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Freezing of Xylem Sap Without Cavitation

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Summary. Freezing of stem sections and entire twigs of hemlock (*Tsuga canadensis*) has been demonstrated to occur without increasing the resistance to the movement of water through the frozen part after rewarming. This was interpreted to mean that freezing did not produce cavitation in the xylem sap even though A) the sap was unquestionably frozen; B) it contained dissolved gases; and C) it was under tension before freezing and after. Freezing stem sections of some other evergreen gymnosperms during the summer again produced no evidence for cavitation of the xylem sap. On the other hand, freezing stem sections of some angiosperms invariably increased the resistance to sap flow leading to wilting and death in a few hours when the sap tension was at normal daytime values at the time of freezing. These results were interpreted to mean that the bordered pits on the tracheids of gymnosperms function to isolate the freezing sap in each tracheid so that the expansion of water upon freezing not only eliminates any existing tension but also develops positive pressure in the sap. Dissolved gases frozen out of solution may then be redissolved under this positive pressure as melting occurs. As the bubbles are reduced in size by this ice pressure developed in an isolated tracheid, further pressure is applied by the surface tension of the water against air. If the bubbles are redissolved or are reduced to sufficient small size by the time the tension returns to the sap as the last ice crystals melt, then the internal pressure from surface tension in any existing small bubbles may exceed the hydrostatic tension of the melted sap and the bubbles cannot expand and will continue to dissolve.

Evidence in support of the cohesion theory for the transport of water through the xylem system has steadily increased since it was first stated by Dixon and Joly (3) and was so eloquently developed by Dixon (4). Not the least evidence has been the recent direct measurements of negative hydrostatic pressures (hydrostatic tensions) in halophytes, tall trees and desert shrubs for which the values obtained were appropriate for the needs of the plant (8, 9). The negative hydrostatic pressure was also shown to be equal to the osmotic pressure of the leaf intracellular sap when turgor pressure was zero. Yet, the theory can still be challenged by simply inquiring about the freezing and thawing of xylem sap which is under tension prior to and after freezing. Evergreens in temperate and Arctic zones contain xylem sap which Scholander (7) has shown by calorimetric measurements to be periodically frozen. Dissolved gases in the xylem sap would thereby be frozen out of solution. If the sap were under tension at the time of freezing or thawing, should it not cavitate and should not cavitation impede the easy flow of sap through the xylem system? The results to follow are interpreted to show that cavitation does not occur in hemlock (*Tsuga canadensis*) and in some other evergreens, possibly due to the expansion of water upon freezing within a closed system.

Three kinds of experiments were undertaken in this study and the methods, material and results will be presented in separate parts. For Part I, evidence for the occurrence or absence of cavitation was sought in detail in a single evergreen species. For Part II, a survey was made of several species of woody trees and shrubs to determine for which species cavitation of the xylem sap was caused by stem freezing. The seasonal effect upon the osmotic pressure of the intracellular sap was examined for Part III. Although the latter effect may be unrelated to the conditions for cavitation, the data obtained provide a basis for estimating the average modulus of elasticity for the leaf cell walls.

Part I. Water Intake and Weight Loss Prior to, During and After Freezing of Twig. All twigs for these tests were taken from 6 hemlock (*Tsuga canadensis*) trees approximately 10 years old and grown in Connecticut. Most were measured in late winter and were still conditioned by many and recent freeze-thaw cycles. Two were measured in mid-June and had 2 to 3 cm new growth.

Only 2 measurements were made as a function of time on each twig; A) the amount of water

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entering the twig through the stem, and B) the weight loss, primarily a result of transpiration. All twigs were removed from the tree (the stem was not under water when cut off) and treated variously before measurements began. To measure the amount of water entering the stem of the twig, the end of the stem was gently scraped to the cambium, cut cleanly off under degassed, distilled water with a razor blade, and immediately joined to a potometer containing distilled, filtered water in its long, calibrated glass tube. A short rubber tubing connected the water in the glass tubing with the stem. Care was taken to avoid trapping gas bubbles in the connection. All water entering the stem from the glass tube was previously filtered through an HA millipore filter (40μ pore size). When saturated water was used, air was bubbled through the water at the stated temperature prior to filtering, otherwise all water was degassed by boiling prior to filtering. All weight changes were measured to the nearest 0.01 g. When sap tensions were measured, small test twiglets (0.4 to 0.8 g by weight) were removed from the twig and enclosed in a pressure chamber where the gas pressure (N_2) was slowly increased until the sap returned to the cut end of the twiglet protruding through a seal to the outside of the pressure chamber (8, 9).

Water intake and weight loss were obtained for twigs which were frozen in 2 ways: A) by freezing a 4 to 5 cm section of the stem with CO_2 snow. The first centimeter of the stem from the end joined to the water in the glass tubing was not frozen. This type of freezing was always accomplished while measurements of water intake and weight loss were in progress. B) By slowly freezing the entire twig for several hours at $-15°$ either prior to measurements of water intake and weight loss, or, in some cases, during the measurements. In the latter case, the water in the glass tubing and in the first 2 to 3 cm of the stem was prevented from freezing by local warming.

Except where noted, all measurements were made at a dry bulb temperature of 20° to 23° and a relative humidity between 20 and 30 %.

Stem Frozen—Water Degassed. A 15 gm hemlock twig was dehydrated until a test twiglet from it measured 265 psi tension in the xylem sap. On 2 successive occasions, while measuring its intake of degassed and filtered water and its weight loss, a 4 cm section of the stem was frozen for about 1 hour (fig 1A and B). As the frozen section rewarmed, the inrush of water was followed by a steady state influx of water equal to the rate of weight loss. This can be interpreted to mean that freezing the section did not introduce an impediment in the path of xylem flow, that is cavitation of the xylem water did not occur. If cavitation had occurred, the inrush of new water into the twig would not have occurred following thawing of the frozen section; nor would the steady state rates of water intake before and after freezing have been equal. Figure 1C illustrates the water intake into a twig when air bubbles such as are produced by cavitation enter into conducting elements. In this case, the stem was separated from the water in the glass tube for 20 minutes and then rejoined. Note that the steady state rates of influx and weight loss after stem freezing at A in figure 1 actually exceed the pre-freezing rates because the twig had just been transferred from a temperature of 2° to 22°, and the rates of influx and weight loss in the warmer environment were not yet established before freezing at A occurred. The steady state rate of intake before stem freezing at B was 55 mg water per hour per gm fresh weight of twig (or simply expressed hereafter as 5.5 %/hr). The steady state influx after freezing at B was 4.3%/hr. The small difference between 5.5 and 4.3 %/hr suggests that some cavitation did occur in this instance. These observations were repeated upon 6 other twigs with little or no evidence of cavitation.

