Plant Physiol. (1973) 51, 859–862

Rehydration *versus* Growth-induced Water Uptake in Plant Tissues[1]

Received for publication November 10, 1972

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ABSTRACT

Experiments show that the rate of water uptake by living tissues external to mature xylem of cotton stems (*Gossypium hirsutum* L. Auburn 7-683) is very similar to the corresponding curves for leaf tissue. In both cases one obtains a two-phase curve with phase I corresponding to passive rehydration and phase II pertaining to active growth.

A theory of water movement in plant tissue first proposed by Philip allows one to make a more rigorous distinction than made previously between phase I and phase II. This theory is applied explicitly to water uptake by leaf disks and results in a simple expression for the time required for phase I completion. Because the time required varies as the square of the disk radius, it is essential to use a standad disk size in water uptake studies of a particular tissue.

Additional analysis indicates that clear temporal distinction cannot be made between phase I and phase II. Different portions of the leaf disk rehydrate at significantly different rates, resulting in a grey zone with phase I and phase II occurring simultaneously in different parts of the disk.

It has been reported that when a nonturgid leaf disk is floated on water, the water uptake curve (plot of water uptake *versus* time) is composed of two phases (1, 2, 5). According to the experiments and analysis of Barrs and Weatherley (1), phase I is in response to the initial water deficit, and phase II results from the continued water uptake due to growth effects. It is not clear when rehydration ends and growth effects begin, and the present writers contend that such a separation cannot be made. For practical purposes, such as the determination of relative turgidity of leaves, it has been suggested that during the first 4 hr of the uptake process, rehydration is completed with little growth contribution (1). However, this must be recognized as a more or less arbitrary criterion.

The first objective of the present paper is to describe experiments which show that a similar uptake phenomenon occurs in the rehydration of water-stressed cotton stems. A second objective is to discuss a theory of moisture movement in plant tissue which allows for a more rigorous distinction than that made previously between phase I and phase II of the water uptake process.

MATERIALS AND METHODS

The basic experimental procedure was to sever the stems of water-stressed cotton plants (*Gossypium hirsutum* L. Auburn 7-683) beneath degassed aqueous solutions, thereby releasing the tension in the xylem. Water would then begin to flow radially into the phloem and associated living tissues, causing them to swell (6, 8). Except for the solutes present in the xylem sap and the geometrical-mechanical differences, this tension release process is analogous to that occurring when a nonturgid leaf disk is placed on a free water surface.

The volumetric water uptake of the phloem and associated tissues was determined as a function of time by monitoring the stem diameter approximately 12 cm below the cut with a linear variable differential transformer as indicated in Figure 1. Molz and Klepper (7) reported that the stem diameter increase following the severing is due almost entirely to the volume of water entering the phloem and associated tissues from the mature xylem. Thus, the volume of water absorbed may be determined directly from the LVDT[3] readings.

In certain experiments, the stems were severed under distilled water, while in others the fluid reservoir contained 10 mM KN_3. In the latter case it was reasoned that the solution would be pulled down into the xylem where it would inhibit metabolic activity at least in the cambial region of the phloem and associated tissues at the height at which the stem diameter was measured. Postexperimental visual examination of the cambial tissues indicated that the KN_3 did penetrate. A third variation of the basic procedure was to sever the stem under −4 bar Carbowax (polyethylene glycol) solutions. The distilled water experiments were also repeated several times at a low temperature.

RESULTS

Figures 2, 3, and 4 show typical plots of the percentage of initial volume of phloem and associated tissues *versus* time. The plants in different experiments were not equally dehydrated, and so the data cannot be presented in precisely the format utilized by Barrs and Weatherley (1). However, dehydration differences were held to a small range. At time zero the stems were severed and the initial temperature of 31 C was maintained throughout the experiment. Figure 2 corresponds to a stem severed under distilled water, while Figure 3 is for a stem severed under the KN_3 solution. The curves are very similar to those obtained by Barrs and Weatherley (Ref. 1,

[1] This study was supported in part by the Engineering Experiment Station and by Alabama Project 5-863, Alabama Agricultural Experiment Station, Auburn University, Auburn, Ala. 36830.

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[3] Abbreviation: LVDT: linear variable differential transformer.

