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THE EFFECT OF LOW STEM TEMPERATURES AND STEM  
INCISIONS ON THE TRANSLOCATION OF WATER<sup>1, 2</sup>

DONALD R. GEIGER<sup>3</sup>

*Department of Botany and Plant Pathology, The Ohio State University, Columbus 10*

The present study was prompted by reports in the literature of phenomena which appeared inconsistent with the tension-cohesion theory of water movement. One such instance was the temperature effect observed by Handley (1939). On several occasions during a study of stem growth at reduced temperatures Handley noted loss of turgor in leaves of experimental plants, a phenomenon which was attributed to the curtailment of translocation of water in the part of the stem cooled with a jacket. Measurements of the air temperature inside the stem jacket indicated that the temperature ranged from 0 to -1 C. Movement of water subsequently resumed as the temperature reached 2 C. In attempting to explain this and similar observations reported in earlier literature, Handley proposed a mechanism of water transport which was dependent upon living xylem cells. According to this explanation, water transport is dependent upon cell metabolism. Thus, at a critical temperature metabolic activity would fall below a certain level and water movement would decline significantly.

Besides this temperature effect, the phenomenon of water flow through stems whose vessels contain vapor or gas bubbles has led some to question the validity of certain of the tenets of the tension-cohesion theory (Preston, 1952). A number of investigators have introduced air into the vessels of the stem by incisions arranged in various patterns (Elazari-Volcani, 1936; Preston, 1952; Scholander et al., 1955, 1957, and 1961; Greenidge, 1955, 1955a, and 1958; and Postlethwait and Rogers, 1958). In all of these experiments water apparently moved up the stem

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<sup>3</sup>Present address: University of Dayton, Dayton, Ohio, 45409.

even though the water columns in the vessels were severed throughout all or most of the stem cross-section.

In the above mentioned experiments no data are given concerning the exact extent to which translocation of water was altered during the course of the experimental treatments. To determine the effects of such procedures as cooling or cutting the stem, water translocation was measured potometrically before, during, and after experimental treatment. By this procedure it was hoped that one could ascertain whether or not the tension-cohesion theory could be used to explain the observations.

#### METHODS

A potometer (fig. 1) was used to measure the rate at which water entered the roots. The sensitivity of this apparatus was sufficient to enable a series of measurements to be made at intervals of from three to five min. The fact that repeated

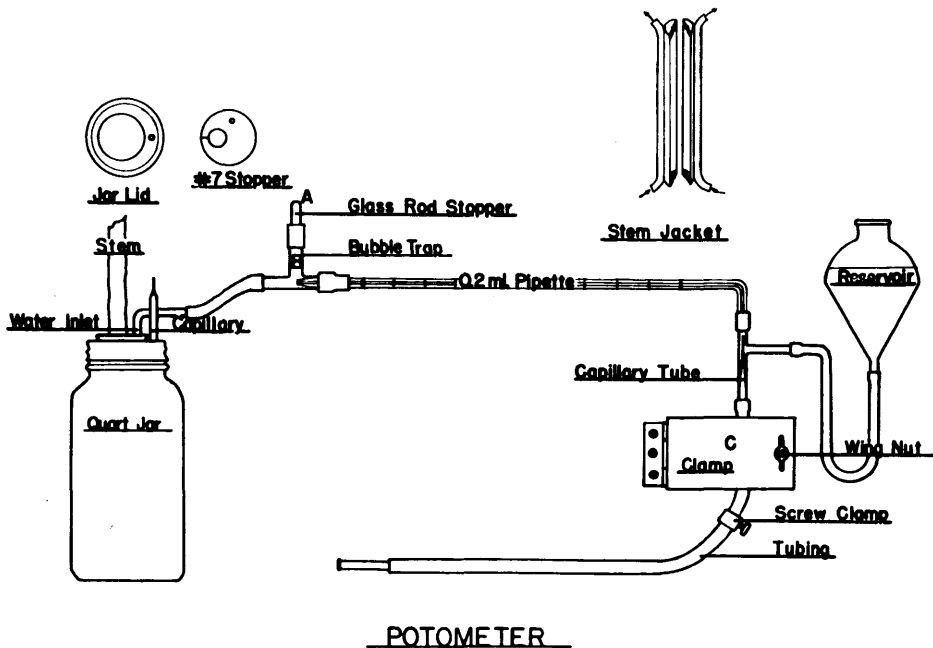


FIGURE 1. Detail of potometer and stem jacket.

measurements could be taken made it possible to study changes in the rate of translocation of water as the stem temperature was varied or as incisions were made in the stem.