Stem and Entire Twig Frozen—Water Degassed. Slowly freezing and holding an entire twig at $-11°$ during the night and then slowly rewarming the twig did not impede the flux of water entering the

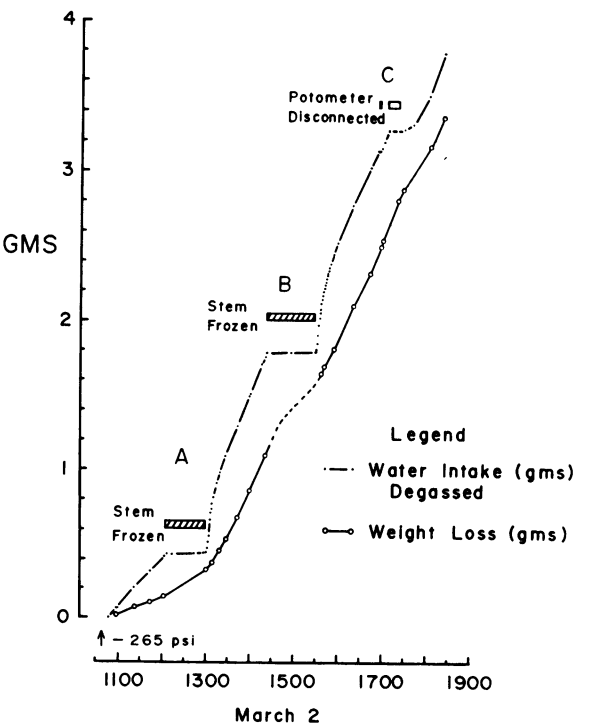

FIG. 1. Water intake (g) and weight loss (g) of hemlock twig as a function of time (initial twig wt = 14.8 g). Prior to measurements of water exchange, twig was held at 2°. At 1040 twig was transferred from +2° to +22°, RH =30 %. 4 cm of stem was frozen for length of time indicated by length of hatched bar at A and B. Stem was removed from potometer for 1 minute and then for 15 minutes (indicated by length of open bar) at C.

stem as illustrated in figure 2. For this test, the twig was initially dehydrated to 165 psi xylem tension before it was joined to the potometer containing degassed, filtered water. It was next tested by freezing a section of the stem, figure 2A. After freezing the entire twig for 14 hours, figure 2B, and while rewarming it, a section of the stem was kept frozen with CO_2 snow until transpiration was reestablished. Following removal of the freeze clamp at C, there was at first an inrush of water followed by a steady state flux of 3.9 %/hr which was equal to the transpiration rate and was only slightly less than 4.2 %/hr, the initial steady state influx before freezing at A. Freezing the entire twig therefore did not impede the flow of xylem water except when the water was frozen.

Entire Twig and Stem Frozen—Water Saturated. A twig was joined to a potometer containing water saturated with air at 1°. The twig and potometer were held at 4° for 7 hours so that 1.5 gms of water entered the twig. The temperature of the 13.5 gm twig was slowly lowered to −15° for overnight. The twig was slowly warmed to above freezing then transferred to 22°. Water intake and weight loss increased rapidly to a steady state rate of 4.8 %/hr.

Entire Twig Frozen Prior to Measurements. Slowly freezing an entire twig for 4 hours on the day before measurements of weight loss and intake of degassed, filtered water were made again did not impede water movement. The steady state influx was 3.6 %/hour. The steady state influx at 22° in another twig that was slowly frozen to −13° for overnight and slowly rewarmed was 5.2 %/hr.

Stem Frozen with Degassed and again with Saturated Water—also Entire Twig Frozen. The above observations are illustrated again by a sequence of tests upon a single twig and lasting for 52 hours, figure 3. Measurements were started 3 minutes after the twig was cut from the tree. In the first test, 4 cms of the stem was frozen with CO_2 snow while degassed water entered the twig, figure 3A. As before, releasing the freeze clamp on the water produced a rapid inrush followed by a steady state influx equal to the transpiration rate, and the influx and transpiration rates were the same before and after freezing. The steady state influx was 5.9 %/hr before and 5.7 %/hr after stem freezing. This test was repeated an hour after switching to water saturated with air at 20°, figure 3B. The steady state influx before freezing was 5.7 %/hr and 5.9 % after. Therefore, when water containing dissolved gases was taken up by the stem, the result was the same as when degassed water was absorbed. In figure 3C, the stream of water entering the stem was interrupted for 10

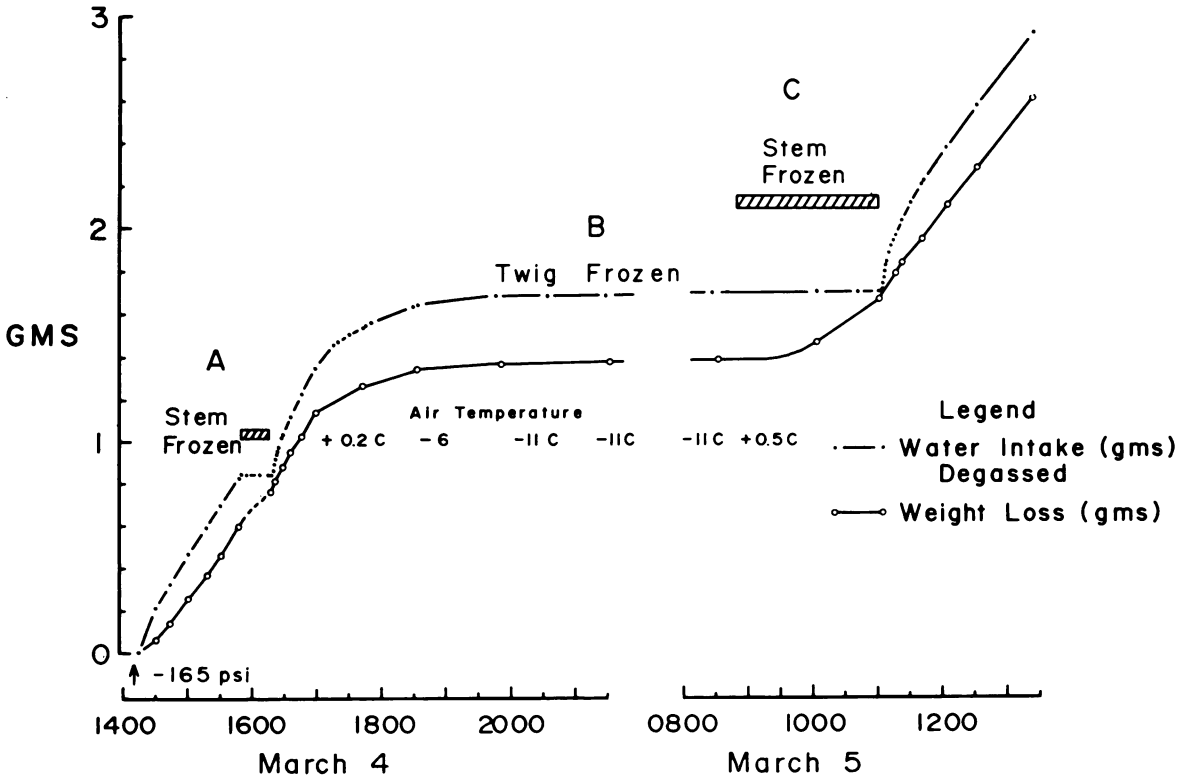

FIG. 2. Water intake and weight loss of hemlock twig with 4 cm of stem only frozen at A, entire twig except end of stem frozen at B, and with 4 cm section of stem only frozen at C. Initial twig weight = 10.9 g. Air temperature at 21° except as noted at B for sub-zero values.

