

Effect of Low Temperature on Ascent of Sap in Trees^{1, 2}

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Although the so-called cohesion theory is widely accepted as the most reasonable explanation of how water is lifted up to the leaves of tall trees, there are investigators who question or even reject it. Among the objections to the cohesion theory, Handley's experiments (5) have been cited repeatedly during recent years (e.g. 4, 8). In 1939 Handley published a paper describing experiments in which he chilled the stems of 4-foot-tall saplings of *Acer Pseudo-Platanus* L. and *Fraxinus excelsior* L. He reported that if the stem was chilled to between 0 and 2°, the leaves wilted; if the temperature was raised again to slightly above 2°, the leaves recovered, provided the low-temperature treatment had not lasted too long. This observation is rather remarkable since no known property of water changes drastically enough at a temperature above freezing so that the wilting could be explained in terms of a physical interference with water movement. Handley concluded therefore that metabolic forces are somehow involved in sap ascent.

Handley's experiments are by no means the only ones in the literature dealing with the effect of temperature on the ascent of sap, but since instrumentation of the older experiments was rather crude when compared to modern standards, it is difficult to evaluate them today. It may suffice here to refer to Handley's own review of these older papers (5).

During the summer of 1959 a study was begun on the effect of locally applied temperature on phloem transport (11). In the course of these experiments the question arose as to the temperature at which xylem sap freezes in the tree.

Methods

The experiments have been carried out with numerous tree species of various size, in Prospect Hill Tract I of the Harvard Forest, in Petersham, Massachusetts. Cooling was achieved in the following way. The cooling coil of a PCC-3 portable cooling unit (Blue M Engineering Co., Blue Island, Ill.) was immersed in a plastic waste basket containing methanol-type antifreeze. The liquid-filled waste basket was fitted into a second one, with an air space between the 2 for insulation. From this reservoir liquid was pumped through rubber tubing (6 mm inside, 10 mm outside diameter), which was closely

wrapped around the tree stem in ascending coils over a length of 40 cm (in freezing experiments) or 100 cm (in wilting experiments). The rubber coil was insulated on the outside with layers of cotton rags and polyethylene film. Temperature control was achieved with 2 thermostats; the temperature of the liquid reservoir was controlled by the thermostat of the cooling unit itself, and that of the tree stem was maintained at the desired level by switching the pump with a Yellow Springs Model 63RA temperature controller. The temperature sensing device for the second thermostat was a No. 661 surface thermistor placed under a bark flap with the sensitive surface in contact with the wood of the stem.

Temperatures were measured and recorded with Yellow Springs instruments. For critical measurements the small, 5-mm diameter, No. 421 surface thermistors were used, for other measurements the somewhat larger No. 409 disks. They were all placed under bark flaps, the sensitive surface in contact with the wood. Care was given, particularly in the case of ring-porous species, to avoid injury to the water-conducting earlywood vessels. All probes were checked from time to time in a water-ice mixture. They did not deviate more than about 0.1° from the calibration point. Temperatures were recorded with a 47TH scanning thermometer and a Model 80 recorder. This combination allows the simultaneous recording of up to 11 different temperatures.

Electricity was brought to the forest with a heavy cable from the nearest outlet. Thus, the radius of action was about 300 m around the greenhouse and the sawmill. All electric contacts, such as the connections of cables, were placed in polyethylene bags and tied (the bag upside down) to trees. All other equipment was similarly protected from rain so that the experiments could be carried out independently of the weather.

Results and Discussion

The Freezing Point of Moving Xylem Sap. Older reports indicate that xylem sap freezes only at temperatures considerably below 0°. Most of the literature on the freezing of xylem sap is more than 50 years old (e.g. 2, 3), though there is a modern investigation, that of Lybeck (7). In her experiments, spruce twigs were brought to a cold room and the wood temperature was measured with a thermocouple. The lowest supercooling observed was

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² All temperatures are given in centigrade.

