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Temperature Effects on Radial Propagation of Water Potential in Cotton Stem Bark[1]

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ABSTRACT

Low temperature affects the lateral movement of water across the xylem-phloem boundary in intact cotton stems. There is a reduction in the effective diffusion coefficient relating free energy flux to water potential gradients with an associated increase in resistance to water flow. Detached phloem and excised leaves do not show this effect of low temperature. Experiments on stem section halves indicate that the effect is probably associated with the cambial region.

Recent experiments on water relations of cotton stems have shown that diurnal changes in stem diameter result almost entirely from lateral movement of water out of and into the phloem and associated living tissues external to mature xylem (12). This water movement can be considered to occur in response to water potential gradients in these tissues (11), and the pattern of stem diameter change brought about by a change in xylem water potential depends in part on the diffusivity for free energy transfer in these living tissues. The tissues of concern are largely phloem and cambium but may include greater or lesser amounts of young xylem elements which still have living protoplasts. For the sake of brevity, the term "bark" will be used to describe the tissues being measured. The "bark" unless otherwise stated includes the vascular cambium, primary and secondary phloem, cortex, and periderm. A distinction has been made (13) between D, the diffusion coefficient relating free energy flux to the gradient in water potential, and D_κ, a similar coefficient relating water flux to water potential gradient. According to the theory of Philip (15), these two coefficients are proportional to each other and measurements of the increase in bark volume during hydration can be used to estimate D, the apparent diffusivity of the bark. During rehydration of a droughted stem, the curve relating bark volume to time is two-phased, similar to curves obtained for leaf discs, with a passive rehydration phase preceding an active growth phase (14). The temperature of 31 C selected for our previously reported experiments on cotton stem rehydration was chosen because it approximates midsummer field conditions. The experiments reported here were done at several temperatures in order to determine the effect of temperature on rehydration characteristics of droughted cotton stem tissues.

MATERIALS AND METHODS

Methods for Experiments on Intact Plants. Soil-grown cotton plants (*Gossypium hirsutum* L. Auburn 7-683) were raised in a growth chamber at 31 C/21 C on a 12-hr photoperiod for approximately 3 months. The experiments were done in a series of growth chambers at different temperatures. For about 24 hr before an experiment, plants were equilibrated at the experimental temperature, with the exception that plants were equilibrated at 8 C only a few hours to preclude tissue damage by such a low temperature. Several hours before each experiment, a plastic reservoir was attached around the stem so that the main stem could be severed under water held in the reservoir. A linear variable differential transformer for continuously monitoring stem diameter (4) was attached below the reservoir as described previously (13). The outer surface of the stem was coated with stopcock grease, and a ring of bark was removed between the reservoir and the linear variable differential transformer. The girdle was filled with stopcock grease to insure that water would move into the bark only laterally by way of the xylem when the stem was later severed. Plants were allowed to transpire until they depleted soil moisture to the point where they were slightly wilted. Shoots were then covered with plastic bags to decrease transpiration, and the covering around the shoot was manipulated to control transpiration so that there was no change in stem diameter for 2 to 3 hr. This was sufficient time for water potential in the bark tissues to equilibrate with the low water potential in the xylem (13). Plant water potential was not always measured at this point; but, from previous experience, these plants were equilibrated to a potential in the range of −10 to −15 bars. The stem was then cut under water. Rehydration of the bark proceeded with xylem water as the source, and xylem water was replenished by free water in the reservoir. At the end of the experiment, final stem diameter and thickness of the fully hydrated bark were measured with a micrometer caliper so that relative displacements given by the linear variable differential transformer could be used to calculate bark thickness as a function of time during the hydration period. Generally, bark separated easily at the cambial layer.

Volume increase during hydration is directly related to the free energy flux into the tissue if one assumes a linear relationship between cell water potential and cell volume over the range of interest (15). Data were analyzed to determine diffusivity using a semi-log plot of fractional volume increase *versus* time, described in detail in a previous paper (13). The log of $(1 - V[t]/V[\infty])$ was plotted against time where $V[\infty]$ is the total volume increase of the phloem and associated tissues when

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hydration is complete and $V[t]$ is the corresponding quantity at any time, t, during the hydration process. The slope of the linear portion of this curve enables one to compute the effective diffusivity. This number measures the rate at which water potential gradients will disappear from the tissue and removes the effect of a particular radial geometry (15). It is also proportional to the coefficient, D_w, relating water flux to water potential gradient and, hence, D^{-1} is proportional to the tissue resistance to water flow (10).

