

Effect of Temperature on Water and Ion Transport in Soybean and Broccoli Systems¹

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ABSTRACT

Steady-state flow rates and exudate osmotic potentials were measured from complete root systems from warm- (28/23 C) or cold- (17/11 C) grown soybean or broccoli (*Brassica oleracea*) plants at various pressures or different temperatures.

In warm-grown soybean roots systems, a break occurred at 14.7 C in the Arrhenius plot of total flow at constant pressure. When plants were grown at lower temperatures, the break point shifted to 8 C. Broccoli, a chilling-resistant species, showed no break for the temperature range used, but cooler growth temperatures decreased the activation energy for water flow through the root system from 18 kilocalories per mole to 9 kilocalories per mole. In both broccoli and soybean, cold-grown plants had lower exudate potentials and greater flow rates at low hydrostatic pressures than the warm-grown plants.

These observations indicate that the rate-limiting site for passive water transport is a membrane which may be modified as the plant acclimates to varying growth temperatures. An additional part of the acclimation process is an increase in activity of root ion pumps.

Since the time of Hales (1727) it has been known that cold soil reduces the absorption of water by plants. Differences among species in reaction to water absorption at low temperatures were reviewed by Kramer (11). Kuiper (13) was among the first to note that at a "critical" temperature, which varied with the temperature at which roots were grown, a sharp drop occurred in water absorption. Some investigators attributed anomalies such as this to discontinuities in properties of surface water at 15, 30, 45, and 60 C (7, 15, 18).

A more likely hypothesis attributes sharp breaks in Arrhenius plots of biological phenomena to phase transitions in membrane lipids in which the hydrophobic core changes from a liquid to a solid or crystalline state at some specific temperature. Such changes alter membrane permeability and enzyme activity (8, 10, 19). Arrhenius plots were used by Clarkson (2) to study exudation rates and ion concentration of the xylem exudate from excised barley and rye roots preconditioned at various temperatures. With rye roots grown at 20 C he found a break in the Arrhenius plot of exudation rate versus temperature at 10 C, but for roots preconditioned at 8 C for 3 days the break occurred at 5 C. There was no break in the Arrhenius plot of barley roots.

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The formal use of an Arrhenius plot is to graph the natural log of a reaction rate constant versus the reciprocal of the absolute temperature. Convenience has usually led to the use of reaction rates rather than rate constants, with the assumption that the reaction rate is linearly related to the rate constant. In a system as complex as the coupled movement of solute and solvent through roots under hydrostatic pressure, this assumption is not necessarily valid. This is evident from the simplified rate equation:

$$J_v = L_p(\Delta P - \sigma\Delta\pi)$$

where J_v is the total rate of root exudation in $\text{cm}^3 \text{cm}^{-2} \text{s}^{-1}$, L_p is the hydraulic conductivity in $\text{cm}^3 \text{cm}^{-2} \text{s}^{-1} \text{bar}^{-1}$, ΔP is the hydrostatic driving force in bars, σ is the dimensionless reflection coefficient, and $\Delta\pi$ is the osmotic driving force in bars. From this relationship it can be seen that not only would a change in L_p affect the flow rate, but a change in the effective osmotic driving force, either by a change in the reflection coefficient or the osmotic gradient, would have similar effects. Before we can relate temperature-induced changes in flow rates to changes in conductivity, a linear relationship between J_v and L_p over the temperatures and pressures used must be demonstrated.

Experiments thus far done with intact plants, bleeding roots, or excised roots under suction have not discriminated between effects on the hydraulic conductivity and driving force, making mechanistic interpretations difficult. Our experiments were designed to provide more information concerning the effects of growth temperature on the permeability of roots of a warm and a cool season plant to water at various temperatures. They were also designed to show whether temperature-induced changes in water flux are caused by changes in the driving force ($\Delta\pi$) or in the hydraulic conductivity (L_p) of the roots.

