

WINTER FREEZING IN RELATION TO THE RISE OF SAP IN TALL TREES¹

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The cohesion theory of Dixon and Joly (4) and Askenasy (1) has generally gained acceptance as the most likely explanation of how water rises to the top of tall trees (12). It may be taken as reasonably well established that in the summertime there are continuous water columns in the xylem, and experimental evidence that cohesive tensions of sufficient magnitude may develop in tall trees and lianas in a period of transpiration, has been furnished by experiments of potometer type (14, 21, 22, 23, 24, 25).

One may assume that leaf shedding does not per se break any such tension, as it has been shown that the walls of the vessels and tracheids will not let through a gas-water interface (29). With exception of the grapevine, and probably some other species which have air-filled vessels in the winter (28), it is known that the water content at all levels in the xylem of several species of trees remains as high or nearly as high in winter as in summer (7, 9, 15, 16, 30).

When water freezes, dissolved gas is forced out as bubbles. This happens whether water freezes in bulk or in glass capillaries (27). It has been shown that the sap of the grapevine is fully saturated with dissolved atmospheric nitrogen (28), and the same very likely holds also for other species. One must, therefore, assume that gas bubbles are formed whenever freezing takes place in sap-filled vessels and tracheids, and a great many of these would hence become emptied and eliminated for the transport system, were the sap under tension at the time of thawing. The only mechanism which might conceivably repair such breaks, would be a sap pressure high enough to redissolve the gas. In the case of the grapevine, which empties its vessels during the winter, the root pressure in the springtime is so high that it may readily refill the conducting system (28). But such pressures are at present known only in a few hardwood species (3, 8, 13, 17, 32).

Sub-zero temperatures with freezing of the sap, therefore, are difficult to fit into the cohesion theory, but this difficulty will not arise if the sap remains supercooled throughout the winter. A few degrees of supercooling has been produced experimentally in different species of trees, but this was observed for a brief period only (20).

In the present investigation the status of the sap has been studied in various species of trees at sub-zero temperatures.

MATERIALS AND METHODS

The following species have been used for the observations and experiments: Spruce (*Picea excelsa* (Lam.) Link.), pine (*Pinus silvestris* L.), linden (*Tilia cordata* Mill.), birch (*Betula odorata* Bechst.), maple (*Acer platanoides* L.), ash (*Fraxinus excelsior* L.), alder (*Alnus incana* (L.) Moench.), asp (*Populus tremula* L.) and willow (*Salix sp.*).

BUBBLE FORMATION WHEN WATER FREEZES IN CAPILLARIES: Water-filled glass capillaries, about 30 μ in diameter, were frozen at -8° C and examined under the microscope. A string of gas bubbles could be seen in the center of the ice, and after thawing, the bubbles persisted in the water for several days. This also happened when both ends of the capillary were sealed with grease, so no air could be drawn in from the outside.

Other glass capillaries (0.03 to 0.3 mm in diameter), bent to a Z shape and filled with water, were spun in the centrifuge by the method of Briggs (2). The pressure at the center was calculated to be -1.8 atmospheres. Freezing of the water by applying dry ice to the center of the spinning capillary (fig 1) resulted in immediate breaking of the water column. These experiments show that gas bubbles are formed and cohesion is broken when water freezes in glass capillaries of vascular or tracheidal dimensions.

Pieces of fresh spruce wood were put in a dish, covered with kerosene and observed with a microscope. The tracheids were water-filled and without bubbles. The dish was placed in a cold room, and the pieces were frozen at -8° C. Upon thawing, gas bubbles could be seen in many of the tracheids, although not in all.

DEMONSTRATION OF ICE IN TWIGS: During sub-zero temperatures twigs of many trees become brittle, and several authors have observed ice microscopically in different parts of living plants in the winter (19, 31, 33, 34): but one may ask to what extent the manipulation may possibly have caused a supercooled plant to freeze.

When ice is present in a plant it can easily be demonstrated by the heat of fusion. Many experiments were performed in the following manner. A thermocouple, covered by cotton, was placed on a twig of linden. The temperature was -6° C. When the twig was severed from the tree near the thermocouple, the temperature remained unchanged, nor did it rise when the cut end was seeded with snow. At room temperature a typical melting curve was ob-

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served, showing that the twig was already frozen while on the tree (fig 2).

