

Temperature Effects on Soybean Imbibition and Leakage

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ABSTRACT

As a part of an analysis of the nature of chilling injury to seeds, measurements were made of the initial linear rates of water entry into and solute leakage out of cotyledons of soybean at various temperatures. Arrhenius plots were approximately linear for water entry into both living and dead cotyledons, with the slope (and activation energy) for entry into living cells being insignificantly higher than for dead cells, suggesting little effect of membrane barriers on water entry. The plots for solute leakage showed 10-fold lower leakage rates from living than from dead tissues; a reversal of slope in the Arrhenius plot at temperatures below 15 C reflected increasing leakage rates, interpreted as a quantitative disruption of membrane reorganization at the temperatures associated with chilling injury.

Evidence has been accumulating that chilling injury to seeds during imbibition might be attributed to disruptive effects on the reorganization of membranes as water enters the dry seed (1, 9). A quantitative means of analyzing the barrier effectiveness of membranes could utilize the initial linear rates of water entry and of solute leakage from soybean cotyledons, and their analysis through Arrhenius plots.

The time course of initial water entry into dry cotyledons shows a period of rapid, nonlinear entry lasting 5-10 min, followed by a period of linear water entry lasting 30 min or more (7, 11). The initial time course of solute leakage from the imbibing cotyledon also shows a period of rapid, nonlinear leakage, followed by a linear phase beginning at about the same time that water entry becomes linear. It has been suggested that the initial rapid phases of each of these events represented a period during which the membranes were relatively disorganized, and the linear phases represented steady-state processes through the reorganized membranes (7, 8, 11). If this interpretation is correct, linear rates of water entry and of solute leakage should reflect the effectiveness of membranes in resisting the passage of water and of solutes, respectively.

The experiments reported here measure the linear rates of water entry and solute leakage of cotyledons at various temperatures to permit construction of Arrhenius plots and estimation of the energy barriers provided by the reconstituted membranes.

MATERIALS AND METHODS

Water entry and solute leakage were measured using cotyledons taken from seeds of soybean (*Glycine max* [L.] Merr. cv. Wayne) screened for uniform size (6.7-8 mm). Some cotyledons were heat-killed at 100 C overnight. Before imbibition, all cotyledons were placed in a saturated atmosphere for 24 h to elevate the initial water content to about 20% on a wet weight basis.

The time course of water entry was measured as the fresh weight of 5 cotyledons immersed in 5 ml water. Weights were taken at 10-min intervals after blotting with facial tissues. Constant tem-

peratures between 1 and 40 C were maintained by immersing the imbibing system in a water bath.

The time course of solute leakage was measured as the rise in conductance of 25 ml of water containing 10 cotyledons. Conductance was measured with a Markson ElectroMark analyzer at 5-min intervals.

Rates were determined from the slope of the 10- to 40-min periods, using calculated regression lines. The logarithms of these slope values were plotted as Arrhenius plots, and activation energies were then calculated using the equation of Stein (13):

$$2.3 \log \frac{k'}{k''} = \frac{A_d}{R} \left(\frac{1}{T''} - \frac{1}{T'} \right)$$

where A_d is the activation energy, k' and k'' are log rates for 40 and 5 C (T' and T'' , respectively), and R is the gas constant.

RESULTS

Representative curves for the time course of water uptake and of solute leakage from soybean cotyledons are shown in Figure 1. The rate for both water uptake and solute leakage is constant after the first few min of imbibition. It can also be seen that dead cotyledons leak solutes at a much higher rate than do live cotyle-