Methods for Experiments on Detached Bark and Leaves. Pieces of cottom stem about 2 cm long and 1.5 cm in diameter were removed from a slightly wilted plant. The stem was split in half longitudinally, and the bark was removed at the cambial layer. Thus the tissue consisted of torn fragments of vascular cambium, intact primary and secondary phloem, cortex, and periderm. The two pieces of bark were used in an experiment, one at a warm and one at a cold temperature. Cut ends as well as the disrupted cambial surface were free to absorb water when sections were floated for several hours on deionized water at the experimental temperatures. One experiment was run in a cold room at 2 C and the other in a laboratory at 22 C. The sections were maintained in darkness but were exposed to light at each blotting and weighing time.

In the second part of this experiment, intact wilted leaves were hung on the balance arm of a Mettler analytical balance in a controlled temperature room, and their petioles were cut under deionized water at 5 C or 24 C. The leaves were paired samples both taken from the same height on the stem of a slightly wilted cotton plant. Each leaf was coated over its entire surface with stopcock grease to prevent water loss.

Methods for Experiments on Stem Section Halves. Cylindrical pieces of stem about 2 cm long were excised and split longitudinally into two equal halves. Cut ends of the bark were sealed with either nail varnish or stopcock grease, and the outer corky layer (phellem) of the bark was removed. In one experiment, the xylem surface was sealed with nail varnish so that water could enter only through the cortical surface. In the other experiment, the cortical surface was coated with stopcock grease so that water would enter the phloem via the xylem across the cambial layer. The stem sections were submerged in water in constant temperature baths of 31 C and 2 C and were periodically blotted and weighed. In a third experiment, the procedure just described was repeated with one stem section being used to measure water uptake into the phloem across the cambial layer and the other being used to correct for uptake into the xylem. In this experiment, the stem was split longitudinally. In one section half, the phellem was removed and the bark ends and cortical surface were coated with stopcock grease. The bark was removed from the other stem section-half, leaving a piece of xylem tissue. This tissue was coated with stopcock grease on the outer (cambial) surface. Both halves were submerged in water at the same temperature (either 2 C or 31 C) and were blotted and weighed periodically. Tissues used at the two temperatures were excised from stem sections adjacent to each other on a slightly wilted cotton plant.

RESULTS AND DISCUSSION

Experiments on Intact Plants. For plants at warmer temperatures, curves relating bark thickness to time were similar to those previously reported (13, 14), with a simple, passive hydration phase during the first several hours followed by a second phase attributable to growth (Fig. 1, curve at 31 C). The growth phase was absent at 8 C and depressed strongly at 17 C. Furthermore, the time required to approach equilibrium was very much longer at colder temperatures than at warmer temperatures. The half-time was about three times longer at 17 C than at 31 C and 20 times longer at 8 C than at 31 C (Table I). Previously, Woolley (16) found that the half-time for equilibration of tritiated water in corn roots was 25 sec at 25 C and 480 sec at 3 C; that is, he found a temperature effect for roots similar to the one reported here for stems.

Half-times depend both on bark thickness and on tissue diffusivity. Diffusivities were calculated using the semi-log plot described previously (13). They ranged from 2.1×10^{-7} cm^2 sec^{-1} at 8 C to 1.7×10^{-6} cm^2 sec^{-1} at 39 C and showed a small temperature coefficient at the temperature extremes and a large temperature coefficient between 17 C and 31 C (Fig. 2). Diffusivity values indicate that between 39 C and 8 C tissue resistance to radial water flow increased by a factor of about 10. The curve in Figure 2 is reminiscent of relationships reported for cotton roots (5, 6), which show a large reduction in permeability between 20 C and 10 C with less temperature effect on either side of these values. Ginsburg and Ginzburg (3) have shown a similar relationship for corn root cortical sleeves (root sections with the vascular cylinder removed), where the greatest temperature effect on permeability occurs between 10 C and 32 C.