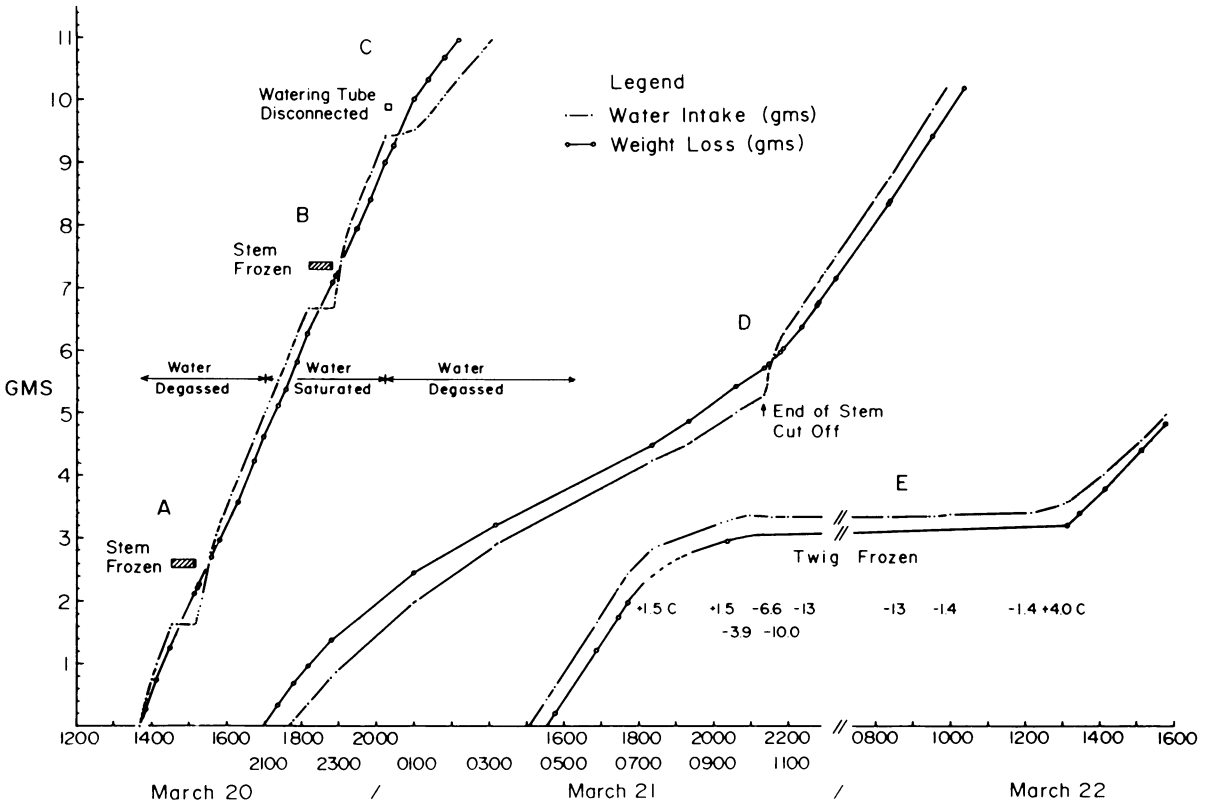

FIG. 3. Continuous record of water intake and weight loss of hemlock twig (initial twig wt = 25.3 g). Note that time scale has been telescoped at 2200 March 20, and at 1500 March 21; a break in time is indicated between 2100 March 21 and 0700 March 22. The ordinate scale goes from 10 to 21 g for the segment of the curves running from 2100 March 20 to 1500 March 21. The scale goes from 20 to 31 g for the curves from 1500 March 21 to end of measurements. Air temperature at 20° except as noted at E for sub-zero values.

minutes while the cut end was exposed to air before the stem was allowed once again to take up degassed and filtered water. As a consequence, the transpiration rate decreased to 1.2 %/hr as though an impediment to flow had been introduced. The rate of influx of water was initially and greatly diminished and only gradually did it become equal to the transpiration rate. The latter was also slowly reduced by the impediment. As shown in figure 3D, the impediment was nearly eliminated by cutting off under water only a few millimeters from the end of the stem. There was now a rapid inrush of water followed by a steady state influx of 3.7 %/hr that matched the increased transpiration rate. Next, the entire twig, except the first few cms of the stem and the potometer, were slowly frozen to −13° for more than 10 hours and then rewarmed, figure 3E. During the freezing phase, no water entered the twig, and transpiration was greatly reduced. Upon rewarming to room conditions, i.e., 20° and an R.H. of 30 to 40 %, transpiration was restarted followed by a matching water intake at 2.5 %/hr or somewhat less than the rates prior to freezing.

Twig Equilibrated to 10 atm N_2 Prior to Freezing Entire Twig and Stem. A twig was equilibrated to 10 atmospheres of nitrogen at 0.5°. Then the pressure was slowly reduced to 1 atmosphere and the entire twig was slowly frozen to −15° for 5 hours. Next the twig was slowly rewarmed and held overnight at 15°. The water influx and weight loss were measured the following day at 22°. The steady state influx after connection with potometer was 4.5 %/hr. After freezing the stem for 1 hour, the influx was reduced only slightly to 4.0 %/hr.

Stem Freezing of Twig with Spring Growth. In 2 experiments, 1 illustrated in figure 4, a twig with 2 to 3 cm of new growth on all tips was dehydrated until the hydrostatic tension in its xylem sap increased to approximately 132 psi. It was then joined to the potometer containing water at 22° saturated with air. About an hour later the tension decreased to 35 psi and the steady state rate of water intake, as well as the rate of weight loss, was 5.2 %/hr. The stem was frozen in the usual way with CO_2 snow for about 30 minutes and the tension increased to about 111 psi, figure 4A. After rewarming of the stem, there followed an

inrush of water and a steady state influx which was the same as before stem freezing; the tension was also reduced to 35 psi. Therefore no change in the resistance to the flow of water through the stem had occurred due to freezing a section.

