. The endotherm is indicated by a reduction, below 0.2°C/min, in the rate of change of stem temperature since heat energy absorbed at this time is consumed in the heat of fusion and not contributing to raising stem temperature. Sap, under positive pressure, began to exude from the stem at this time. Peak flow rate during efflux was generally less than during absorption but usually persisted for a longer time. Total volume of efflux was generally equal to or slightly less than the total absorbed (0.1 + 2.0 + 0.1 = 2.2 ml absorbed and 2.1 ml exuded). Occasionally after several perfusions exudation volume was greater than absorption volume immediately prior to thawing. However, there was always a net absorption leading to increased stem density over the entire experiment.

The response of the previous stem after perfusion with 10 mM NaCl, *i.e.* in the absence of sucrose is shown in Figure 4B. Absorption associated with cooling (without freezing) was again observed; however, absorption associated with freezing was clearly reduced (1.4 ml) and of shorter duration. It is especially important to note that additional absorption occurred during the thaw (1.0 ml). The volume of sap absorbed during the thaw was consistently less than during freezing.

These observations are consistent with those of Marvin (8). Specific exudation volumes in ml per kg dry weight of wood are similar. We extended Marvin's experiments by reperfusing the same stem with NaCl and 2.0% sucrose. These results are shown in Figure 4C. Absorption characteristics are similar to those of the native response. In addition, exudation has been restored on thawing. Stems could be repeatedly reperfused with or without

sucrose with consistent results: the presence of sucrose results in larger absorption volumes and in the exudation response. However, absorption/exudation volumes generally decreased slightly with subsequent perfusions as water was retained in the stem (determined by increased stem density).

Similar experiments were performed using sycamore maple stems and the results are summarized in Figure 5. Patterns similar to sugar maple were obtained during the freezing and thawing cycles. Specific absorption and exudation volumes were generally slightly less than for sugar maple. This difference may be the result of different tissue types: *e.g.* branches of mature sycamore trees *versus* small diameter sugar maple saplings. Again exudation occurs only in the presence of sucrose. Sucrose also affects absorption volume. Red and Manitoba maples showed the same response to sucrose.

Freezing cycles were run on samples collected throughout the year. Responses fall into two distinct groups: (a) leafy or summer and (b) winter/early spring responses. The response of winter stems has already been described. Stems collected after bud break show greatly reduced absorption volumes on freezing (Fig. 6A). These stems also absorb sap during the thaw, contrary to O'Malley's (15) observations. Native sucrose content is negligible at this time. Perfusion with sucrose leads to slightly increased absorption on freezing and to some exudation on thawing (Fig. 6B). The amount of sap exuded is small, less than 50% of that absorbed. These stems quickly become saturated and cease functioning. However, it is clear that sucrose has the same effect on these stems as it does on winter stems.

Winter and summer responses for both sugar and sycamore maple are summarized in Figure 7 for comparative purposes. Both the native response and the response after perfusion (NaCl for winter and 2% sucrose for summer) are shown. Freezing response is indicated with 'F,' thawing with 'T.' Empty bars indicate absorption; hatched indicate exudation. Although responses between stems are variable there is a consistent effect of sucrose concentration on exudation volume in individual stems.

In Figure 8 the effects of concentration on four sugar and two sycamore maples collected in late winter are shown. Perfusion with increasing sucrose concentration leads to increased exuda-

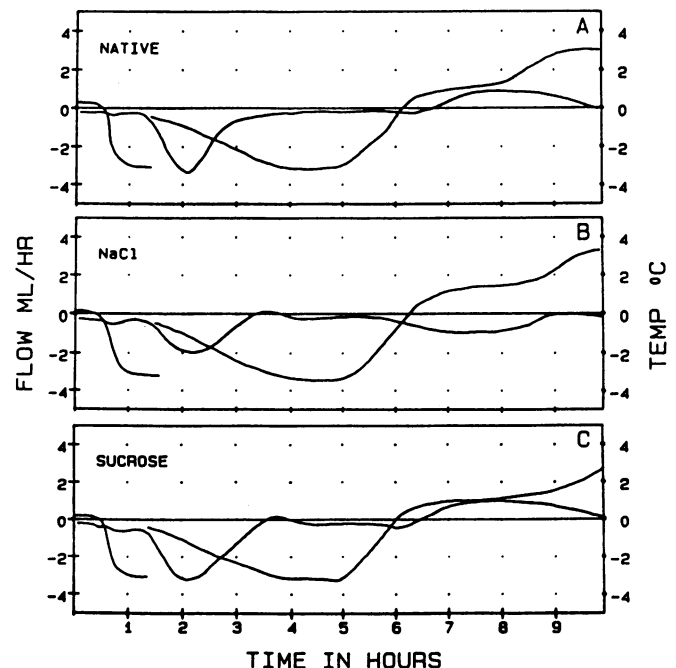


FIG. 5. Response of sycamore maple to freezing cycles. Note the similarity in response to sugar maple.