MATERIALS AND METHODS

Soybean (*Glycine max* L. cv. Ransom) and broccoli (*Brassica oleracea* var. *botrytis* L. cv. Green Sprouting) were grown hydroponically in 3-liter plastic pots containing half-strength Hoagland solution for 30 to 60 days in controlled environment chambers at the Duke University Phytotron. Solution level was kept constant by filling the pots with distilled H_2O daily and nutrient solution weekly. Every 2 weeks the growth solution was replaced. All plants were kept under a long day photoperiod of 14 h light, and thermoperiods of 28/23 C for warm-grown plants and 17/11 C for cold-grown plants. Both regimes kept soybeans from flowering; broccoli occasionally flowered in the cold temperature regime. No differences were observed in root properties between those that flowered and those that did not.

Plants were detopped early in the morning and sealed in a pressure chamber containing 10.3 liters of half-strength Hoagland solution. The chamber was pressurized with compressed air (80% N_2 , 20% O_2) through an aquarium-type "air stone" in the bottom

of the pot. A bleed-off valve regulated flow and allowed for constant agitation of the solution. A copper circulating coil in the bottom of the pot enabled temperature control within ± 0.5 C. An injection port was used to keep the solution level constant, as well as to allow for the injection of compounds while maintaining a constant pressure.

Root systems were either held at a constant temperature and the pressure varied in steps between 0.2 and 5.0 bars, or held at a constant pressure and the temperature varied between 25 and 4 C in steps of about 3 C. Steady-state values of total root exudation rates (Q) in $\text{cm}^3 \text{s}^{-1}$ and the osmotic concentration (π) in mosmol kg^{-1} of the exudate were determined for each increment. Osmotic concentration was determined with an Advanced Instruments osmometer. The potential in bars was then calculated, assuming an ideal solution, by the Van't Hoff relation.

Several root systems were sealed in the pressure chamber and treated with 2 mM sodium azide for 12 h at 25 C, at 0.2 bar pressure. The pressure was then increased to 5 bars and the temperature lowered in 3 C increments to 7 C in the same manner as with live root systems.

The hydraulic conductance (L) of the root systems was calculated from the slope of the curve for total flux versus applied hydrostatic pressures between 2 and 5 bars where the relationship becomes linear (9). Since surface area measurements were not made, all experiments were comparisons within root systems. Consequently, the L and Q values are for the whole root system and have not been converted to a unit area basis. In those cases data have been normalized to the flow rate at 5 bars and 25 C so that relative changes may be seen.

In the temperature experiments, flow rates are plotted in the Arrhenius form with the activation energies (E_a) calculated from the slope of the straight line portions of the curves.

RESULTS

Temperature Effects on Total Flow. The shapes of the Arrhenius plot or the activation energies were not dependent on the direction of the temperature change. Figure 1 is an Arrhenius plot

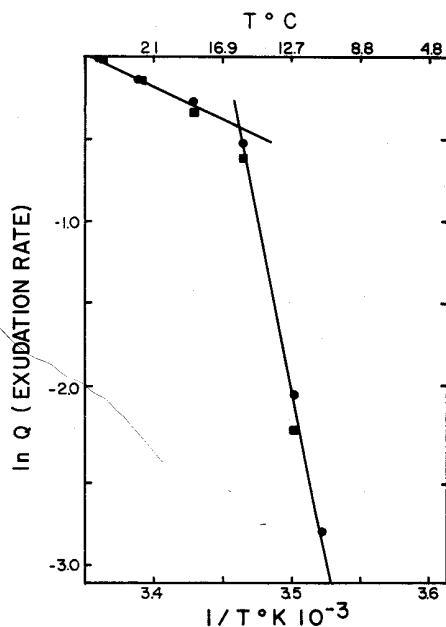


FIG. 1. Comparison between descending (●) and ascending (■) temperatures on Arrhenius plot of flow versus reciprocal temperature for soybeans grown at 28/23 C. Temperature was lowered from 25 to 7 C and then raised back to 25 C in approximately 3 C steps.

of flow rate versus temperature for a warm-grown soybean. The temperature was first decreased in steps to 7 C and then increased in steps to 25 C. Points from the ascending and descending temperature fall along the same lines and the break in the plot occurs at about 14 C.