The potometer consisted of a 0.2 ml pipette, a container in which the root system of the plant being studied was sealed, an arrangement for introducing a small bubble of air into the pipette, and a reservoir of water. The time which elapsed while the bubble was drawn through the volume of the pipette was used to determine the rate of entrance of water into the roots. During the experiment the plant stem was supported in a slit rubber stopper and the stem-stopper junction was sealed with caulking compound. The height of the reservoir was adjusted to keep water in the tip of the capillary at all times.

To make a measurement, the rubber tubing was compressed slightly with a large clamp. The bubble which forms at the tip of the capillary tube rises into

the end of the pipette and is carried along in the stream behind the water entering the plant. The time points plotted on the graphs are the times when the bubble began moving through the 0.2 ml volume of the pipette.

Because of the local water deficits in the plants, water translocation rates and water absorption rates may not always be in one-to-one correspondence. However, it was found that water absorption quickly ceased when translocation of water was stopped by severing the stem. In view of this observation and because of the moderate transpiration rates, it seems reasonable to infer that the water deficits in the plants during the present experiments were at a minimum and that the rate of absorption of water closely approximated the rate of its transport up the stem. The close agreement between gravimetric determinations of transpirational loss and the potometrically measured water uptake further supports this reasoning.

A copper jacket of the type described by Swanson and Whitney (1953) was used to regulate the temperature along the 10 cm portion of the stem. Methyl alcohol, which was used as the coolant, was circulated through coils of copper tubing placed in a refrigerator freezer chest. A small impeller pump forced the coolant through the circuit. Temperatures ranging down to  $-10^{\circ}\text{C}$  were attained in the stem by varying the temperature setting of the refrigerator.

The stem temperature was measured with an L & N potentiometer using a 30-gauge copper-constantan thermocouple positioned in the phloem-cortex region of the stem. Temperature was recorded as the bubble began to move through the pipette. A reference temperature bath consisting of a thermos bottle filled with water at a known temperature in the range being measured served as a check on the thermocouple-potentiometer calibration.

All experiments were performed with six-month-old plants of *Ricinus communis* var. Red Spire and with rooted two-to-four-year old branches of *Salix* sp. The plants of both species grew in solution culture. The castor bean plants were about 12 inches tall and the willow plants were about 2 ft tall.

## RESULTS AND DISCUSSION

### *Effects of Stem Cooling*

Results of a representative experiment showing the effect of low stem temperature on the water translocation rate as measured by the rate of water absorption are given in figure 2. Similar results were obtained in all six experiments of this type. The rate of water translocation did not vary significantly with the change in stem temperature until freezing had occurred, after which a rapid decline in the rate of water movement took place. It is evident from the temperature curve shown in figure 2 that the water in the stem supercooled about  $6^{\circ}\text{C}$  prior to freezing. When crystallization was initiated the temperature rose abruptly to  $-2^{\circ}\text{C}$  and this value was taken to be the freezing point of the phloem-cortex tissues in which the thermocouple was positioned. In *Ricinus* the rate of water movement began to decline sharply coincident with freezing. In *Salix* there was a lag period of several minutes and the decline was more gradual. Probably the slower propagation of ice crystals and the larger cross-section of the transpiration stream in the latter species caused this difference in the time course.

An aqueous solution with a freezing point of  $-2^{\circ}\text{C}$  has an osmotic pressure of 24 atmospheres, a reasonable value for the phloem-cortex tissue. The transpiration stream, on the other hand, would be expected to have an osmotic pressure of approximately one atmosphere and a corresponding freezing point of  $-0.08^{\circ}\text{C}$  (Meyer and Anderson, 1952). Since the transpiration stream was moving through the chilled portion of the stem its temperature probably would not have been in equilibrium with that of the adjacent tissues. Thus freezing may well have occurred at about the same time in these two portions of the stem even though their freezing points were different.

The present experiments confirmed the stoppage of the translocation of water at a temperature near 0 C. However, the critical temperature which was found to be effective in the experiments reported here was one to two degrees lower than the 0 to -1 C range that Handley reported. Because the temperature of the air in the stem jacket was approximately 2 C when resumption of translocation of water occurred, Handley concluded that "chilling the stem from about 2 C to about 0 C cuts down the flow of water to the leaves to an almost negligible amount." For this reason he states that "living cells are involved in the ascent of sap." However, one can infer from the undercooling and the -2 C critical temperature observed in the current study, that the stoppage of the transpiration stream resulted from the freezing of water in the stem rather than from a sudden decrease in metabolic activity. The fact that the temperature which Handley found to be effective was somewhat higher than that necessary to stop water movement in the present studies may be due to differences in the experimental technique. Handley