Both movement of water from xylem to phloem of stems and water entry into root xylem from external solutions show reductions with cold temperatures whereas leaf tissues give similar water uptake equilibration curves at cold and at warm

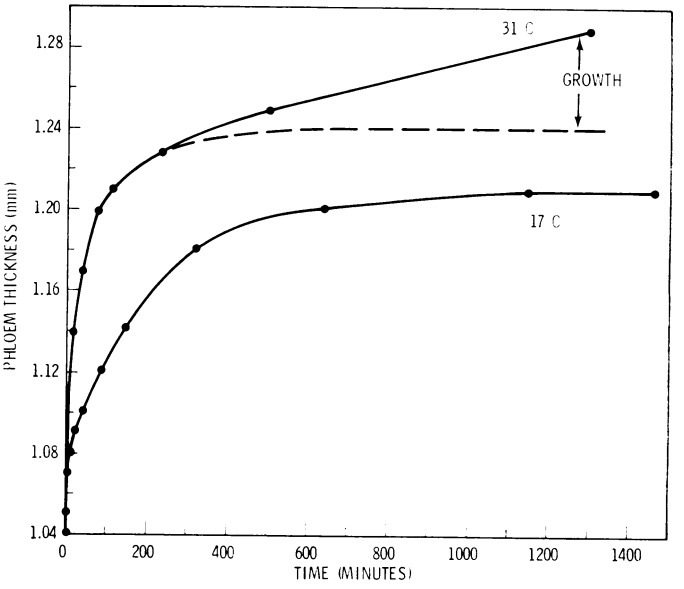

FIG. 1. Time course of bark thickness during hydration of cotton stems at two temperatures. The dotted line on the curve at 31 C shows the presumed curve which would have been obtained in the absence of growth.

Table I. *Values of Half-time of Equilibration for Intact Cotton Bark at Several Temperatures*

Temperature	$t_{1/2}$	Final Bark Thickness
C	min	mm
8	565	1.32
8	402	1.15
17	110	1.21
31	24	1.35
31	28	1.23
31	30	1.29
39	20	0.92

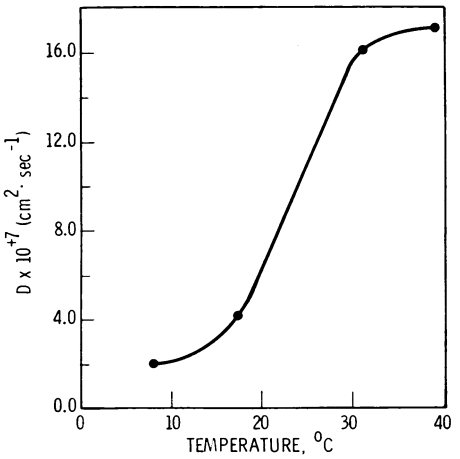

FIG. 2. Relationship to temperature of the diffusivity of intact cotton bark tissues.

temperatures (1, 9). Temperature does affect leaf disc growth so that water uptake associated with growth is reduced by low temperature, but the rate of approach to equilibrium is similar for a range of temperatures even though the equilibrium water content is lower at colder temperatures (8, 9). Thus, stem bark and leaves apparently differ with respect to the effect of temperature on permeability. Further experiments were done on excised tissues to investigate these effects.

Experiments on Detached Bark and Leaves. Figure 3 shows water uptake curves for cotton leaves and for isolated pieces of bark tissue. The curves show essentially identical water uptake patterns for the two tissues with a half-time for each at both temperatures of about 8 to 10 min. This is similar to the free energy transfer half-times (about 6 min) reported for sunflower leaves in a similar experiment (2).

Both types of tissue absorbed more water at the warmer temperature, but cold tissues approached their equilibrium water content as rapidly as did warm tissues. These results agree with those obtained by Millar (9) for *Pinus radiata* needles; namely, they show a decrease in the amount of water taken up at colder temperatures but a similar rate of approach to equilibrium.