EXTENT OF SUPERCOOLING: Fresh twigs of spruce and pine (1 × 10 cm) were kept for several hours in a cold room at 5° C. Thermocouples were placed on the outside of the bark underneath a piece of cotton and in bore holes in the wood. Both ends of the twigs were greased to prevent drying and to avoid accidental seeding with ice in the tracheids. The twigs were transferred to -10° C. Supercooling was regularly observed, but spontaneous freezing took

place within one half hour. Lowest supercooling temperature observed was -8.7° C (fig 3).

Branches of spruce, pine, birch, maple, ash, alder, asp and willow were sawed off at ground level or near the top of trees that were 12 to 14 meters high. The temperature in the wood was measured immediately with a thermocouple in bore holes and found to be the same as the air, i.e. -7° C. The wood around the thermocouple was then crushed with a pair of tongs and bent and broken, but the temperature remained unchanged. The same results were obtained

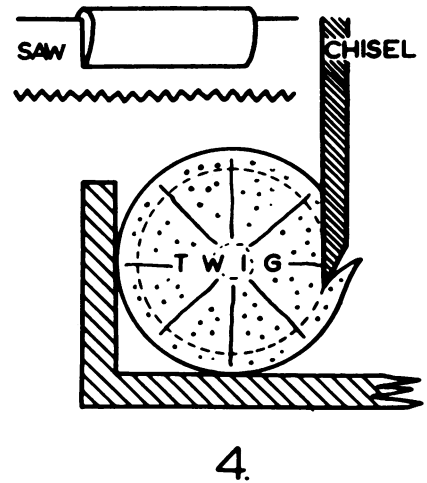
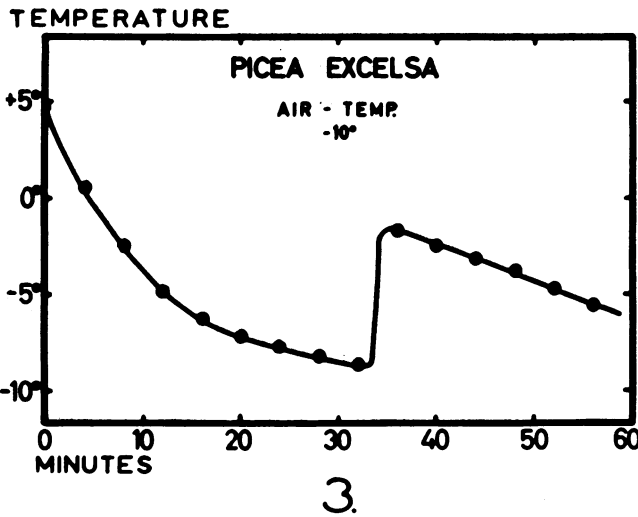
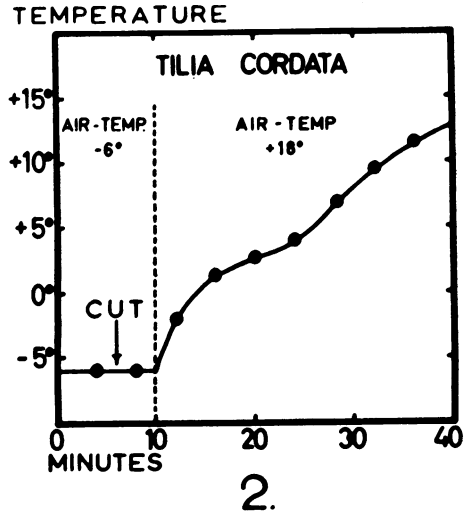
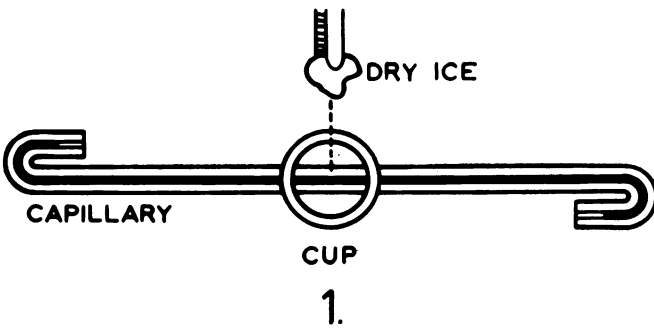


FIG. 1. Z-bent capillary mounted horizontally in the centrifuge (Briggs 1950). Center of the capillary at the spinning axis. Dry ice may be dropped in the cup while spinning.