On the other hand, a marked increase in resistance to flow was produced at the cut end of the stem by separating the potometer from the stem for 15 minutes, figure 4B. After rejoining the stem to the potometer, there was no inrush of water but only a very gradual increase in the influx as the tension increased to greater than 170 psi. The impediment was removed by cutting off 7 mm from the end of the stem under water and again joining the stem to the potometer. Now there followed an inrush of water, figure 4C, and the tension dropped to 21 psi. The steady state influx and rate of weight loss were less than initially (from 5.2 to 2.9 %/hr), possibly due to the partial collapse of some of the conducting tissue in the new growth

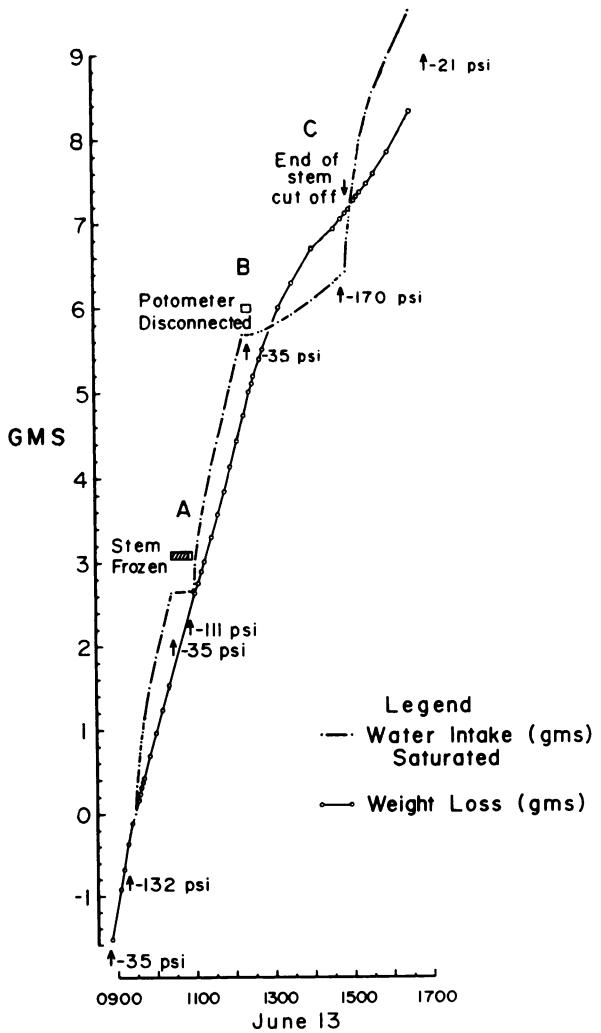

FIG. 4. Water intake and weight loss of a 35.7 g twig that had 2 to 3 cm of new growth on each tip.

when the tension exceeded 170 psi. The reduced influx cannot be attributed to residual impediment left in the stem because the tension, after steady state influx, was not increased. On one occasion involving no freezing, a twig with new growth was dehydrated until the tension exceeded 210 psi. When it was joined to the potometer in the normal way, the influx of water was very low; probably due to collapse and damage to the conducting mechanism in the new growth.

Stem Freezing with Partial Cavitation. Partial cavitation can be produced by stem freezing, especially when the sap tension is allowed to increase in excess of 300 psi by prolonged stem freezing when the transpiration rate is high. Partial cavitation did occur in 1 twig. Prior to stem freezing, the water influx was 8.0 %/hr. During stem freezing, the hydrostatic tension increased from 63 to 348 psi before rewarming occurred. Although there followed an inrush of water, the steady state influx did not return to the prefreezing rate; both the steady state influx and transpiration were reduced to 3.9 %/hr. On 2 other occasions, a similar partial cavitation was produced even when degassed water was used in the potometer.

Part II. Hydrostatic Tension in Twigs on Some Evergreen and Deciduous Trees and Shrubs Before and After Freezing Stem in Situ. A 2 to 3 cm section of the stem of twigs on several trees and shrubs was frozen for 5 minutes with CO_2 snow. Just prior to freezing the stem (still attached to the tree), the hydrostatic tension in an adjacent twig was determined. The experimental stem was often frozen in the morning when the sap tension was still low. The tension in another adjacent twig was measured in the afternoon when transpiration was greatest. One to 2 hours after sundown, the tension was measured in yet another adjacent twig and in a twiglet from the experimental twig whose stem was frozen. The experimental twig was examined daily for signs of wilting and in some instances more twiglets were tested for hydrostatic tension. All of these tests were made in July.

Table I summarizes the results obtained when freezing for 5 minutes the intact stem of several trees and shrubs. When cavitation occurred, as in the American elm, grey birch, black oak, sugar and red maple, black cherry and dogwood, the tension in the cavitated twig continued to rise to values in the high hundreds of psi with consequent wilting, withering and even crisping of the leaves within a few hours of cavitation on a dry day. The tension in the cavitated twigs was not relieved after darkness when the tension normally drops, as in the control twigs.

When cavitation did not occur (eastern hemlock, white pine, juniper, and yew), the tension in the twig with frozen stem followed the normal diurnal variation in tension observed in the control twigs. Irreversible damage was done, however,

Table I. *Hydrostatic Tensions in Xylem Sap of Twigs. Before and After Freezing in situ a 2 to 3 cm Section of Stem for 5 Minutes*

Species	Pre-freeze control Psi	Pre-freeze control Time	Time Frozen	Post-freeze control Psi	Post-freeze control Time	Post-freeze experimental Psi	Post-freeze experimental Time
Tsuga canadensis	231	1400	1400	130	2100	137	2100
	184	1400	1400	103	2230	109	2230
	129	0800	0800	225	1500		
				142	2200	142	2200
Pinus strobus	132	0800	0800	185	1500		
				106	2230	108	2230
Juniperus communis	35	0800	0800	200	1530		
				68	2300	65	2300
	182	1200	1200	178	1830	185	1830
	417*	1900					
Taxus canadensis	66	0800	0800	190	1600		
				38	2300	37	2300
	176	1200	1200	37	2200	38	2200
	386*	2200					
Meta sequoia glyptosprobodies	34	0830	0830	152	1530		
				17	2200	25	2200
	166	1200	1200	30	2100	270	2100
	475*	2100					
Kalmia latifolia	6	0800	0800	175	1500		
				4	2130	4	2130
	104	0900	0900	153	1500		
				17	2100	195	2100
						9	0630
	140	1230	1230	2	2230	78	2230
	>600*	2230					
Buxus sempervirens	52	0900	0900	150	1430		
				14	2130	9	2130
	153	1230	1230	23	2300	192	2300
	310*	1930					
Betula populifolia	164	1500	1500	17	2230	300	2230
	26	0830	0830	195	1500		
				8	2200	355	2200
Quercus velutina	255	1430	1430	130	2200	340	2200
Quercus coccinea	202	1130	1130	48	2130	480	2130
	710*	2130					
	4	0630	0630	275	1600		
				15	2200	60	2200
Fraxinus americana	50	0630	0630	290	1630		
	590*	2300		65	2230	600	2230
Prunus serotina	23	0630	0630	300	1600		
	780*	2300		53	2200	210	2200
	288	1300	1300	62	2200	395	2200
	>900*	1930					
Cornus florida	212	1130	1130	160	1830	378	1830
	500*	1840					
	24	0630	0630	175	1600		
				67	2200	77	2200
Acer saccharum	265	1500	1500	125	2130	465	2130
	32	0800	0800	195	1600		
				52	2230	570	2230
Acer rubrum	252	1500	1500	42	2200	420	2200
Ulmus americana	242	1530	1530	70	2100	690	2100