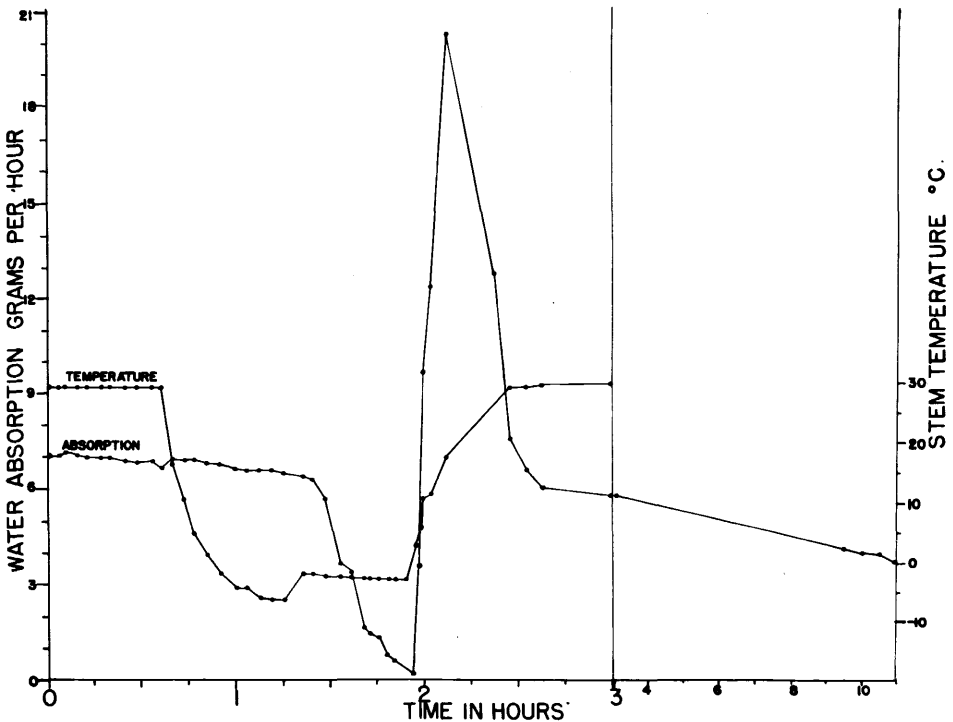


FIGURE 2. Effect of stem cooling on translocation of water in *Salix*.

cooled the entire length of the tree stem for periods of many days. The greater length of the cooling jacket and the longer duration should have resulted in a greater uniformity of temperature in the stem. There would have been less difference between the temperature of the air in the jacket and that of the moving transpiration stream. As a result, an air temperature slightly lower than the freezing point of the xylem sap presumably would have caused the transpiration stream to freeze. The temperature gradient between the air in the jacket and the transpiration stream would be expected to be greater with the 10-cm jacket employed in the present study. A higher transpiration rate under the conditions of Handley's experiment may have resulted in a lower osmotic pressure in the transpiration

stream and consequently a higher freezing point. Because of these considerations precise temperature measurements taken at various sites throughout the cooling jacket and stem would be required to confirm that freezing has not occurred during the experiments reported by Handley.

The present findings confirm the observations of Johnston (1959) made on *Pinus radiata*. After concluding that the cessation of water transport in the chilled stem of *P. radiata* was due to freezing, Johnston stated that "it appears quite possible that the freezing points of the stems used by Handley were much closer to 0 C than was assumed by him and that the temperature did in fact fall to the freezing point at some part of the stem."

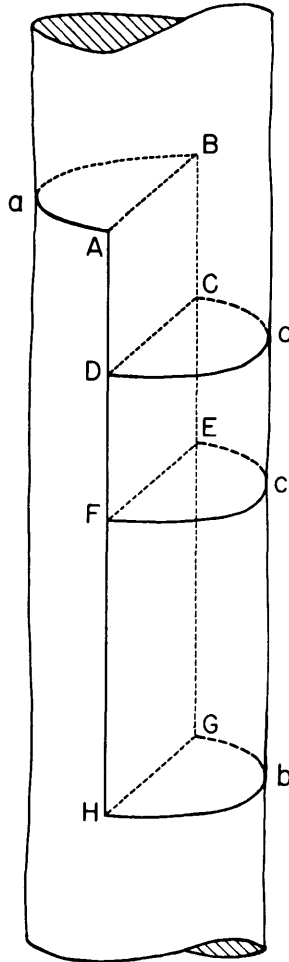


FIGURE 3. Configuration of stem incisions.