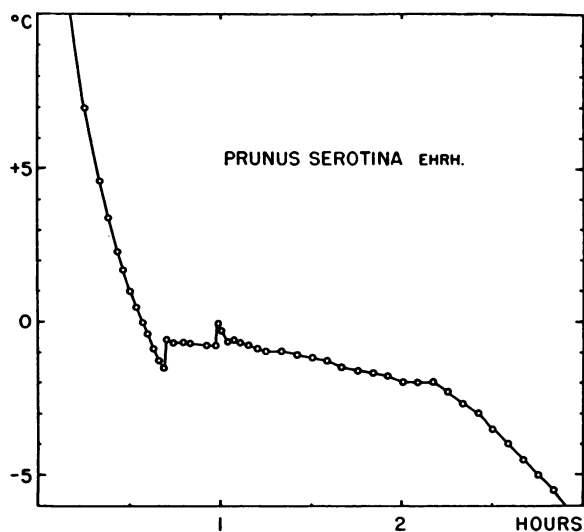


FIG. 1. Temperature of the wood, recorded during the freezing of moving xylem sap in an intact tree.

-8.7° and the subsequent temperature jump brought it up to about -2° . Since these experiments were performed with detached twigs of a conifer, it was decided to repeat them with intact, living dicotyledonous trees.

The freezing-point depression of xylem sap due to solute concentration can only be minute. Bollard gives approximately 0.4% solids as a maximum concentration of xylem sap (1). This corresponds to a freezing-point depression of not more than 0.2° . Our measurements with xylem sap extracted from *Ulmus americana* L. yielded values only about one-tenth of this. The question then arose whether the freezing point is lower when the sap is in contact with the wood.

Freezing-point determinations were made on standing trees of the following species: *Acer rubrum* L., *Acer saccharum* Marsh., *Betula papyrifera* Marsh., *Fraxinus americana* L., *Prunus serotina* Ehrh., *Quercus rubra* L., and *Ulmus americana* L. In these experiments a tiny area of wood (about 6 mm diameter) was exposed by cutting away the bark, and a No. 426 surface thermistor was taped to the exposed wood. A matched No. 409 thermistor, immersed in a water-ice mixture, served as a control. The thermistor on the tree stem was insulated with cotton wool, and the area wrapped over a length of 40 cm with the cooling coil. The coil was then insulated with cotton rags and polyethylene film as described before. Chilling was done with full power and without thermostatic control. Temperatures thus obtained were below -20° in the liquid reservoir and ca. -12° in the liquid returning from the tree. The chilling temperature at the stem surface was thus between -15 and -17° .

Chilling of a dead, dry branch gave an uninterrupted temperature curve. Chilling of a living tree stem, however, resulted in a curve clearly shaped by

the freezing of water (fig 1). The lowest supercooling observed during this series of experiments was -3° ; however, in the majority of cases freezing began at about -1.5° . Once the freezing started the temperature rose to between 0 and -1° for a brief period, then it dropped again to the value previously reached where it stayed for an hour or so. The horizontal part of the curves corresponds to the time it takes to freeze all the freezable water in the neighborhood of the thermistor. This time varied somewhat from tree to tree, apparently indicating the amount of freezable water in the stem. It is, of course, also correlated with the rate of cooling, which, in turn, is higher in cold weather than on a warm day. Comparative investigations on the amount of freezable water in different species would be interesting, but no attempt was made to do this.

We could ask why earlier investigators have measured much lower temperatures of freezing xylem sap. The answer undoubtedly lies in the spatial relationships. If a freezing-point determination is made with a standard cryoscopic device, the thermometer is immersed in the freezing liquid. This situation cannot easily be duplicated in trees. The wood has to be cooled from the outside, and the measuring device is usually placed between the chilling agent and the moving xylem sap. A temperature intermediate between that of the chilling agent and that of the xylem sap is therefore measured. The higher the rate of sap movement the more drastic a rate of external chilling has to be applied, lest the ascending sap escape. Even a thermocouple is not ideal because it does not, like a surface thermistor, measure on the wood side only. From these considerations we are forced to the conclusion that even our own data represent minimum values; the actual freezing point of xylem sap may even be higher than the measurements indicate. On the other hand, the measurements of our freezing experiments are comparable with those of our wilting experiments described below, because they were taken in the same way.