* The pre-freeze control twig was suspended near the experimental twig and tested again at the later time.

even to these frozen stems so that after a few weeks the leaves and stems were dead, but the disfunction cannot be attributed to cavitation in the xylem sap. In 2 instances, hemlock stems frozen in mid-April, before new growth had appeared, went on to develop 1 to 2 cm of new growth which appeared normal but which was dead by mid-June.

If the tension was high when freezing took place, cavitation was also observed in the dawn redwood and partial cavitation was observed in the mountain laurel and in the box. On the other hand, only slight cavitation occurred in the scarlet oak when the xylem tension was very low, 4 psi, when frozen; similarly for the dogwood when freezing the sap at only 24 psi.

Part III. Hydrostatic Tension vs. Volume of Water Expressed from Twig. These tests were made only upon twigs from the 10 year old Connecticut grown hemlock. The initial hydrostatic tension in the twig was measured with the twig in the pressure chamber (8, 9). Then a small amount of sap was expressed from the twig by over-pressure in the chamber; the amount removed was weighed; and the new hydrostatic tension was determined. Successive removals of sap were made and weighed, and the hydrostatic tension was determined after each removal.

An example of the relationship between the reciprocal hydrostatic tension and the volume of water removed from an autumn (October) hemlock twig is illustrated in figure 5A. Pressing out the first 0.4 ml of water from this 7.4 gm twig dropped the turgor pressure within the cells to zero and, at the same time, increased the osmotic pressure of the cell sap. Pressing out more water can only increase the osmotic pressure which now is balanced only by the hydrostatic tension in the sap outside the cells. The linear part of the curve in figure 5A is the zero turgor line for which osmotic pressure and hydrostatic tension are equal for every volume. To the right, it extrapolates to the intra-

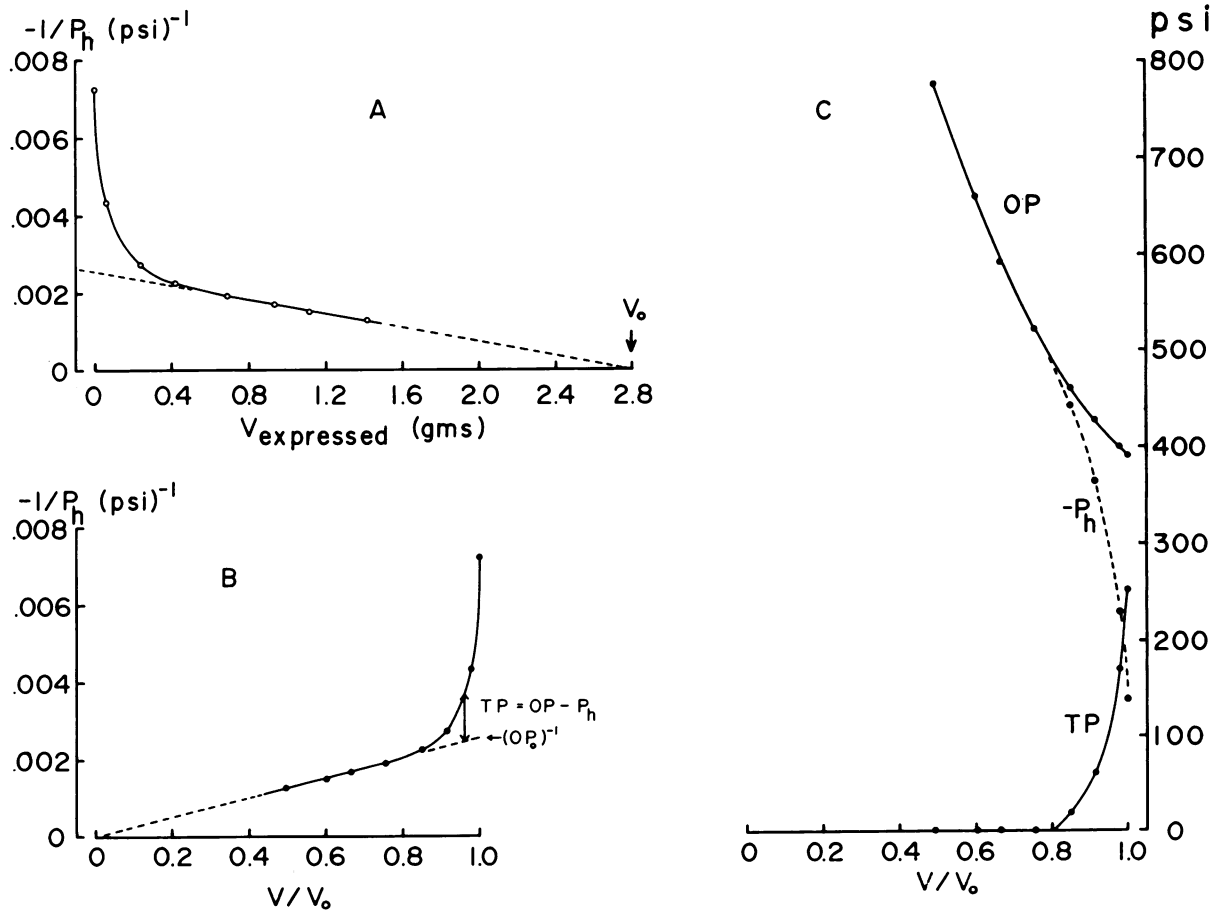

FIG. 5. A) Reciprocal of negative hydrostatic pressure of xylem sap vs. volume of water expressed from the twig (initial wt = 7.4 g). Broken line extrapolates to the right to the initial intracellular volume of leaf water, V_o. B) Reciprocal of hydrostatic tension vs. fraction of intracellular water. $V = V_o - V_{expressed}$. Since the linear portion of the curve pertains to cells at zero turgor pressure so that the balancing hydrostatic pressure equals the osmotic pressure of the cell sap, the line extrapolates to the initial osmotic pressure, OP_o. C) The turgor pressure, the intracellular osmotic pressure and the hydrostatic tension (negative hydrostatic balancing pressure) are plotted as a function of V/V_o.

cellular water in the leaves of the twig, V_o. Ideally, infinite tension is required to suck out all of the cell water. To the left, it extrapolates to the initial osmotic pressure, OP_o, of the intracellular sap before any water was removed. For this twig and another October twig, OP_o was 391 and 365 psi respectively (for the same turgor pressure) and V_o was equal to 80 % and 77 % of the total water content of the twig. Five twigs pressed in January after they had experienced several recent freezing periods yielded values of OP_o and V_o which were respectively 410, 407, 365, 385, 400 psi and 76 %, 83 %, 80 %, 91 %, 83 %. These values average to OP_o = 393 psi and V_o = 83 % of total leaf water content and are not different from the October values. One summer twig (July) had a significantly lower initial OP_o at the same turgor pressure, namely, 277 psi while the volume of its intracellular sap was 77 % of total water content of leaf.