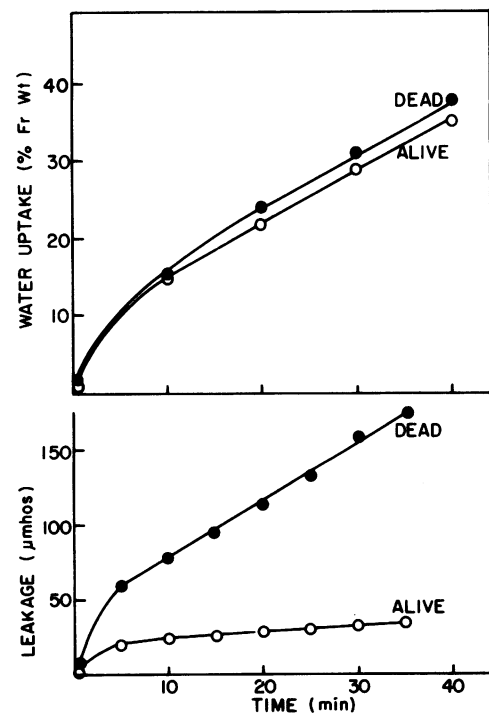


FIG. 1. Time course curves for water uptake (upper) and solute leakage (below) for living and dead cotyledons of soybean. Water uptake as cumulative fresh weight; leakage as a cumulative conductance of ambient solution; 23 C.

dons.

The log rates of water uptake are plotted against the inverse of the temperature to obtain an Arrhenius plot in Figure 2. Dead cotyledons exhibit approximately a linear slope (-1.1) over the temperature range tested. Living cotyledons show a slightly steeper slope over the same temperature range (-1.4), though the Student *t* tests did not indicate that the difference was significant. Over the lower temperature range, dead cotyledons have a slightly higher rate of water entry than do living cotyledons.

An Arrhenius plot of solute leakage rates over the same temperature range is shown in Figure 3. Dead cotyledons show an approximately linear plot over the entire temperature range (slope = -1.6), but living cotyledons show a striking break in the plot, with a linear region above 20 C having a slope of -1.7, and a completely different slope (+1.8) below 20 C. Although the slopes of the plots for dead and for live cotyledons at temperatures above 20 C are roughly similar, the rates of leakage from dead cotyledons are an order of magnitude faster than those from living tissue.

From these Arrhenius plots, one can calculate apparent activation energies for water uptake and for solute leakage. Water entry into both dead and live cotyledons shows apparent activation energies in the range of 4 and 5 kcal mol⁻¹ (Table I), which is expected for the free diffusion of water and other small molecules (13). Activation energies for solute leakage from both dead and living cotyledons from 20 to 40 C is roughly 7 kcal mol⁻¹. The activation energy for leakage in the temperature range of 1-20 C is a negative number, indicating that in this range an increase in temperature results in a decrease in the ease with which solutes can leak from the tissue.

DISCUSSION

Kinetic analysis of the passage of water and solutes into and out of cotyledon tissue has been used to make inferences about the nature of chilling damage. The use of linear rates of initial water entry, and linear rates of solute leakage for living and dead cotyledons permits comparison of the passage of substances into and out of the tissue under conditions where membrane reorganization can and cannot be effected. Arrhenius plots of water

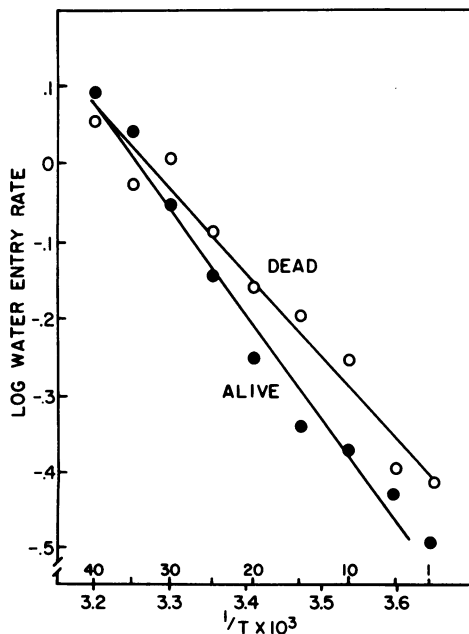


FIG. 2. Arrhenius plots for water uptake rates (log %/min) by living and dead cotyledons. The two slopes are not significantly different as measured by the student *t* test.