FIG. 2. Temperature curve showing heat of fusion in a twig of linden, which was cut off and warmed to room temperature.

FIG. 3. Temperature curve showing supercooling of twig of spruce when transferred to a -10° C cold room.

FIG. 4. Twig end with bark shaved off. Saw with depth guide. Pieces used for the calorimetric measurements were broken off with a chisel.

when the thermocouple was put into the trunk of small trees which were similarly crushed and bent. This shows that the trees at all levels were frozen and not supercooled.

QUANTITATIVE DETERMINATIONS OF ICE IN THE XYLEM: Branches from different levels above ground or small stems of spruce, birch and maple were taken in March and brought without thawing directly to the cold room.

The amount of ice in the xylem was measured by a calorimetric method introduced by Müller-Thurgau (11, 18, 20, 26). A thermos bottle with 250 ml distilled water was used as calorimeter. A thermometer which could be read within $\pm 0.02^\circ \text{C}$ was inserted through the paraffined cork stopper. The heat capacity of the thermos with water was determined by dropping into it pieces of ice of known weight and temperature. Eight such measurements agreed within $\pm 1.5\%$.

After the branches or stems (3 to 4 cm thick) had remained at least 24 hours in the cold room at a desired sub-zero temperature, the following operations were conducted, taking care not to heat the samples. The twig ends were discarded, because of possible drying, and the bark trimmed off. Shavings, 5 to 6 cm long, were cut with a chisel from the outer 3 to 4 mm of the functional xylem (fig 4). About 20 g of the shavings were put into a wide-mouthed plastic funnel. The calorimeter, containing water of near 20°C , was brought into the cold room, received the shavings and was then immediately returned to room temperature. In one half minute, or less, the temperature became steady 2 to 3 degrees lower, and was read. The shavings were placed in an oven of 110°C for about 24 hours to obtain dry weight and total water content.

The amount of ice in the sample was calculated from the equation:

$$M_i = \frac{C(T_2 - T_3) - (S_d M_d + S_w M_w)(T_3 - T_1)}{H_i - (S_w - S_i)(T_0 - T_1)}$$

where C is heat capacity of the calorimeter; M_d , dry weight of the sample; M_w , total water content in the sample; M_i , amount of ice in the sample; S_d , specific heat of wood; S_w , specific heat of water = 1.0^2 ; S_i , specific heat of ice = 0.5 ; H_i , heat of fusion for ice = 79.9 ; T_0 , freezing point of the sap in the samples; T_1 , initial temperature of the sample; T_2 , initial temperature of the calorimeter; T_3 , final temperature of sample in calorimeter.

The specific heat of wood (S_d) does not vary measurably with species, habitat or anatomical part, but has been corrected for temperature according to the equation

$$S_d = 0.266 + 0.00116 \frac{T_3 + T_1}{2} \quad (\text{Dunlap 1912})$$

² The specific heat of the unfrozen water very likely lies between that of ice (0.5) and that of free water (1.0). If it were 0.7, we get a decrease of about 10% in the calculated amount of unfrozen water.

We have here calculated with a mean value for $S_d = 0.27$. The freezing point of the sap has been taken as 0°C . Even if it were -2°C , it would give no more than 3% increase in the calculated amount of unfrozen water.

RESULTS

The amount of unfrozen water has been calculated in relation to the dry weight of the samples (figs. 5, 6 and 7). It will be seen that it constitutes some 30 to 40% of the dry weight and decreases slightly with lowering of the temperature. This relation is independent of the total water content of the sample and shows that the unfrozen water is essentially that which is bound to the cellulose walls. About one tenth of the unfrozen water can be accounted for by the increased osmotic concentration of the sap as water freezes out.

The water content of a colloidal system depends upon the relative humidity, but is influenced very little by temperature (10). In the presence of ice, however, the relative humidity decreases with temperature. Our data for unfrozen water at sub-zero temperatures can, therefore, be compared with data for water content in white spruce at different relative humidities (6). As will be seen from figure 8, the curves are very similar, although showing slight species differences.

From these results we conclude that in winter time practically all sap within vessels and tracheids of the trees investigated freezes, forcing the dissolved gases out as bubbles at all levels.