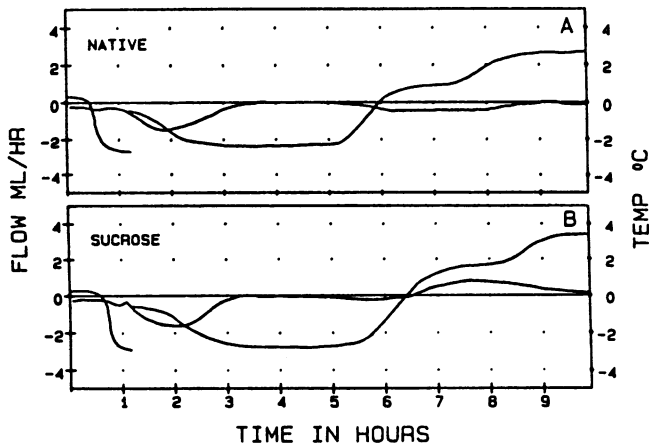


FIG. 6. Response of sugar maple collected in summer. The native response is shown (A). Native sucrose concentration is negligible. Perfusion with 2% sucrose (B) leads to typical absorption and exudation.

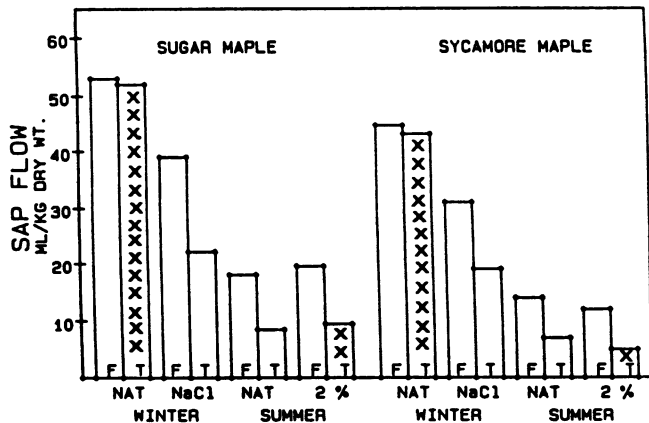


FIG. 7. Sap flow summary. Results for both sugar and sycamore maple stems from summer and winter are shown. Sap flow on freezing is indicated by F and on thawing by T. Absorption is indicated by empty bars and exudation by hatched bars. The unperfused response is indicated by NAT; response after perfusion with NaCl or 2% sucrose are indicated by NaCl and 2%.

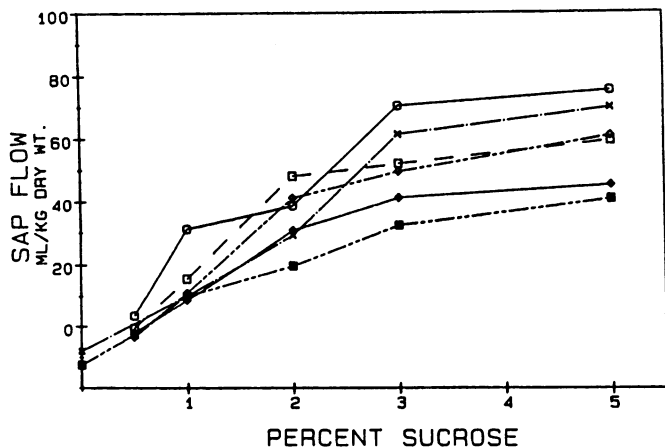


FIG. 8. Concentration dependent exudation in sugar maple. Exudation in ml/kg dry weight of frozen stem tissue increases with increasing concentration of sucrose in the vessel sap.

Table II. Response of Sugar Maple to Thawing

Sugar	Marvin	Us	Specific Volume ml/kg <sup>a</sup>
Glucose	Abs <sup>b</sup>	Abs	29 ± 3
Fructose	Abs	Abs	31 ± 3
Mannitol	Abs	Abs	15 ± 2
Inositol	Exu <sup>c</sup>	Exu	9 ± 2
Sucrose	Exu	Exu	66 ± 8
Maltose	Exu	Exu	61 ± 5
Lactose	Exu	Exu	58 ± 8
Raffinose	Exu	Exu	47 ± 5

<sup>a</sup> Mean and standard deviation of 3 to 5 stems. <sup>b</sup> Sap absorbed on thawing. <sup>c</sup> Sap exuded on thawing.

tion. This response appears to saturate at 5% sucrose.