Attempts to determine whether changes in conductance or the driving force are responsible for the decreases in Q due to a decrease in temperature are summarized in Figures 2 and 3. Soybean roots were pressurized at 3, 4, and 5 bars at five temperatures between 25 and 10.8 C (Fig. 2). The slope of each of these lines is the conductance of the root system at that temperature (9). Figure 3 shows the results of plotting the normalized total flow

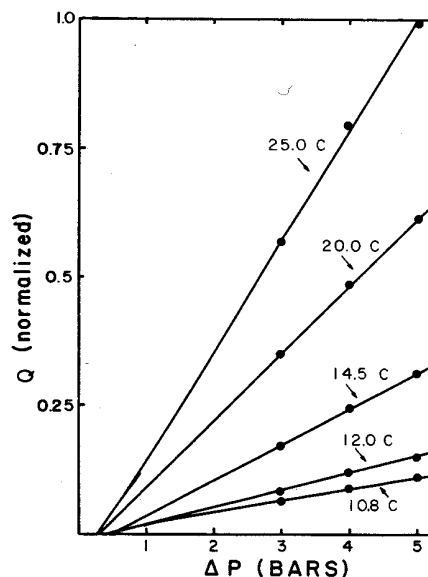


FIG. 2. Effect of temperature on flow curve (Q versus ΔP) of a soybean root at 3, 4, and 5 bars hydrostatic pressure. All flow rates were normalized to the rate at 25 C and 5 bars. Data are from one of four root systems, all of which responded similarly. Slope of each line is L_p of root system at that temperature.

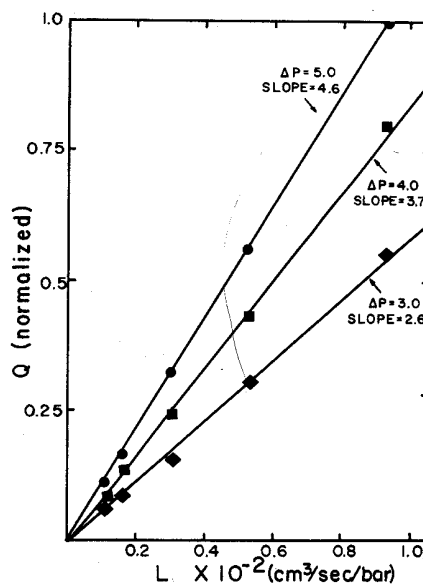


FIG. 3. Secondary plot from Figure 2 of flow rate versus L at each hydrostatic pressure. Slope in bars, of each line, is equal to $(\Delta P - \sigma\Delta\pi)$.

(Q) versus the conductance (L) at each hydrostatic pressure. In each case there was a linear relationship between Q and L, intercepting at the origin, and having a slope equal to $(\Delta P - \sigma\Delta\pi)$.

The effects of growth temperature on the Arrhenius plot of flow rate versus temperature are summarized in Figures 4 and 5. A single break was apparent in the curves for both the warm- and cold-grown soybean plants (Fig. 4). The break for warm-grown plants was about 14 C and, for cold-grown plants, about 9 C. Growth temperatures had no effect on the activation energy (Ea) above the break point, where Ea = 5 kcal/mol, or below the break point, where Ea = 35 kcal/mol.

Warm- and cold-grown broccoli both had straight line Arrhenius plots (Fig. 5). The Arrhenius activation energy, however, did decrease with lowered growth temperatures. The warm-grown plants had an Ea of 19.7 kcal/mol, while the cold-grown plants had an Ea of 9.3 kcal/mol.

Broccoli and soybean root systems treated with 2 mM sodium azide lost both the ability to accumulate ions and their semipermeability. The solution flowing from the stump was identical to the ambient solution. An Arrhenius plot of flux versus temperature showed no break and had an Ea of 3.6 kcal/mol.