Discussion

The results reported here are interpreted to mean that freezing can and does occur in stems and in entire twigs of hemlock and in stems of some other evergreens without increasing the resistance to the flow of water through the frozen part after rewarming has occurred. This, in turn, is interpreted to mean that freezing has not produced cavitation in the xylem sap even though A) the sap was unquestionably frozen; B) it contained dissolved gases; and C) it was under tension before freezing and after.

If the dissolved gases contained in the sap do come out of solution when the sap freezes (because the solubility of gas in ice is so very low), then perhaps the apparent lack of cavitation can be explained in 4 steps: A) describing the construction of the conducting tube which permits the normal hydrostatic tension to exist in the sap without collapsing the wall of the tube, B) analyzing the relationship between the hydrostatic tension in the sap and the physical properties of the wall of the conducting tube, C) applying the analysis to estimate how much sap water must freeze in order to reduce the tension in the unfrozen sap to zero, and D) suggesting the unique property of gymnosperms to which freezing without cavitation applies. The analysis is based upon a model of a tracheid as depicted in figure 6A for a late growth, thick walled tracheid in the secondary xylem of a gymnosperm.

In the tracheary elements of most vascular plants, the physical and chemical properties of the structural components of the cell wall are presumably selected and arranged to prevent collapse of the lumen when the water contained therein is under 10 to 20 atmospheres of suction pressure during transpiration. The high tensile strength of the cellulose microfibrils, the way they are arranged, and the hydrophilic properties of cellulose, hemicellulose and pectic substances in the cell wall strongly suggests that the cell walls of the tracheids and vessel elements are pre-stressed by an amount which exceeds the maximum suction pressures encountered during transpiration.

The tracheary elements of the secondary xylem consist of a thin, outer primary wall and an inner and thick secondary wall in which forms the specialized perforated plate and/or bordered pits for the free fluid communication between adjacent elements (5, 6). The primary cell wall, when first formed, shows a predominantly transverse orientation of microfibrils, but as the cell matures the orientation becomes more disperse and the microfibrils appear interwoven. After the primary wall ceases to increase in surface area, a much thicker 3-layered secondary wall is laid down consisting of cellulose, hemi-cellulose, pectic substances and gums and is heavily lignified. The outer layer of the secondary wall develops as annular or helical thickenings with the microfibrils of cellulose running transverse to the axis of the cell. The fibrillar orientation of the inner layer may vary between transverse and helical. The comparatively wide central layer of the secondary wall consists of laminations of microfibrils oriented between longitudinal and steeply pitched helical. The microfibrils are groupings of cellulose molecules arranged such that crystalline regions of cellulose molecules (micelles) are longitudinally separated from regions where the molecules are less perfectly joined and allowing water to attach to the hydroxyl groups in this amorphous region. Other constituents of the wall, gums, hemi-celluloses, pectic substances are also highly hydrophilic and swell when absorbing water.

These details of the wall of a tracheid have been briefly reviewed to suggest that the transverse microfibrils of the primary wall and of the outer layer of the secondary wall are pre-stressed (under tension) by the swelling of the hydrophylic molecules of the central layer which must be under compression.

Hooke's law may now be applied to this working model of the cell wall of a tracheid in order to estimate the decrease in hydrostatic tension caused by an increase in volume when sap water expands upon freezing. Referring again to figure 6A, the change in tension of a unit length of the outer elastic band of thickness t composed of the primary wall and outer layer of the secondary wall may be described by Hooke's law,

$$\frac{dL}{L} = \frac{}{r_o} = \frac{l}{E} dT_o$$

where L is the circumference of the outer elastic band of radius r_o and E is its Young's Modulus. The modulus of elasticity, E, of this cellulose ma-

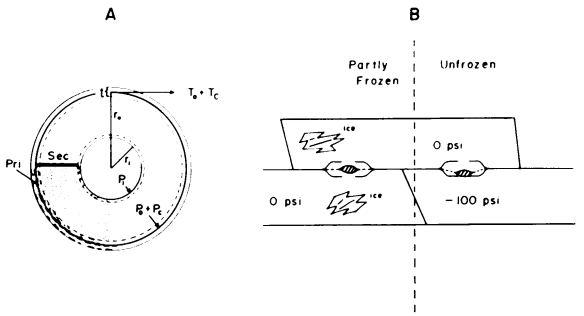

FIG. 6. A) Model of a tracheary element in cross section showing the construction of primary and secondary walls and showing the compression pressure P_c generated by the hydrophylic central layer, the hydrostatic pressures P_i and P_o all acting to produce the tensile stress ($T_o + T_c$) of the outer thin elastic bank of thickness, t. B) Model of tracheids at the boundary between partially frozen and unfrozen cell sap showing the hydrostatic tensions and positions of the tori in the bordered pits.

terial will probably equal or exceed the longitudinal modulus of elasticity of green wood which is about 1.2×10^6 psi. The relationship between the pressure, $P_o + P_c$, exerted by the swollen central layer and the tension, $T_o + T_c$, in the outer layers of thickness t is

$$(T_o + T_c)\ t = (P_o + P_c)\ r_o$$

where T_c is the pre-stressed tension due to the compression P_c of the middle layer. Assuming that P_c and therefore T_c do not vary with changing P_o and r_o, Hooke's law can be written

$$\frac{dr_o}{r_o} = \frac{l}{Et}\ [(P_o + P_c)\ dr_o + r_o\ dP_o]$$

or

$$\left[1 - \frac{(P_o + P_c)\ r_o}{Et}\right] \frac{dr_o}{r_o} = \frac{r_o}{Et}\ dP_o$$

For a unit length of cylinder, the ratio of the lumen volume, V_i, to the total volume, V_o, is also the inverse ratio of a change of pressure in the lumen to the change of pressure on the outer shell, i.e.,

$$A = \frac{V_i}{V_o} = \frac{r_i^2}{r_o^2} = \frac{\Delta P_o}{\Delta P_i} = \frac{P_o}{P_i}$$

These ratios are based on the assumption that the bulk modulus of elasticity of the thick secondary wall is infinite.