Experiments on Stem Section Halves. The previous experiment led us to conclude that isolated bark tissues themselves were not the site of the increased resistance to water flow during the hydration process at cold temperatures. Consequently, we modified the experimental procedure to allow us to study water uptake into bark with the xylem-cambium-phloem boundary intact and with a geometry as similar as possible to the geometry of an intact stem. We treated stem section halves so that water entered bark either through the exposed cortex or through the xylem-phloem boundary.

The half-time of equilibration for stem sections hydrated via the cortex was about 42 min at 31 C and 50 min at 2 C. For the sections hydrated via the xylem and cambium, half-times were around 20 min at 31 C and 125 min at 2 C. The two experimental half-times cannot be compared for a given temperature because of differences in the geometry of hydration in each case. In addition, some leakage through varnished surfaces did occur. However, the fact that there was a large temperature effect in one case and not in the other is significant and is in agreement with observations on intact plants. Apparently, when water penetrates the phloem via the xylem-cambium pathway, there is a large resistance to transfer at cold temperatures.

In sections hydrated via the xylem-cambium pathway, a portion of the water taken up probably was in the xylem and not in the phloem. Furthermore, the previous experiment did not rule out the possibility that the temperature effect is located somehow in mature xylem tissues. Therefore, a modified experiment was done to allow values to be corrected for water in the xylem. Sections were coated with stopcock grease so that water entered via the xylem-phloem boundary.

Results are presented in Figure 4. At 31 C, equilibration was fairly rapid with a half-time of around 20 min; for 2 C, the curve shows a half-time of about 140 min. Since xylem weight changes have been subtracted out in this experiment, it appears that the site of the low temperature resistance is not in the xylem, but in the boundary tissue between mature xylem and mature phloem.

CONCLUDING DISCUSSION

There is a marked increase in the resistance to water flux into cotton bark at low temperatures. This effect appears to be similar to low temperature effects previously reported for cotton root systems (5, 6) and for tritiated water equilibration in

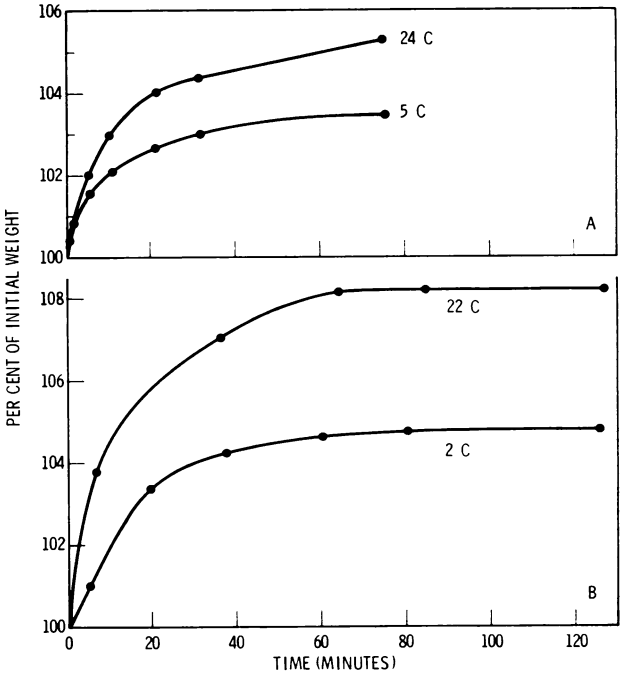

FIG. 3. Time course of hydration for cotton leaves (A) and detached bark of cotton stems (B) at different temperatures.