Effect of Growth Temperature on Pressure Curves. Soybean root systems from cold- and warm-grown plants were pressurized in steps between 0.2 and 5.0 bars at 25 C. The pressure was then dropped to 0.2 bar and the temperature dropped to 10 C. When steady-state conditions were reached the roots were again pressurized in steps to 5.0 bars. In Figure 6 the measured flow rates have been normalized to the rate at 25 C at 5.0 bars, and are the averages of data for three root systems. Both the warm and cold soybeans had a decrease in the L from 25 to 10 C; the cold-grown plants decreased 34%; the warm-grown plants decreased 55%.

Differences were also apparent for the warm- and cold-grown soybean plants at low hydrostatic pressures. In the warm-grown plants the flow rate at 0.2 bar at 25 C was 2% of the flow rate at 5.0 bars, whereas for the cold-grown plants it was 5.5% (Fig. 6).

The change in L between the 25 and the 10 C pressure series

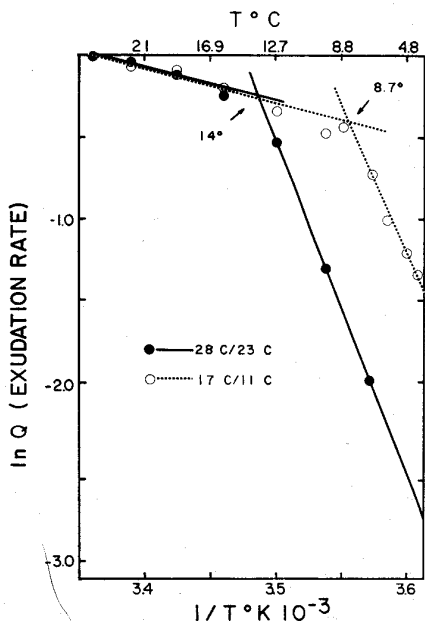


FIG. 4. Effect of growth temperatures on water flow through soybean roots at constant hydrostatic pressures of 5 or 6 bars and decreasing temperatures. Each curve is average of four experiments for plants grown at 28/23 C (●—●) and 17/11 C (○---○). Flow rates for each root system were normalized to the rate at 25 C and the relative rates averaged.

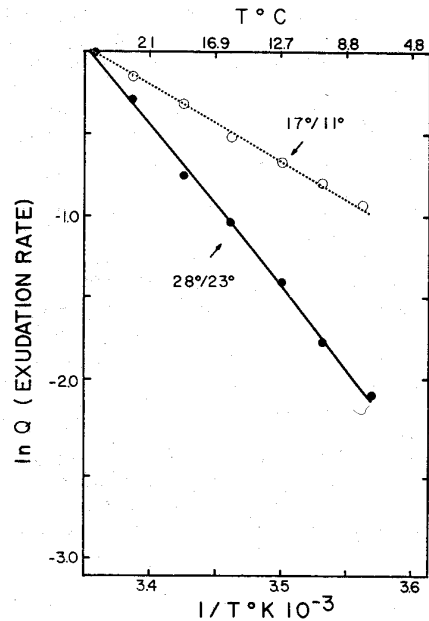


FIG. 5. Effect of growth temperatures on water flow through broccoli roots at constant hydrostatic pressures of 5 bars and decreasing temperatures. Each curve is mean of three experiments for plants grown at 28/23 C (●—●) and plants grown at 17/11 C (○---○). Flow rates for each system were normalized to the rate at 25 C. Relative rates averaged and plotted in the Arrhenius form of natural log of flow versus reciprocal of absolute temperature.

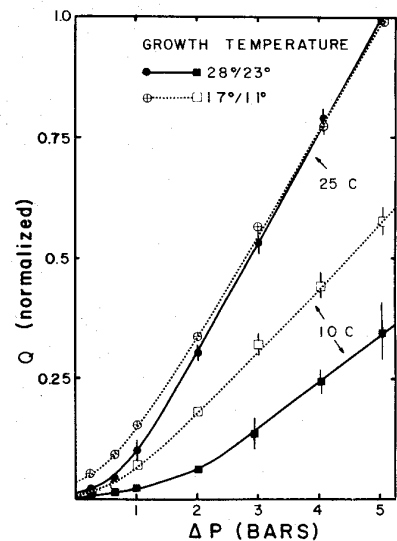


FIG. 6. Effect of growth temperature on flow curve (Q versus ΔP) of soybean root systems at 25 C and then at 10 C. Flow rates for each root system were normalized to the rate at 25 C and 5 bars. Means \pm SEM are plotted for 28/23 C-grown plants measured at 25 C (●—●) and 10 C (■—■), and for 17/11 C-grown plants measured at 25 C (○---○) and 10 C (□---□).

was not due to aging. When two pressure series were run consecutively at 25 C the flow curves were almost identical between 2 and 5 bars with a conductance change of not more than 5% in any one experiment.