DISCUSSION

It has been demonstrated in the present investigation that the free sap in the xylem of several species of trees freezes during the winter. All or the main part of the sap left unfrozen is bound to the cellulose in the wood. It is known that when water freezes in capillaries, dissolved gases are forced out as bubbles. The sap in the vessels of grapevine has been found to be fully saturated with atmospheric nitrogen (12 to 13 mm^3/ml) and more than half saturated with atmospheric oxygen (4 to 5 mm^3/ml) (28). Very likely, this holds also for other species. If all this gas were forced out by freezing of the sap, it would amount to about 2% of the ice volume.

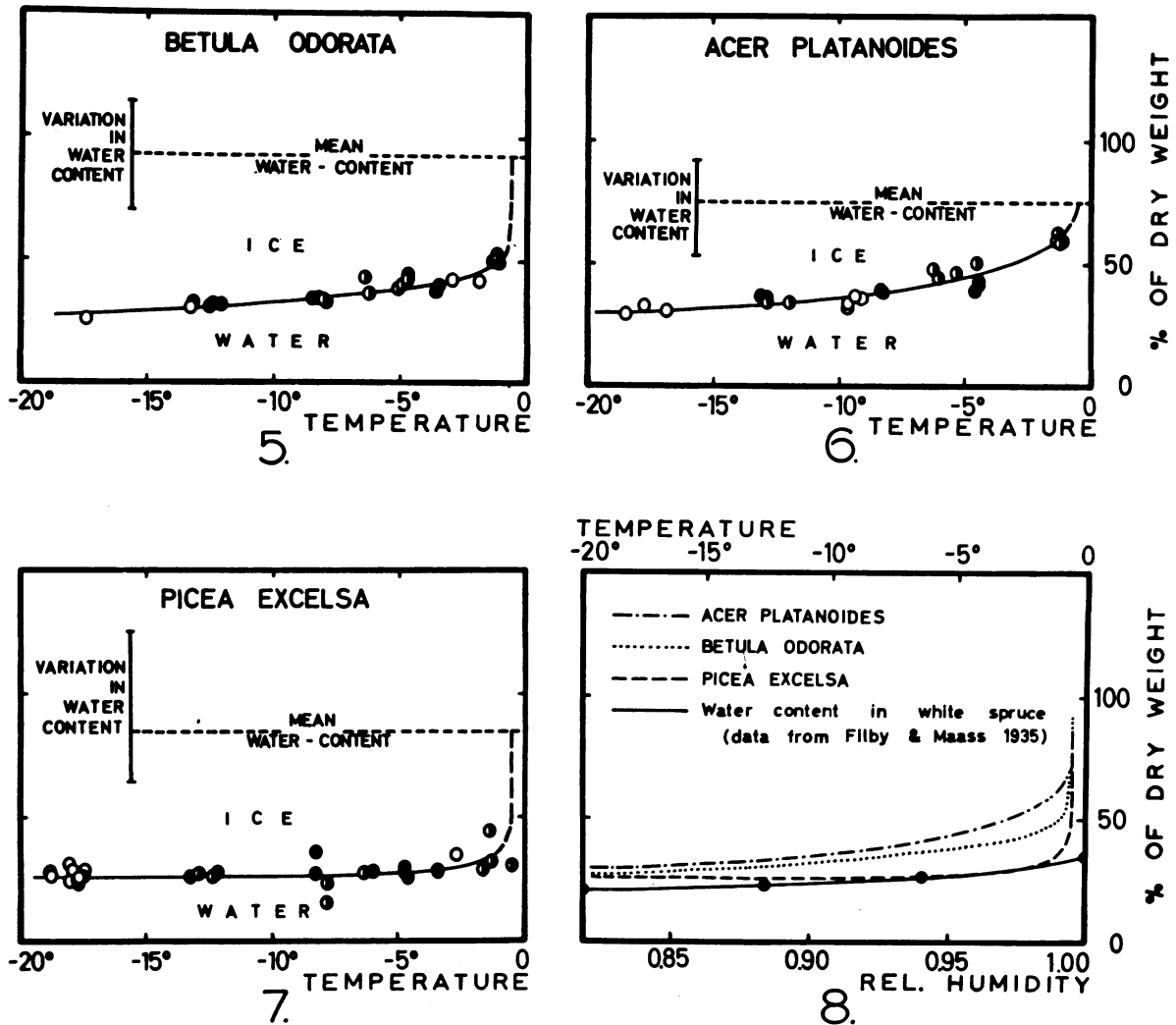
Were the sap under tension during freezing or thawing, the gas bubbles would expand and fill the units in which they were formed, and if the gas evolution were evenly distributed, bubble formation would destroy the cohesion in all tracheids and vessels. It was shown in our spruce experiments, however, that some tracheids did not contain bubbles after thawing. Evidently, therefore, some diffusion may take place through the walls. Migration of gas over great distances is unlikely, however, because supercooling will generally precede freezing, which then becomes a relatively even and rapid process.