The Wilting of Trees at Low Stem Temperatures. Handley's experiments, carried out originally with *Acer Pseudo-Platanus* L. and *Fraxinus excelsior* L. (5), were duplicated with *Acer saccharum* Marsh., *Fraxinus americana* L., *Prunus serotina* Ehrh., and *Quercus rubra* L. The size of the trees ranged from a height of 1.5 to 15 m, and from a diameter at chilling height of 1 to 15 cm. Treated trees were always standing beside control trees so that wilting could be recognized immediately. The stems were chilled over a length of 100 cm. Wood temperatures were measured at 8 points along the stem; below, within, and above the chilling jacket (fig 2). The thermistor of the thermostat controlling the pump was placed at a point of anticipated lowest temperature; in diffuse-porous species (low sap velocities) this was in the lower part, in ring-porous species (high sap velocities) in the upper part of the chilled section.

The experiments were all started by rapid chilling of the stem to between $+1^{\circ}$ and 0° , in later experi-

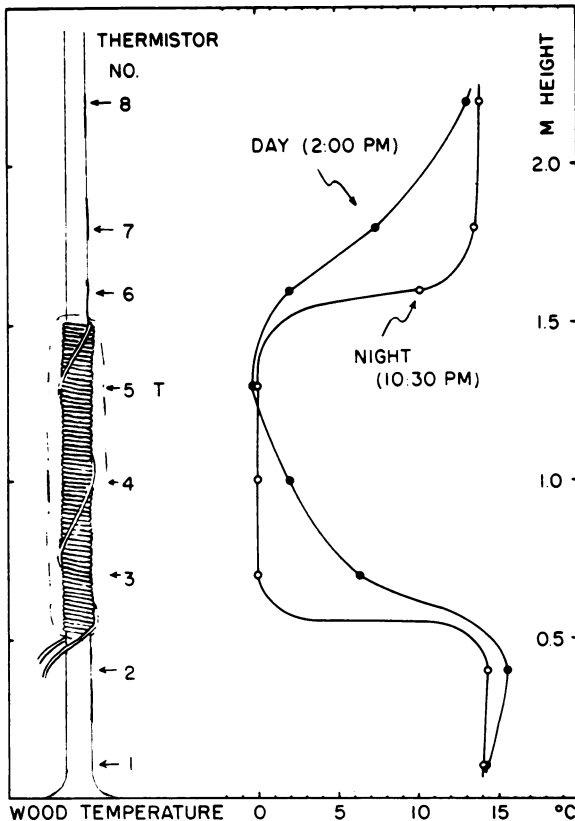


FIG. 2. Two temperature profiles along the stem of *Quercus rubra* L. during a chilling experiment. The night profile indicates that the xylem sap is (nearly) stationary. During the day, relatively warm water moves into the chilled section from below and chilled water moves out of it above. The stem temperature normally fluctuates with the ambient air temperature, of course. It is somewhat stabilized in the lower part of the stem by xylem sap moving up from the roots. To avoid needless confusion, the profiles of this figure were chosen from 2 times when the air temperatures were similar.

ments to between 0° and -1° . The temperature was then maintained at this level continuously for several days, and after about a week further lowered in very small steps until the tree wilted. Wilting never occurred above -1° , although the temperature in all species was maintained between 0 and -1° for many days. Wilting did occur, however, in all species as soon as the temperature was lowered appreciably below -1° . The reversibility of wilting could be observed beautifully with *Prunus serotina*, because this species has a very distinct drooping position of the leaves.

The temperature profile along the stem of chilled trees was a very effective means of monitoring the relative velocity of water ascent, especially in the ring-porous species. It is, in principle, the same phenomenon that Vieweg and Ziegler (10) described in their modification of Huber and Schmidt's (6) thermoelectric method.

Sap movement up the stem was very slow during the night. The temperature of the wood within the cooling jacket was then quite uniform and corresponded to the temperature set on the thermostat. Above and below the jacket, temperatures were similar to the air temperature. Soon after sunrise water began to move up; this was indicated by an influx of relatively warm water into the jacketed area from below and an efflux of chilled water out of the upper end of the chilled area (fig 2). The temperature profile was a sensitive measure of water movement. It showed distinct differences between sunny and partly cloudy days (fig 3a, b). Less water moved on cloudy days, but the rate of movement increased each time the sun appeared from behind the clouds (fig 3b).