Since $V_o = \pi r_o^2$ and $\frac{dV_o}{V_o} = 2\frac{dr_o}{r_o}$, the above equation of Hooke's law can now be written

$$\frac{1}{2}\left[\frac{Et\sqrt{A}}{r_i} - (AP_i + P_c)\right]\frac{dV_o}{V_o} = A\,dP_i$$

or

$$\Delta P_i = \frac{1}{2}\left(\frac{Et}{\sqrt{A}\ r_i}\right)\frac{\Delta V_i}{V_i},$$

since both P_i and P_c are negligible compared with

$$\frac{Et\sqrt{A}}{r_i}.$$

Approximate values for the constants in this expression are:

$$E = 5.2 \times 10^6 \text{ psi};$$

this value was obtained by Barkus (1) as the longitudinal Young's modulus for the cell wall material of Sitka spruce. The same modulus for the gross wood was about one third, or 1.8×10^6 psi.

A = 0.6 or ranging from 0.4 to 0.7 (1)

r_i = 5μ as measured in a hemlock stem

t = 0.05μ, the wall thickness was estimated to be about 10 % of the cell radius and the outer transverse layer was estimated to be about 10 % of the total wall thickness.

For these values

$$\Delta P_i = 3.4 \times 10^4\ \frac{\Delta V_i}{V_i}.$$

Water expands by about 9 % when it freezes. Therefore, from this application of Hooke's law to an idealized tracheid, there is an estimated decrease of 30 psi in the hydrostatic tension when 1 % of the sap in the tracheid freezes. To change the tension in the sap from 100 psi to zero, only 3 % of the sap would need to freeze. As more of the sap in an isolated tracheid freezes, what was once negative hydrostatic pressure (tension) before freezing started now becomes positive hydrostatic pressure. As most of the tracheid sap becomes frozen, the yet unfrozen sap containing the dissolved gases may be at a pressure of 1000 psi or more assuming that the structural cellulose of the wall does not yield, i.e., does not undergo plastic flow and permanently lengthen. Upon warming and melting, the pressure changes would follow in reverse order so that not until 97 % of the sap ice had melted would the sap pressure return to zero and only as the last 3 % of the ice melted would

the hydrostatic tension of 100 psi return to the melted sap.

Although the values of the constants used in Hooke's law to compute the change in pressure associated with the expansion upon freezing of the sap were selected to be most representative for hemlock and other conifers, they may be in some error. Worrel (10) correlated the diameter of trees measured by a cathetometer with the hydrostatic tension measured by the pressure chamber method (8, 9). Over a period of 1 week, he slowly dehydrated an initially well watered greenhouse grown *Pinus radiata* (3 cm in diameter and 4 m in height) and compared diameter with the hydrostatic tension as tension increased. From his data

$$\triangle P = 1.5 \times 10^4 \quad \frac{\triangle D}{D} \text{ psi.}$$

Since $\frac{\triangle D}{D} = \frac{1}{2} \frac{\triangle V}{V}$ (assuming only the diameter changes with dehydration), then

$$\triangle P_i = 0.75 \times 10^4 \frac{\triangle V}{V}.$$

The constant obtained from this experimental data is only about one-fifth the value obtained from the above application of Hooke's law to an idealized tracheid. The smaller value obtained from Worrel's data may reflect the modulus of elasticity for the gross wood which is usually about one third the modulus for the cell wall material (1).

Regardless of the precise relationship between a change in pressure and the corresponding change in lumen volume of a tracheid, the analysis suggests: A) whatever tension there was in the sap prior to freezing, it would have been reduced to zero when a small amount of the sap water froze; B) any additional freezing would compress the sap and retain the dissolved gases in solution until the last water froze; C) when melting was again possible, the outgassed bubbles would be under great pressure to redissolve; D) only when the last of the ice had melted would tension develop in the melt water again.

These pressure changes which are here suggested to occur in the tracheids of a frozen stem section could not take place in open ended conducting tubes extending through the frozen section. As the water began to freeze and expand, it would flow out of the freezing zone through the open tubes into the unfrozen regions where the tension remains high. Thus, as all of the sap froze in one zone, the tension there could be only slightly reduced and certainly no positive pressure could develop. Upon melting, the melt water would be under tension and the outgassed bubbles would expand and cavitate the xylem sap. Therefore, there must be something about the tracheids found in gymnosperms and in abundance in other woody plants which isolate the pressure changes occurring in a freezing zone from the tension remaining in the adjacent unfrozen sap. A mechanism is suggested in figure 6B. On the partly frozen side of the boundary, enough sap has frozen to reduce the tension to zero in all adjacent tracheids including the one that straddles the boundary. Therefore there is no tension difference between these tracheids, and the tori of the bordered pits remain in open position. There is, however, a tension difference between the tracheid straddling the boundary and the adjacent tracheids on the unfrozen side. This gradient closes the bordered pit and isolates the frozen tracheids from the unfrozen ones. These bordered pits remain closed until the tension returns after all the ice has melted.

An observation was made which can be easily interpreted by the above model. A hemlock twig was enclosed in the pressure chamber with the cut end of the stem extending through the pressure seal to the outside. The pressure was increased to 20 psi and the chamber was slowly cooled to $-10°$ while the stem extending through the seal was maintained above 0° by a small heater around the stem. As freezing occurred, no sap appeared at the cut end of the stem even though the sap froze inside the chamber and did not freeze in the stem outside. Presumably, the expanding sap was isolated in the tracheids where the freezing occurred.

Partial cavitation was observed with freezing of a section of hemlock stem, especially when the tension was allowed to increase to high levels before melting occurred. If the bordered pits in the tracheids straddling the boundary between partially frozen and unfrozen tracheids were to become leaky, then any outgassed bubbles in the partially frozen tracheids would not be under high pressure and may not be redissolved when the tension returns upon rewarming. The sap in these tracheids would then cavitate.

Extensive cavitation leading to wilting, withering, and death by dehydration within a few hours in the several angiosperms tested may also be explained by suggesting that these species rely heavily upon vessel members rather than tracheids for conduction of water from root to leaf. Unlike the short tracheids with bordered pits which may seal when a pressure gradient develops between adjacent elements, the vessel members are long and may be continuous from root to leaf. When not continuous, they are terminated with perforated plates which will not pass an air-water interface but also will not isolate 2 end-to-end vessels; the perforated plate cannot maintain a pressure gradient in the liquid between 2 connected vessels. Therefore, when melting occurs, the melt water may not be under pressure and the gas bubbles which were frozen out of solution may not be

redissolved by the time the melt water must undergo tension as the last ice crystals melt.

Those angiosperms which are evergreen in the temperate zone and withstand winter freezing, apparently rely upon their tracheids for water conduction even in the summer time since they show much less tendency to cavitate when a section of stem is frozen.