With repeated freezing and thawing throughout the winter, one would expect an increasing number of elements to be set out of function by gas bubbles, but regular winter freezing is neither fatal nor even injurious to trees in cold climates. Therefore, one may assume that some mechanism exists which is capable of repairing gas breaks, and the only known mechanism which could accomplish this is a high hydrostatic pressure in the xylem. However, high stem pressures in the spring are known for a few hardwood species only, and the question still remains open: How are the gas breaks repaired? Accurate measurements of hydrostatic pressure gradients can give a clue to the solution of this problem.

SUMMARY

The status of sap in the xylem of tall trees during sub-zero temperatures has been investigated. Preliminary experiments showed that when water was frozen in fine glass capillaries, gas bubbles always separated out. By spinning the capillary in a centrifuge, the water was put under tension, and freezing resulted in breaking the cohesion. Gas bubbles were also formed in many tracheids of spruce wood when water-filled pieces were frozen and thawed under kerosene.

Twigs and stems of several species of trees were investigated at freezing temperatures. When crushed, broken and seeded with snow, their tempera-



FIGS. 5 to 7. Percent unfrozen water in xylem at various temperatures. Open circles: Material taken frozen in winter and transferred to cold room. Filled circles: Material taken in summer and transferred directly to cold room. Half filled circles: Material taken in summer, transferred to cold room after a few days at 5° C.

FIG. 8. Unfrozen water in spruce, birch and maple at sub-zero temperatures compared to water content in wood of white spruce at various relative humidities (Filby and Maass 1935). Abscissas give sub-zero temperatures and the equivalent relative humidities.

ture did not change, and at room temperature a typical melting curve was observed. It was concluded that the sap of the trees examined was not supercooled, but frozen. A slight degree of supercooling in cut twigs of spruce and pine could readily be produced experimentally, but persisted for a short time only.

The amount of unfrozen water in the xylem of spruce, birch and maple was measured calorimetrically and amounted to a constant fraction (30 to 40%) of the dry weight. It decreased with temperature, but was independent of the total water content. This shows that the unfrozen water was bound to the wood leaving all sap within tracheids and vessels frozen. Freezing causes release of the dissolved gas as bubbles in the conducting elements. If the sap is being transported by cohesive lift, a mechanism must exist which can mend such breaks, but observational clues are lacking as to how this comes about.