The L of soybean root systems did not change at high pressures during the 48 h required to complete some experiments, but the

roots of the broccoli plants were less stable. This decreased the reliability of comparisons between 25 C pressure series and 10 C pressure series on the same root system, although comparisons could still be made between the 25 C pressure series run on both warm- and cold-grown plants (Fig. 7). In warm-grown broccoli, the flow rate at 0.2 bar was 7% of the 5-bar rate, whereas in the cold-grown plants the rate at 0.2 bar increased to 14% of the rate at 5 bars.

Effect of Temperature on Exudate Potential. Exudate potentials were measured for each steady-state flow rate in all experiments. To facilitate comparisons with the flow curves, the sap potentials were plotted against T^{-1} or hydrostatic pressure for the temperature and pressure series, respectively. Except where otherwise indicated, all data points were the averages of four experiments.

When held at a constant 5-bar hydrostatic pressure the cold-grown broccoli and soybeans had lower exudate potentials than their warm-grown counterparts at most temperatures (Fig. 8). Sap potentials from the cold-grown broccoli and soybean decreased with decreasing temperature, with the trend being much more pronounced in broccoli than soybean. Exudate potential was relatively constant in warm-grown broccoli and soybean plants except for the soybean pressurized at 7 C, where the potential decreased.

The exudate potential from warm- and cold-grown broccoli and soybean increased rapidly with increasing pressure between 0.2 and 2.0 bars, and reached a plateau between 3 and 5 bars (Figs. 7 and 9). Although the general shape of the curve was similar in warm- and cold-grown broccoli, the cold-grown plants had a lower exudate potential at all pressures. The difference was most pronounced at the higher pressures.

When the same root system was pressurized twice at 25 C the exudate at low pressures was more dilute in the second pressure series than in the first. At higher pressures this difference disappeared, both series having exudate potential between -0.1 and -0.15 bar. As in the case of the flow rates, this discrepancy at the low pressures must be kept in mind when interpreting the sequential 25 C then 10 C pressure series.

The cold- and warm-grown soybeans responded differently to the 10 C pressure series after a 25 C (Fig. 9). The exudate from the cold-grown plants was much more dilute in the 10 C series

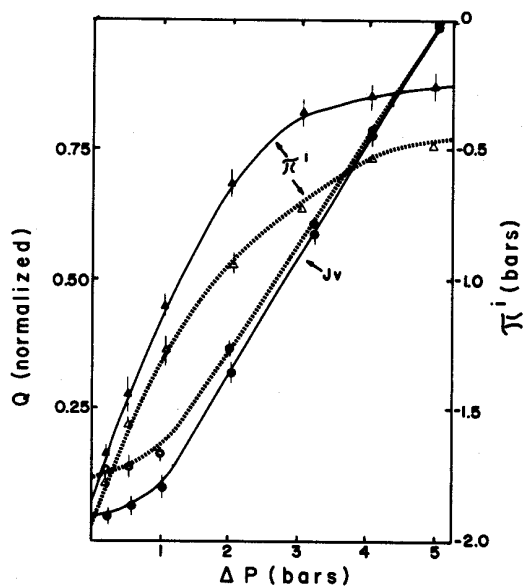


FIG. 7. Effect of growth temperature on normalized flow rates (J_v) and exudate potential (π^i) of broccoli pressurized at 25 C. Means and SEM of three experiments for 28/23 C (●—●, ▲—▲), and 17/11 C (○—○, △—△)-grown plants are presented.