The temperature profile was a far more sensitive indicator of water movement than the phenomenon of wilting. When the stem temperature, toward the end of the experiment, was lowered in steps of about 0.3° each day, one could anticipate wilting by at least

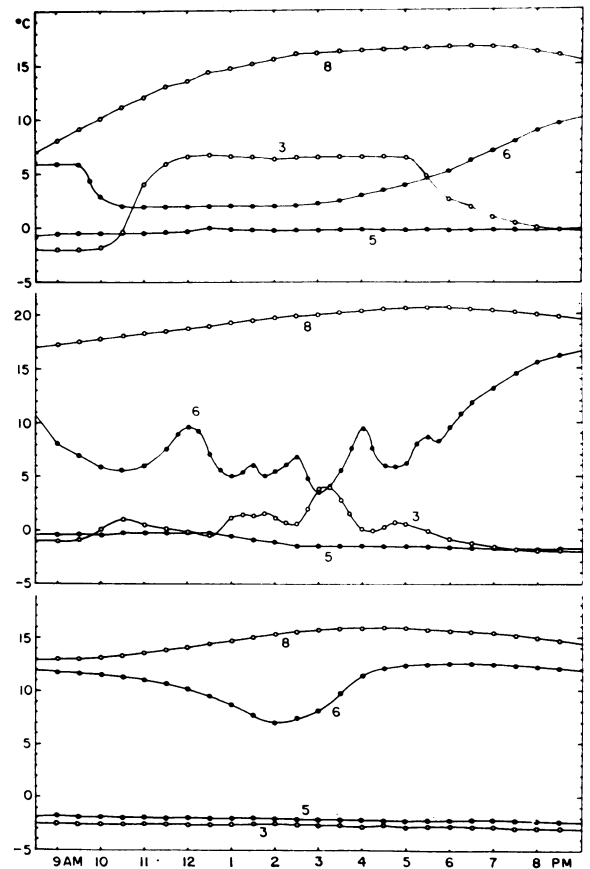


FIG. 3. Temperatures at 4 different points along the stem of *Quercus rubra* L. during a chilling experiment. Thermistor numbers are the same as in figure 2. (a, top) Sunny day. (b, middle) Cloudy day with occasional sunshine. (c, bottom) Sunny day, xylem sap partly frozen. Ice formation restricted sap ascent, but the tree did not yet show any sign of wilting.

24 hours. In other words, when the stem temperature was lowered to *below* -1° one could see, from the temperature profile, that something interfered with sap ascent. From the freezing experiments described above, it is quite clear that this something was ice formation. Ice formation could thus be detected with the temperature profile before the tree began to wilt (fig 3c).

Wilting could not be achieved at temperatures above the freezing point of water. Wilting did occur, however, at temperatures low enough to cause freezing of the moving xylem sap. We are then left with the problem of explaining Handley's findings. An analysis of his publication can only allow us to speculate; a final elucidation of the situation would require a repetition of his experiments with the equipment then used, and with additional devices which permit the remote measurement of temperatures. Handley used mercury thermometers (Handley, personal communication). If we assume that these were properly calibrated, there seems to remain only one possible explanation of his results, namely that Handley was unable to reach the coldest point within the chilling jacket without realizing it. The distance between the temperature-sensing bulb and the zero mark is always quite short in standard thermometers. It is possible that heat flux along the thermometer itself and along the tree stem prevented his thermometer bulb from registering the lowest temperature achieved with the cooling device. This assumption is supported by the fact that Handley's equipment *was* capable of achieving temperatures below 0° . He reported that ice formed on the cooling tubes when he used full cooling power (when the leaves wilted); this ice often persisted for an appreciable length of time after the temperature was raised again (5).

The Effect of Freezing on Subsequent Sap Ascent. It has been pointed out by Scholander (9) and Lybeck (7) that the freezing of xylem sap during the winter represents a serious limitation for the water conducting system of tall plants during the following vegetation period. Any air that happens to be dissolved in the xylem water will come out of solution when the tree freezes. Thus the continuity of the water columns is interrupted when the ice melts in the spring. This phenomenon is undoubtedly one of the factors responsible for the distribution of tall species on the surface of the earth. In other words, the reason why some trees do not grow in regions with cold winters is because their water-conducting system would fail. In northern latitudes where there are severe winters, the trees are all specialists that have solved the problem of winter freezing in one way or another. Three different groups seem to be able to recover from cold winters: A) *Conifers*. Upon thawing of the xylem water, each bubble is confined to a single cell (tracheid). B) *Diffuse-porous trees (and some vines)*. Metabolic forces produce positive pressures in late winter and early spring. This can refill part of the conducting tissue. C) *Ring-porous trees*. These form a new growth ring with very large earlywood

vessels early in the spring before the leaves expand, that is, before substantial water conduction is called for. Water conduction in these species takes place almost exclusively in these newly-formed vessels at very high velocities (6). Older vessels, embolized by winter freezing, remain gas filled.