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LITERATURE CITED

1. ASKENASY, E. Ueber das Saftsteigen. *Verh. naturh.-med. Ver. Heidelb.*, N.F. 5: 325-345. 1895.
2. BRIGGS, L. J. Limiting negative pressure of water. *Jour. appl. Phys.* 21: 721-722. 1950.
3. BURSTRÖM, H. and KROGH, A. Bleeding and bud development in *Carpinus*. *Svensk Botan. Tidskr.* 41: 17-44. 1947.
4. DIXON, H. H. and JOLY, J. On the ascent of sap. *Ann. Bot.* 8: 468-470. 1894.
5. DUNLAP, F. The specific heat of wood. U.S.D.A. Forest Service Bull. 110. 1912.
6. FILBY, E. and MAASS, O. The sorption of water vapor on cellulosic materials. *Canad. Jour. Research B.* 13: 1-10. 1935.
7. GIBBS, D. Studies of wood. II. On the water content of certain Canadian trees and on changes in the water-gas system during seasoning and flotation. *Canad. Jour. Research* 12: 727-760. 1935.
8. GIBBS, D. Studies of wood. III. On the physiology of the tree, with special reference to the ascent of sap and the movement of water before and after death. *Canad. Jour. Research* 12: 761-787. 1935.
9. GIBBS, D. Studies in tree Physiology. I. General introduction. Water contents of certain Canadian trees. *Canad. Jour. Research. C.* 17: 460-482. 1939.
10. GORTNER, R. A. *Outlines of Biochemistry*. Third ed. Pp. 1-537. Wiley and Sons, N. Y., Chapman and Hall, London 1949.
11. GREATHOUSE G. A. Unfreezable and freezable water equilibrium in plant tissues as influenced by sub-zero temperatures. *Plant Physiol.* 10: 781-788. 1935.
12. GREENIDGE, K. N. H. Ascent of sap. *Ann. Rev. Plant Physiol.* 8: 237-257. 1957.
13. KRAMER, P. J. The forces concerned in the intake of water by transpiring plants. *Amer. Jour. Bot.* 26: 784-791. 1939.
14. KÖCKEMANN, A. Vergleichend-messende Untersuchungen von Saugspannungen, Saugleistungen und Widerständen bei der Wasserleitung in Pflanzen. *Planta* 17: 667-698. 1932.
15. LANGNER, W. Die Wasserverteilung im Stammholz und ihre Veränderungen. *Bot. Arch.* 34: 1-80. 1932.
16. LUNDEGARDH, H. The transport of water in wood. *Arkiv. Bot. ser. 2, 3:* 89-119. 1954.
17. MERWIN, H. E. and LYON, H. Sap pressure in the birch stem. *Bot. Gaz.* 48: 442-458. 1909.
18. MEYER, B. S. Further studies on cold resistance in evergreens, with special reference to the possible role of bound water. *Bot. Gaz.* 94: 297-321. 1932.
19. MÜLLER-THURGAU, H. Ueber das Gefrieren und Erfrieren der Pflanzen. I. Theil. *Landwirtsch. Jahrb.* 9: 133-189. 1880.
20. MÜLLER-THURGAU, H. Ueber das Gefrieren und Erfrieren der Pflanzen. II. Theil. *Landwirtsch. Jahrb.* 15: 453-610. 1886.
21. NORDHAUSEN, M. Zur Kenntnis der Saugkraft und der Wasserversorgung transpirierender Sprosse. *Jahrb. wiss. Bot.* 58: 295-335. 1919.
22. NORDHAUSEN, M. Weitere Beiträge zum Saftsteige-problem. *Jahrb. wiss. Bot.* 60: 307-353. 1921.
23. RENNER, O. Experimentelle Beiträge zur Kenntnis der Wasserbewegung. *Flora* 103: 171-247. 1911.
24. RENNER, O. Versuche zur Mechanik der Wasserversorgung. I. Der Druck in den Leitungsbahnen von Freilandpflanzen. *Ber. dtsh. bot. Ges.* 30: 576-580. 1912.
25. RENNER, O. Zum Nachweis negativer Drucke im Gefässwasser bewurzelter Holzgewächse. *Flora* 118-119: 402-408. 1925.
26. SAYRE, J. D. Methods of determining bound water in plant tissues. *Jour. Agr. Research* 44: 669-688. 1932.
27. SCHOLANDER, P. F., FLAGG, W., HOCK, R. J. and IRVING, L. Studies on the physiology of frozen plants and animals in the Arctic. *Jour. Cellular Comp. Physiol.* 42 Suppl. 1: 1-56. 1953.
28. SCHOLANDER, P. F., LOVE, W. E. and KANWISHER, J. W. The rise of sap in tall grapevines. *Plant Physiol.* 30: 93-104. 1955.
29. SCHOLANDER, P. F., RUUD, B. and LEIVESTAD, H. The rise of sap in a tropical liana. *Plant Physiol.* 32: 1-6. 1957.
30. STARK, A. L. Unfrozen water in apple shoots as related to their winter hardiness. *Plant Physiol.* 11: 689-711. 1936.
31. STEVENS, C. L. and EGGERT, R. S. Observations on the causes of the flow of sap in red maples. *Plant Physiol.* 20: 636-649. 1945.
32. WHITE, P. R., SCHUKER, E., KERN, J. R. and FULLER, F. H. "Root Pressure" in gymnosperms. *Science* 128: 308-309. 1958.
33. WIEGAND, K. M. The occurrence of ice in plant tissue. *Plant World* 9: 25-39, 107. 1906.
34. WIEGAND, K. M. Some studies regarding the biology of buds and twigs in winter. *Bot. Gaz.* 41: 373-424. 1906.