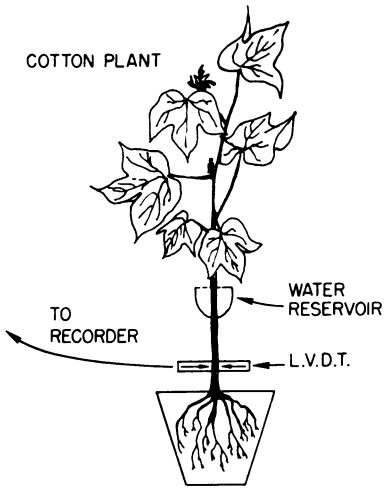

FIG. 1. Schematic diagram of an experimental arrangement in which a water-stressed cotton stem was severed below a water solution in a reservoir and the subsequent stem swelling monitored with an LVDT.

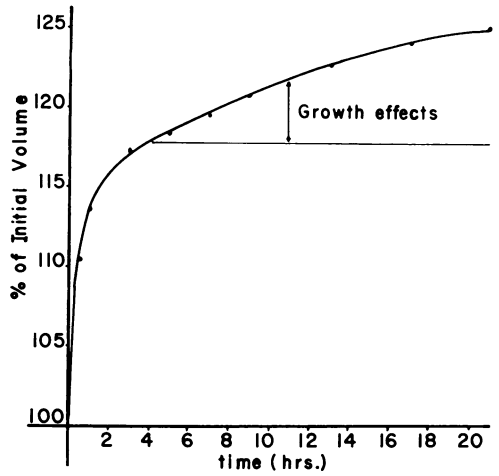

FIG. 2. Increase in volume of phloem and associated tissues as a percentage of the initial volume for a cotton stem severed under distilled water. After approximately 4 hr, the volume increase is due mainly to growth.

Fig. 3) for leaf disks. The first 3 or 4 hr would correspond to passive rehydration (phase I) while the remainder would correspond mainly to growth (phase II). If we accept the convention that within 4 hr phase I is completed (the stem swells back to its original fully turgid diameter in about 4 hr), then it is seen that after 21 hr at least 26% of the total volume increase shown in Figure 2 can be attributed to growth effects. With the stem cut under the solution of the metabolic inhibitor (Figure 3) phase II at 21 hr was held to about 12% of the total volume. Growth of leaves is limited severely when cells are subjected to water potentials in the range of −3 to −4 bars (2). Hence phase II is essentially eliminated in Figure 4 when the stem was severed under the Carbowax solution which allowed the stem to swell only until it equilibrated with −4 bars. Experiments performed at 8 C showed that growth was eliminated by a low temperature. All of these results are consistent with those obtained by Barrs and Weatherley (1) for leaf tissue.

Separation of Phases I and II. Philip (9) developed a theory, subject to certain assumptions, which describes the spatial and temporal dependence of passive water relations of plant tissue. The fundamental parameter of the theory is the diffusivity of plant tissue which is given by Philip (9) as

$$D = \frac{\alpha KL\ (\epsilon + P_0)}{2} \quad (1)$$

where D = diffusivity, α = a shape factor, K = permeability of cell wall to water, L = length of typical cell, ϵ = elastic modulus of cell wall, and P_o = osmotic potential of cells at zero turgor. On theoretical grounds, Philip (12) estimated that D is on the order of 5×10^{-6} cm^2 sec^{-1} for plant leaf tissue, which in the present application includes mesophyll cells and vascular strands. Philip (11) also quoted data which yielded a D value for *Avena* coleoptile tissue of 2.3×10^{-5} cm^2 sec^{-1}. Employing an experimental approach, Molz *et al.* (8) obtained a D value at 31 C of 1.6×10^{-6} cm^2 sec^{-1} for the phloem and associated tissues of cotton stems. This value is approximate since it is based on the simplifying assumption that stem tissue external to xylem is homogeneous. Evidently, the diffusivity can vary considerably for tissues of different species.