Preliminary dye-ascent experiments have been performed by us with ring-porous trees after experimental freezing. These seem to indicate that large vessels are, indeed, embolized and that water conduction after freezing is forced through auxiliary water-conducting cells such as small latewood vessels and tracheids. Further experiments on this problem are under way.

Summary

Handley's experiments according to which the leaves of a tree wilt when the stem is chilled to between 0 and $+2^{\circ}$ have been repeated with precise and sensitive control and measurement of wood temperatures. No wilting was observed even if tree stems were maintained between 0 and -1° for many days. A stem temperature of between -1 and -2° however did interfere with the sap ascent and it was demonstrated that ice formation takes place at this temperature. The temperature profile along the stem was a sensitive indicator of the relative rate of water movement.

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Note. After the preparation of this manuscript the author corresponded with Professor P. E. Weatherley of the University of Aberdeen, Scotland. In his letter of October 23, 1963, Professor Weatherley wrote: ". . . Dr. D. J. F. Bowling and Mr. John Milburn in my laboratory demonstrated with *Ricinus* plants that when the stems are cooled down to a little above 0° , not only was there no wilting of the leaves but the transpiration rate of the plant was totally unaffected, indicating that there was no stomatal response and hence presumably no change in water potential in the leaves and we could safely conclude no change in the resistance of the xylem of the stem." Professor Weatherley also called my attention to an Australian paper that was unknown to me (Johnston, R. D. 1959. Control of water movement by stem chilling. *Australian J. Botany* 7: 97-108.) In this paper, Johnston determined the freezing point of stem water of *Pinus radiata* D. Don. Supercooling proceeded to -3° , the temperature then rose to about -2° . When the stem temperature was kept above -2° the transpiration rate was unaffected, when it was maintained just below -2° for 7 days the leaf

water deficit of the shoot rose from 10 to 47 %, but returned to normal values within 4 days after freezing was stopped; the plant showed no signs of damage due to freezing. Johnston's interpretation of Handley's observations are very similar to our own.

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Modification of Plant Transpiration Rate with Chemicals^{1, 2, 3}

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Minshall (12, 13) described a reduction in transpiration rate in excised bean leaves after treatment with monuron, diuron, and fenuron (3-phenyl-1,1-dimethylurea) all of which are inhibitors of the Hill reaction *in vitro* (5).

Wills, Davis, and Funderburk (20) described a reduction in water loss after treatment of plants with atrazine. They found a significant decrease in transpiration and in stomatal aperture from both excised and intact plants of cotton, corn, and soybean. Walker and Zelitch (19) also reported a greatly decreased stomatal aperture after treatment with atrazine and other enzyme inhibitors.

Other workers recently have reported a decrease in stomatal aperture and transpiration rate after treatment with α -hydroxysulfonates (21), 8-hydroxyquinoline sulfate (18, 21) and phenylmercuric compounds

(22, 23). Blandy (1) reported a decreased transpiration rate after treatment with phenylmercuric compounds, but no studies were made of stomatal response.

This paper describes the transpiration response of several species of plants to treatment with several chemicals, most of which are herbicides. A preliminary report on this work was published earlier (17).

Materials and Methods

Transpiration measurements were made with a modified potometer described by Grobbelaar (6) which holds the entire root system. Total capacity of the potometer cup was about 70 ml. The side-arm was a 0.1 ml pipette graduated in μ liters. Treatments were replicated 4 times. All potometer experiments were run in a growth chamber with a light intensity of 2500 ft-c at the leaf level. The light period was 16 hours, and day and night temperatures alternated between 26 and 21°, respectively.

Plants were grown in vermiculite in small plastic flats. The young plants were watered with tap water and with Hyponex nutrient solution when necessary. Depending upon the species, plants were usually from 2 to 5 weeks old (approximately 15-25 cm tall) at the time that they were used in the potometers. They were removed from the flats, the roots were washed

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