In the present experiments the stem was allowed to return to room temperature after absorption of water had ceased. Translocation resumed to varying extents; *Salix* usually recovered fully while *Ricinus* typically failed to recover to any appreciable extent. The effects of freezing on stem structure were not investigated extensively. Some freehand sections of the frozen *Ricinus* stem were studied, and

it was noted that these sections contained numerous gas bubbles. Presumably, gas bubbles were also present in the *Salix* stem as a result of freezing. It is conceivable that cavitation of the water in the lumens of the tracheae and tracheids was brought about by gas which came out of solution during freezing. These cavities would restrict the path available to mass movement of water. The resulting decrease in the cross-section of the transpiration stream would increase the resistance to movement of water through the stem. Differences in stem morphology between the two species could account for the observed differences in the effects of freezing and cavitation. One such anatomical difference is the presence of a narrow ring of xylem in the hollow *Ricinus* stem in contrast to the multiple annual rings in the *Salix* stem. Another difference is the somewhat herbaceous nature of the *Ricinus* stem in contrast to the woody *Salix* stem. Mechanical damage resulting from gas bubbles and the expansion of water during crystal formation would be expected to be less in the willow stem. As a result, there would presumably be less of an increase in resistance to flow in the willow stem.

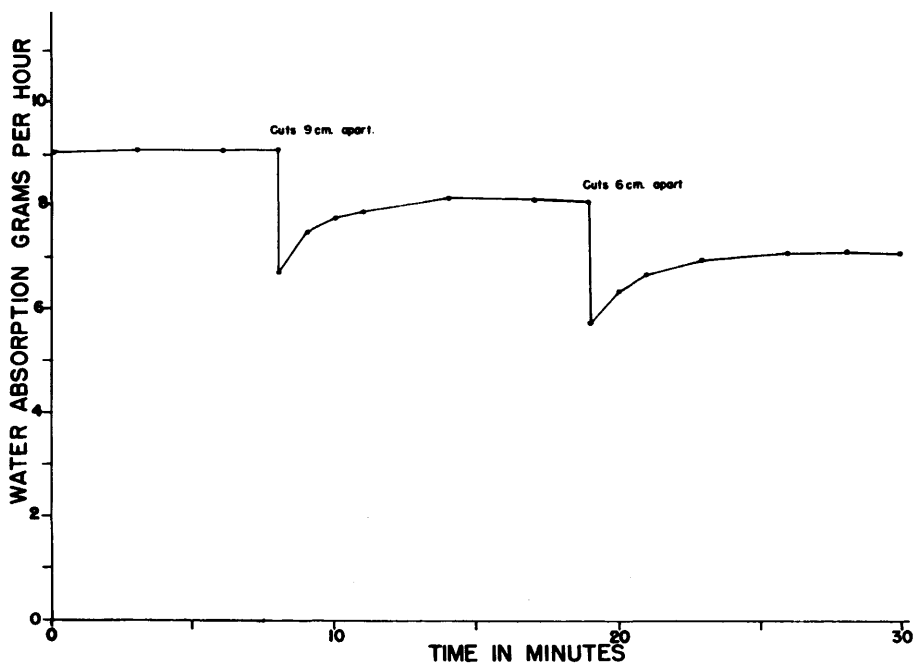


FIGURE 4. Time course of translocation before and after making an incision in the stem of *Salix*.

As may be noted in figure 2, after the stem temperature rose above the freezing point, water absorption increased to a value two to three times the rate prior to freezing. Presumably this surge restored the water deficits which accumulated in the tissues after translocation was stopped.

From the results of the stem cooling experiments, it was concluded that lowering of the stem temperature had no pronounced effect on the translocation of water until the contents of the transpiration stream froze. Further, the phenomena associated with stem cooling appear to be amenable to explanation without discarding or seriously modifying the tension-cohesion theory.

#### *Effects of Stem Incisions*

In order to evaluate the effects of severing vessels on the translocation of water up the stem, a series of incisions extending halfway through the stem was made

in the pattern shown in figure 3. A thin piece of polyethylene plastic was inserted in each cut to prevent water transfer. The cuts were made in the sequence a, b, c, d. The first set a-b was positioned midway up the stem. The distance a-b was 21 cm. Each successive cut was made closer to a. The water absorption rate was measured for a period of time before each incision was made. Measurements were repeated at frequent intervals after each cut until the rate had become stabilized.

The results of a representative experiment are given in figures 4 and 5. Similar results were obtained in seven other such experiments. Water continued to move into the roots and up the stem in considerable quantity after the double cuts were made. In general, the quantity of water conducted was not significantly changed until the initial and final cuts were quite close together; water movement stopped when the cuts were closer than a critical minimum. In *Ricinus* this distance was approximately 3 cm and in *Salix* approximately 0.5 cm, under the conditions of these experiments.