If a value for D is known along with certain other geometrical and boundary properties, then the time required for a dehydrated tissue to reach full turgidity can be estimated. The elaboration of this point which follows will pertain ex-

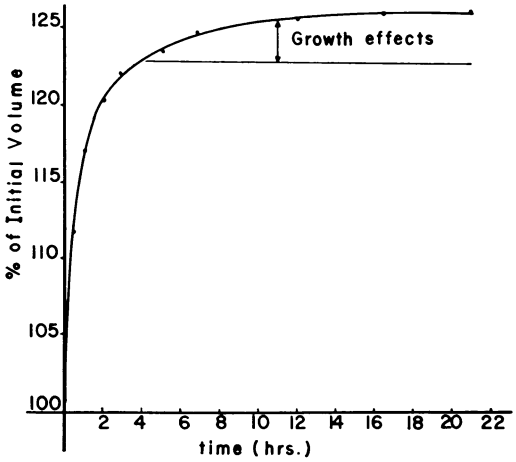

FIG. 3. Increase in volume of phloem and associated tissues as a percentage of the initial volume for a cotton stem severed under 10 mM KN_3. After approximately 4 hr, the volume increase is due mainly to growth.

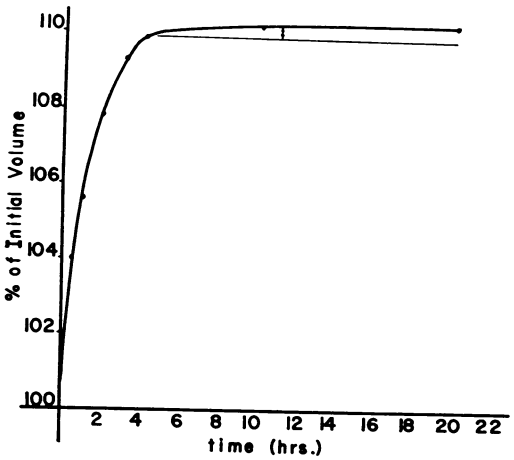

FIG. 4. Increase in volume of phloem and associated tissues as a percentage of the initial volume for a cotton stem severed under a −4 bar Carbowax solution.

plicitly to leaf disks because of their practical importance in relative turgidity determinations. However, a similar analysis can be applied to passive water uptake in other types and shapes of plant tissue. An analysis of the hollow cylindrical case may be found in Richards and Richards (13).

Experiments discussed by Slatyer (Ref. 14, p. 188) have shown that a leaf disk floating on a water surface will behave as a cylinder so far as water and free energy uptake are concerned (8). This is because most of the water would be expected to enter through the cylindrical cut edge as opposed to the cuticular leaf surfaces, which would be relatively impermeable. For a leaf disk of radius, a, initially at a water potential of ψ_0 and floated on water at time zero, the equation governing the water potential distribution in the leaf, $\psi(r, t)$, (6) may be written as:

$$\frac{\partial \psi}{\partial t} = D \frac{\partial^2 \psi}{\partial r^2} + \frac{D}{r} \frac{\partial \psi}{\partial r} \tag{2}$$

with auxiliary conditions

$$\psi(a, 0) = 0 \tag{2a}$$

$$\psi(r, 0) = \psi_0 \tag{2b}$$

where ψ = water potential, t = time after floating, r = radial distance from disk axis, a = disk radius, and ψ_0 = initial water potential. The solution of equation 2 may be obtained from Crank (Ref. 4, p. 66), and Figure 5 shows ψ as a function of r and t for a 0.5-cm floating leaf disk. If the reasonable assumption is made that the water flux into the leaf is proportional to the water potential gradient at the cut surface, then the fractional uptake of water is given by Crank (Ref. 4, p. 66):

$$U = \frac{M(t)}{M(\infty)} = 1 - \sum_{n=1}^{\infty} \frac{4}{a^2 \alpha_n^2} \exp\left[-D\alpha_n^2 t\right] \tag{3}$$

where U = fractional uptake, $M(t)$ = cumulative mass of water that has entered by time t, $M(\infty)$ = total mass of water which enters owing to passive rehydration. The α_n values are tabulated roots of the equation (the roots are available in standard mathematical tables):

$$J_0(a\alpha_n) = 0 \tag{4}$$

where J_0 = Bessel function of the first kind and of order zero. An expression similar to equation 3 for the hollow cylindrical case may be obtained from Richards and Richards (13). From equation 3 it is evident that at a given temperature, U is dependent only on the quantities a^2, D, and t. It can be shown (Ref. 4, p. 72) that when the dimensionless variable Dt/a^2 is equal to approximately 0.6, the ratio $M(t)/M(\infty)$ is for practical purposes unity (*i.e.*, passive rehydration is 98% complete). For a leaf disk of 0.5 cm radius and the diffusivity value suggested by Philip (12), the time required to complete passive rehydration would be given by