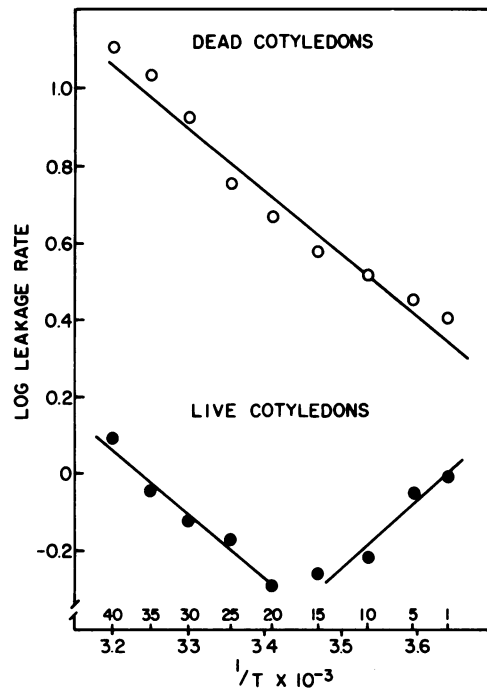


FIG. 3. Arrhenius plots for solute leakage rates (log μ mos/min) from living and dead cotyledons.

Table I. Calculated Activation Energies for Water Uptake and Solute Leakage from Soybean Cotyledons

Tissue	Activation Energy	
	Water Uptake	Solute Leakage
	kcal mol ⁻¹	
Dead cotyledons	4.7	7.3
Live cotyledons	5.8	7.6 (20-40 C) -8.0 (1-20 C)

entry (Fig. 2) show only a slightly steeper slope for entry into living than into heat-killed tissue, which is consistent with the concept that the passage of water through tissues is only slightly hindered by membranes (*cf.* 3, 15). Temperatures which cause chilling injury (16 C and below) do not bring about dramatic changes in the Arrhenius plot of water entry. In contrast, Arrhenius plots of solute leakage (Fig. 3) indicate that living tissues leak solutes at rates about 10-fold lower than the dead tissues. This is consistent with the idea of using leakage rates as indicators of the barrier effectiveness of membranes in the tissue (1, 7, 11). The inversion of the slope of the leakage rates below 15 C provides dramatic evidence that, at these lower temperatures, membranes may be reorganized in a faulty manner and show lessened effectiveness as a barrier.

The concept that water entry into dry tissues leads to a reorganization of membranes and that chilling temperatures interfere with this reorganization, was first suggested for yeast rehydration by Herrera *et al.* (4). The fact that chilling temperatures during the first imbibition of water damage many kinds of seeds was noted by Pollock and Toole (9). Simon (11) attributed the chilling damage to an interference with a supposed transition from a hexagonal organization of phospholipids in the dry state to the lamellar organization in the aqueous state. Van Steveninck and Ledebouer (14) suggested on the basis of Arrhenius plots that the damage from chilling rehydrating yeast cells was possibly related to a phase transition which caused interference with lipid organi-

zation at low temperatures. The data reported here show large differences in the slope of Arrhenius plots of leakage rates between the warm and the chilling temperatures of living cotyledons, and pronounced changes in the apparent activation energies within these two temperature ranges, providing quantitative support of the concept that chilling temperatures interfere with the normal reorganization of membranes during water entry.

The entry of water into dehydrated tissues such as dry seeds must be expected to result in extensive reorganization of cell components, especially membranes (11, 16). Early work by Brown (2) indicated that rates of water entry increased exponentially with temperature, although Shull (10) asserted that the temperature effects were more complicated than Brown had described. Waggoner and Parlange (15) have shown that water entry proceeded at similar rates for living and dead pea seeds. Water entry into several types of seeds has activation energies between 6 and 7 kcal mol⁻¹ (6). Since water entry into soybean cotyledon tissue shows no significant differences in the Arrhenius plots for living and dead tissues, and only slightly higher activation energies for living than dead tissues, it seems reasonable to conclude that water entry into seeds is not appreciably limited by the reforming membranes. The rate of water entry can be considered to be principally a function of the diffusion of water and the hydration of polyelectrolytes in the tissue.

Solute leakage from imbibing seeds is greater at temperatures below 20 C (5, 11, 12). Bramlage *et al.* (1) established that imbibition temperatures which cause chilling damage to germination and growth rates are associated with increased leakiness from soybean embryos, and the Q_{10} of leakage rates dropped to values less than one in the temperature range where chilling injury occurs. The 10-fold lesser leakage rates from living soybean cotyledons than from dead ones (Fig. 3) may be a consequence of the profound limitation of solute leakage by cellular membranes; the upswing of leakage rates in the Arrhenius plot at temperatures

below 20 C is consistent with the interpretation that successively lower temperatures cause increasing damage to the reorganizing membranes, which then approach the degree of solute leakiness of the dead tissues at 1 C.

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