Scholander and his colleagues (1955) found that alternate introduction of air

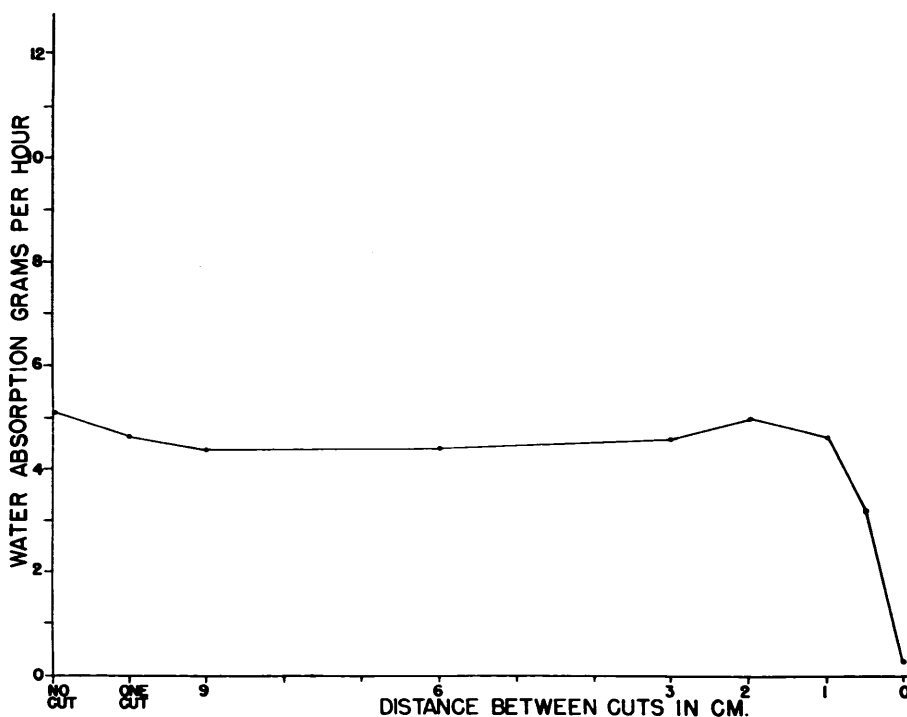


FIGURE 5. Effect of distance between incisions on translocation rate in *Salix*.

and water into a severed grapevine did not greatly diminish the movement of water up the stem. Transpirational flow was maintained although the pressure drop along the stem increased as a result of increased resistance to flow. These observations are in accord with the tension-cohesion model. In such a model tension generated at the leaf-wall surface is transmitted throughout the continuous water columns in the plant. The xylem functions as a continuous system of water-filled pores. These pores range in size from the large lumens of the vessels to the micropores constituting the interstices within the cell walls. Most of the water presumably would flow through the larger pores which offer less resistance to flow.

Using this classical tension-cohesion model, it is possible to interpret the events shown in figures 4 and 5. As a pair of incisions (e.g., a and b) was made in the stem, air entered the vessels and tracheids above and below the cuts and moved to the first intact crosswalls. At these points menisci formed across the micropores of the cell walls and contained the advance of the air. As a consequence of the receding water columns, tension in the water-filled portion of the conducting system decreased. Because of the cavitation in the larger pores, water movement was diverted in part to smaller pores with a corresponding increase in resistance to flow. The drop in tension and increased resistance to flow caused a temporary reduction of translocation of water immediately after an incision was made (fig. 4). Over the next several minutes continuing evaporation of water from the leaves caused an increase in tension in the system of water-filled pores. As a result, translocation gradually increased to a steady rate approximately equal to that prior to the cut. Since the direct longitudinal water columns were interrupted, water moved laterally through the area ABGH. The least resistance to flow occurred in the water-filled lumens of tracheids and of those tracheae which were not cavitated because of intervening cross walls. Where these larger pores were air-filled, the water presumably moved in the micropore system of the cell wall. With each succeeding incision the cross sectional area of the path of lateral movement decreased. As the area decreased the resistance increased. The rate of flow was maintained by the ever increasing tension. With further incisions the resistance to flow became so great that translocation of water almost stopped.

Although it is possible to interpret the results of the stem cooling and ringing experiments by using the tension-cohesion hypothesis, many problems remain. More work is required to determine the exact path taken by water when it moves around obstructions. Also, the structural and flow characteristics of the cell wall micropore system are in need of further study.

#### ACKNOWLEDGMENTS

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