$$t = \frac{(0.6)(0.5)^2}{5 \times 10^{-6}} = 30{,}000 \text{ sec} \tag{5}$$

or about 8.3 hr. The time for 90% completion would be around 4.6 hr. Apparently theory yields values that tend to be a little higher than the 4 hr suggested by experiment. Assuming the experimental estimate to be correct, the discrepancy could be due to cuticular leakage, a low diffusivity value, the presence of air-free xylem strands, nonhomogeneity effects, or a combination of all. However, the growth data of Boyer (2), along with the water potential distribution shown in Figure 5, indicate that growth would be occurring throughout the disk within 4 hr. If the "4 hr criterion" was more indicative of the beginning of growth as opposed to the end of phase I, theory and experiment would be in better agreement.

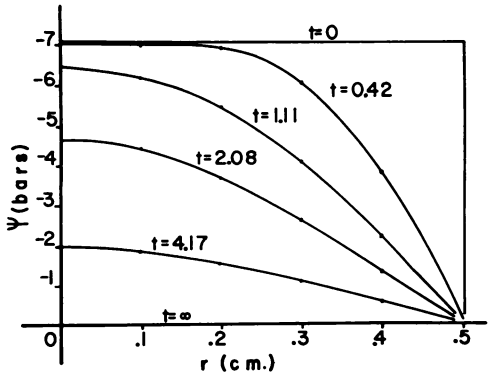

FIG. 5. Water potential, ψ, as a function of radius, r, at various times, t, for a 0.5-cm floating leaf disk initially at a water potential of −7 bars.

CONCLUSIONS

The qualitative aspects of water uptake curves for the phloem and associated tissues external to the xylem of cotton stems are essentially identical to the corresponding curves for leaf tissue. In both cases one obtains a two-phase curve with the first phase corresponding to passive rehydration and the second phase related to active growth effects. (The writers do not wish to imply that growth and phase II are identical.) This conclusion is consistent with the observation that phase II can be suppressed or eliminated by metabolic poisons, osmotic solutions, or low temperatures.

A theory of moisture movement in plant tissue first proposed by Philip (9) offers the possibility of making a more rigorous distinction than made previously between phase I and phase II in water uptake studies. For leaf disks in which the cuticular surfaces are impermeable as compared to the cut edge, the time required for phase I to be essentially completed is given by Crank (Ref. 4, p. 72):

$$t_c = \frac{0.6a^2}{D} \tag{6}$$

where t_c = time for completion of passive rehydration, a = radius of leaf disk, and D = leaf tissue diffusivity in the plane of the leaf. Equation 6 indicates that it is important to use a standard disk size in water uptake studies since t_c varies theoretically as the square of the disk radius. (In actuality, the radius squared dependence is probably an upper bound.)

Presently, the main difficulty in utilizing equation 6 to improve the method for determining relative turgidity of various tissue is in obtaining accurate values for the diffusivity of different tissues. While both theory and experiment indicate the probability that 4 hr is a reasonable time for completion of phase I in most leaf tissue, it is very possible that considerably more or less time is required for certain types of tissue. This can be resolved theoretically if and when more reliable values for diffusivity become available.

The effect of leakage through the cuticular surfaces of leaf disks would be to reduce t_c below the value indicated by equation 6. Should such leakage be important, the equation can be suitably modified if the permeabilities of the surfaces are

determined. Expressions analogous to equation 6 can be obtained and utilized for other shapes and types of plant tissue provided the required geometric and hydraulic properties are known.

It is almost certain that a perfectly clear temporal distinction between phase I and phase II cannot be made. The solution of equation 2 shown in Figure 5 indicates that during water uptake different parts of the tissue will rehydrate at significantly different rates. Therefore, phase II would be expected to begin in the tissue near the cut edges of a leaf disk while the interior portion was still undergoing phase I. This would lead to a "grey zone" in which phase I and phase II occurred simultaneously. However, because of the relatively slow advance of phase II with respect to phase I, it is probable that the "grey zone effect" leads to tolerable errors in relative turgidity determinations. Most likely a good estimate of the end of phase I will serve to separate the two effects sufficiently. If growth can be approximated as a function of water potential or turgor pressure, however, it would be possible to utilize the theory under discussion to analyze further the distinction between phase I and phase II in the water uptake of plant tissues. This would enable a more quantitative conclusion concerning the importance of the grey zone effect.

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