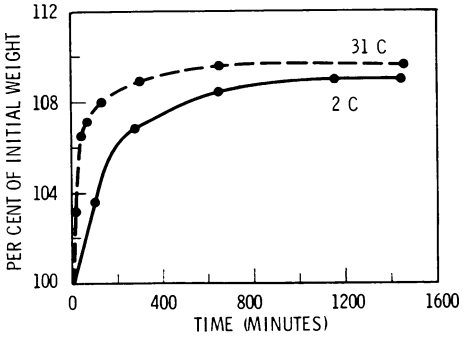

FIG. 4. Time course of water uptake into the bark of cotton stem segments at two temperatures. Values have been corrected for the weight of the xylem at each time.

corn roots (16). However, neither in the literature nor in the present experiments do leaves show the same effect. If they did, it would be tempting to think that the effect is in cell membranes, as Kuiper (7) has suggested. It seems likely that the effect, whether it is associated with cell membranes or with some other structure, is located in only part of the tissue, that is, a layer or ring of tissue present in roots and stems but absent in leaves. Since we have shown that detached bark with the cambial layer disrupted does not show the effect and intact bark does, we have been led to consider the cambial layer as the probable site of the high resistance to lateral water transfer in stems at low temperatures. This layer is chemically and anatomically different from the cambial derivatives on either side and is being investigated further in ongoing work in this laboratory to determine whether it is the site of high resistance at cold temperatures.

It is doubtful that the tissue of concern in young roots is the cambium. It is absent or does not form a complete ring in young roots so as to act as an effective barrier. Furthermore, Ginsburg and Ginzburg (3) have shown a high temperature coefficient over the range from 10 C to 32 C for cortical sleeves of corn roots. These sleeves were bounded on the inside by disrupted endodermis and did not have a cambial layer.

All of the tissues investigated show a decrease in fully turgid water content with reduced temperature. This relationship is also evident in the work of Barrs and Weatherley (1), Millar (9), Milburn and Weatherley (8), and others. Apparently, effects of temperature on cell wall elasticity, cell extensibility, and cell water potential are such that cells assume a smaller final volume when hydration occurs in the cold. This effect may or may not be distinct from effects of cold on growth and should be investigated further to allow more careful distinction between reductions in final volume of nongrowing cells and reductions in the expansion of growing cells caused by cold temperatures.

LITERATURE CITED

1. Barrs, H. D. and P. E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15: 413–428.
2. Boyer, J. S. 1969. Free-energy transfer in plants. Science 163: 1219–1220.
3. Ginsburg, H. and B. Z. Ginzburg. 1971. Radial water and solute flow in roots of *Zea mays*. III. Effect of temperature on THO and ion transport. J. Exp. Bot. 22: 337–341.
4. Klepper, B., V. D. Browning, and H. M. Taylor. 1971. Stem diameter in relation to plant water status. Plant Physiol. 48: 683–685.
5. Kramer, P. J. 1942. Species differences with respect to water absorption at low soil temperatures. Amer. J. Bot. 29: 828–832.
6. Kramer, P. J. 1969. Plant and Soil Water Relationships: A Modern Synthesis. McGraw-Hill, N.Y. 481 p.
7. Kuiper, P. J. C. 1972. Water transport across membranes. Annu. Rev. Plant Physiol. 23: 157–172.
8. Milburn, J. A. and P. E. Weatherley. 1971. The influence of temperature on the process of water uptake by detached leaves and leaf discs. New Phytol. 70: 929–938.
9. Millar, B. D. 1966. Relative turgidity of leaves: temperature effects in measurement. Science 154: 512–513.
10. Molz, F. J. 1972. Comments on the paper entitled "Resistances to water transport in soybean, bean, and sunflower." Crop Sci. 12: 400–401.
11. Molz, F. J. and B. Klepper. 1972. Radial propagation of water potential in stems. Agron. J. 64: 469–473.
12. Molz, F. J. and B. Klepper. 1973. On the mechanism of water-stress-induced stem deformation. Agron. J. 65: 304–306.
13. Molz, F. J., B. Klepper, and V. D. Browning. 1973. Radial diffusion of free energy in stem phloem: an experimental study. Agron. J. 65: 219–222.
14. Molz, F. J., B. Klepper, and C. M. Peterson. 1973. Rehydration versus growth-induced water uptake in plant tissues. Plant Physiol. 51: 859–862.
15. Philip, J. R. 1958. Propagation of turgor and other properties through cell aggregations. Plant Physiol. 33: 271–274.
16. Woolley, J. T. 1965. Radial exchange of labeled water in intact maize roots. Plant Physiol. 40: 711–717.