Marvin (8) had determined that certain sugars could substitute for sucrose. Table II compares the responses of sugar maple to thawing after perfusion with several sugars in equiosmolar concentrations as reported by Marvin and ourselves. Marvin (8) recorded only qualitative data, either absorption or exudation. We include specific volume as the mean and standard deviation of two to five stems. In general, hexoses do not contribute to exudation while disaccharides do, inositol being the only exception. These results indicate that sucrose does not play a simple osmotic role since equiosmolar concentrations do not give similar results.

DISCUSSION

Our initial experiments are in agreement with those of Clark as reported by Wiegand (19). Clark's experiments consisted of excising frozen branches and observing them in the laboratory during the thaw. Only maple and to a much lesser extent buttonwood (probably *Cladrastis leutea*) exuded under these conditions. In other experiments excised branches and tree stumps were observed in the field on warm days. In this case exudation was also observed in birch, grape, and several other species. However, Wiegand (19) clearly separates the exudation response into two groups: (a) those which bleed early in the spring and are temperature dependent and (b) late spring bleeders which are not closely dependent on temperature. Birch and grape fall into the later group. We have been primarily interested in the response of the first group.

The maple response to freezing and thawing appears to be quite unique. These stems show the unusual property of absorbing sap during freezing (Fig. 3). The O'Malley-Milburn (13) model provides a good physical basis for absorption during freezing. But as yet, we have been unable to positively confirm or refute this aspect of the model nor have we been able to explain the uniqueness to maples (see below).

Other results (Figs. 4, 5, and 6) clearly indicate that sucrose is required for the maple sap response. The experiments have shown that absorption volumes increase and exudation occurs only in the presence of sucrose. Only those trees with native sucrose in their vessel sap show the response. Concentrations of 2 to 5% sucrose occur routinely in late winter and early spring (6, 11, 16). Summer stems typically contain negligible amounts of sucrose and do not exude. However, stems collected at any time of the year can be induced to exude when thawing by perfusing with sucrose prior to freezing. This appears to be a general requirement as all *Acer* species tested showed a similar response to sucrose. These results do not necessarily imply metabolic control of the process. Our experiments testing a role for living cells have so far been inconclusive.

Although sucrose affects absorption volume, absorption occurs during freezing regardless of the composition of vessel sap.

Absorption supplies soil solution to the stem during the 'cooling period' of Marvin (8). This freeze-induced absorption as characterized by O'Malley (13, 15) appears to be the unique property contributing to the maple sap response. Many species exude slightly when frozen (19; and Fig. 2). Exudation is the expected response since sap expands on freezing. Therefore sap must be expanding into gas-filled spaces outside of the vessels. Jones *et al.* (7) reported that intercellular spaces are absent in maples. O'Malley and Milburn suggest gas-filled fibers as the possible site of absorption. Wiegand (19) examined the distribution of gas in a wide variety of tree stems and reported very little gas in the vessels of maple while fibers contained large amounts of gas. Other trees showed more gas in vessels and many showed gas-filled vessels over several centimeters. These anatomical and physical characteristics appear to be important in maple sap flow and require further investigation. We are currently investigating factors which contribute to this particular distribution of gas and sap.

Exudation occurs only in the presence of sucrose and appears to be independent of the absorption response. High yields have been correlated with high sucrose concentration (10, 11, 14) and in fact we show a quantitative relationship (Fig. 8). O'Malley (15) considered that this correlation was indirect and that the presence of sucrose was not "crucial to the sap exudation mechanism." However, we believe that O'Malley may not have tested adequately the role of sucrose (see below). Sucrose is not unique in promoting exudation. Stems perfused with lactose, maltose, and raffinose have also exuded during the thaw at slightly reduced specific volumes (Table II). Because stems perfused with hexoses consistently absorbed during the thaw, a purely osmotic role for those sugars that promote exudation has been ruled out. We do not imply that the differential response is due to living cells since other physical properties may account for the differences. Cortes and Sinclair (2) have also ruled out an osmotic role for sucrose in generating sap pressures.

O'Malley's claim that sucrose is not required for sap flow is based on the response of summer stems to freezing cycles. O'Malley did not attempt perfusion experiments to remove or replace sucrose. Summer stems typically contain no detectable sucrose, yet in his experiments these stems exuded. We suspect that the experimental pretreatment may have influenced the results. Stems were allowed to absorb water at 15°C for 12 h to standardize water status prior to freezing. A further 6 to 12 h was required for equilibration after cooling to near 2°C. We have evidence that a significant amount of sucrose is released into vessel sap within this time course. However, 36 h were required for summer stems of sugar maple before exudation occurred on

thawing. These aspects are still under investigation in our laboratory.

We have shown that maples in general have a unique property of absorbing sap during a freezing cycle. All other species tested exuded sap while freezing. This property alone does not account for the exudation of sap from maples. The exudation process depends on the presence of sucrose and appears to be independent of the absorption process. The specific role of sucrose has not been determined but a simple osmotic role has been ruled out.

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