There is another pressure to consider which may possibly assist in redissolving into the melt water any bubbles frozen out of solution. This pressure results from the surface tension of water compressing the enclosed gas in the bubble. Its magnitude in dynes per cm^2 is

$$P = \frac{2S}{r}$$

where the surface tension of water against air at 0° is 75.6 dynes/cm and r is the bubble radius in cm. To estimate the magnitude of r, first assume that all the water in a tracheid is saturated at 0° and when frozen it outgasses to form a single bubble. The radius of such a bubble for a tracheid which is 0.5 mm long and having a lumen radius of 5μ is 6.5×10^{-4} cm. The internal pressure in this bubble due to surface tension would be 0.23×10^6 dynes/cm^2 or 3.4 psi. On the other hand, if the gas were to form a series of bubbles each containing the gas contained in a length of water equal to 1 diameter of the tracheid, then the bubble radii would be 0.86×10^{-4} cm and the internal pressure would be 12.5 psi.

Of course, the actual size of the bubbles which freeze out of solution is unknown and even more uncertain would be their size when tension returns to the melt water as the final crystals melt. If cavitation does not occur, it is reasonable to suppose that either the gas is redissolved or that the bubble size is so small that the internal pressure exceeds the tension in the xylem sap and the bubble cannot expand. Therefore the resolution pressure may result primarily from hydrostatic pressures in the melt water due to the expansion of ice upon freezing in a closed system. To this pressure may be added a not insignificant pressure due to surface tension if the bubbles which freeze out are sufficiently small. The bubbles may be rendered small both by the way they are frozen out of solution at time of freezing and by the primary resolution pressure due to the expanding ice. In those angiosperms which appear to have cavitated xylem sap when frozen, the out frozen bubbles may be large in size due to the greater volume of lumen water per unit length of vessel so that there is less internal bubble pressure due to surface tension. Due also to the absence of an isolating mechanism, the sap may not develop pressure from the expanding ice and therefore the bubbles may not be reduced to sufficient size and increased to sufficient internal pressure to withstand expansion by the hydrostatic tension in the sap; or as suggested by Dimond (2), the undissolved bubbles may simply obstruct the pores in the perforated plate producing increased resistance as if cavitation had occurred.

The experiment illustrated by figure 5 was introduced into this study of freezing without cavitation in hemlock to show the seasonal effect upon the osmotic pressure of the intracellular sap of mature leaves. Apparently the absence of cavitation in frozen xylar sap in the stem is not related to the *OP* of the cell sap which appears to be higher in the winter. There is also in figure 5 some information regarding the rheology of parenchyma cells within the leaf.

The above discussion of an approximate relationship between stress and strain for tracheary elements pertains to tissue composed of dead cells with thick lignified secondary cell walls and designed to perform mechanical functions. *Parenchyma* tissue, on the other hand, is composed of living cells with only thin primary cell walls. The rheology of these cells clearly differs from that of the tracheary elements in that the hydrostatic pressure in parenchyma cells is usually positive, seldom zero and rarely negative (it may be negative only when the suction pressure of the xylem sap exceeds the osmotic pressure of the cell sap which can occur as a transient condition and perhaps during death by dehydration).

An approximate statement of Hooke's law for parenchyma cells may be derived on the assumption that the cells are approximately spherical, at least, when turgid. For a sphere, a change in tension, $\triangle T$, of an elastic element of the wall of width $rd\phi$ and thickness t will be given by Hooke's law as

$$\frac{dL}{L} rd\phi = \frac{1}{E} dTrd\phi$$

or

$$\frac{dr}{r} = \frac{1}{E} dT$$

where L is the length of a great circle and r is the radius.

Since, for a sphere, the relationship between internal hydrostatic pressure, P, and tension, T, in the wall is

$$T = \frac{Pr}{2t},$$

and

$$dT = \frac{1}{2t} (rdP + Pdr),$$

and since the volume is $V = 4/3\ \pi\ r^3$ and

$$\frac{dV}{V} = 3\frac{dr}{r},$$

Hooke's law can be rewritten

$$\frac{dV}{V} = \frac{3\ r}{2\ Et}\left(dP + \frac{P}{3}\ \frac{dV}{V}\right).$$

Rearranging,

$$\triangle P = \frac{1}{3}\left(\frac{2\ Et}{r} - P\right)\frac{\triangle V}{V}.$$

Returning again to figure 5B, the turgor pressure, TP, within the leaf cell may be obtained for each fraction, V/V_o, of the initial volume remaining in the cell. Since $TP = OP - P_h$, the turgor pressure for each V/V_o may be obtained by subtracting the reciprocal of the value on the solid line (reciprocal of external hydrostatic tension) from the reciprocal of the corresponding value on the broken line (reciprocal of the osmotic pressure). In figure 5C, the turgor pressure and the osmotic pressure within the cell and the negative hydrostatic pressure external to the cell are plotted for each fraction of the intracellular volume. The slope of the turgor pressure line may be used to estimate the modulus of elasticity of the average cell wall of the leaf parenchyma since the slope is equal to

$$\frac{1}{3}\left(\frac{2\ Et}{r} - TP\right)$$

in the above equation of Hooke's law. For the leaf cells of the hemlock twig of figure 5, the modulus of elasticity, estimated from the initial slope, was

$$E = 6{,}000\ r/t \text{ psi.}$$

The complex structure of the hemlock leaf, consisting of transfusion tissue around the vascular and epidermal tissue around the transfusion tissue (5), introduces 2 major errors in estimating the average modulus of elasticity from the above equation. The cells are not spherical as was assumed for the analysis. Had the cells been assumed to be cylindrical, the turgor pressure and transverse tension would be related by $T = \frac{Pr}{2t}$, the same as for a sphere, and the turgor pressure and longitudinal tension would be related by $T = \frac{Pr}{t}$. Shape, therefore, would affect the estimation of E by less than a factor of 2. A greater error will be made in estimating the average ratio of cell radius to wall thickness, r/t. An approximate ratio for a species of pine, see plate 78, Esau (5), appears to be 10 to 1 so that the modulus of elasticity of the cell walls for the hemlock leaf is approximately $E = 60{,}000$ psi. This is not an extraordinarily high value for a structure composed of cellulose. In fact, it is very low compared with the modulus of elasticity of strands of flax fiber, 11 to 15 $\times$ 10^6 psi (6). By inference, however, this value for the elastic modulus of the micellar structure of the living cell wall of the hemlock is adequate to withstand full turgor pressure without plastic flow. By the same calculation, the moduli of elasticity for the January and the July twigs were 44,000 and 25,000 psi respectively.

A remarkable fact to be noted in figure 5A is that the data for zero turgor is so nearly linear and extrapolates to 80 % of the total water content of the leaf. This indicates that most of the cells of the leaf behave like osmometers and most of them contribute water from intracellular sap.

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