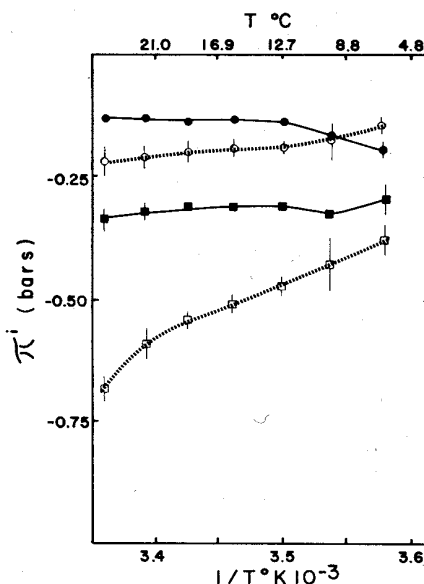


FIG. 8. Effect of growth temperature on exudate potential (π^i) from broccoli (■—■, 28/23 C; □—□, 17/11 C) and soybean (●—●, 28/23 C; ○—○, 17/11 C) roots at a constant pressure of 5 or 6 bars and decreasing temperatures. Data points are mean \pm SEM of four soybean and three broccoli root systems.

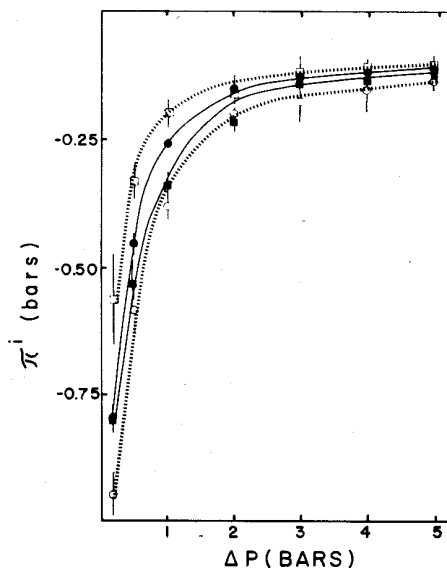


FIG. 9. Effect of growth temperatures on exudate potential (π^i) of soybean roots at hydrostatic pressures between 0.2 and 5.0 bars. Mean \pm SEM for three experiments are plotted for 28/23 C-grown plants at 25 C (●—●) and 10 C (■—■), and for 17/11 C-grown plants at 25 C (○—○) and 10 C (□—□).

than the 25 C series. This difference was most dramatic at the lower pressures, and was apparent though not significant at the higher pressures. The warm-grown plants, however, showed just the opposite response. The exudate potential was lower in the 10 C series than in the 25 C series. The difference was most evident at the low pressures, and negligible at high pressures.

DISCUSSION

Several investigators have shown that absorption of water is decreased less by low temperature if the plant root system has

been preconditioned for a few days or grown at low temperatures (2, 12). These experiments, however, did not permit accurate evaluation of the various components of water flow such as hydraulic conductivity nor of the relative importance of osmotically and hydrostatically driven flow. This left uncertain the relative importance of temperature effects on root permeability as compared to effects on the driving force.

Our experiments have resolved this uncertainty by showing that there is a linear relationship between total flow (Q) and root conductivity (L), at high flow rates (Fig. 3). This justifies the conclusion that the change in total flow seen in Figures 1, 4, and 5 is caused primarily by a change in root conductivity rather than in the magnitude of the driving force.

Numerous investigators have implicated the endodermis as the major site of resistance to water flow through roots (4, 6, 11). In mature root systems, however, secondary root development results in perforation of the endodermis (11), and a considerable number of root tips lack the formation of an identifiable Casparian strip (5). Furthermore, in roots that have undergone secondary thickening the endodermis disappears (11). Therefore, in mature root systems the specific structure that limits water flow remains in question. The candidates to fill this role are: cell walls with a suberized Casparian strip, a cell membrane, and structured water either in the cell wall or along a membrane interface. Our results with mature soybeans suggest that the rate-limiting step is a lipid-containing membrane or membranes.

Breaks in Arrhenius plots similar to those in Figure 1 have been correlated with a membrane phase change, but could also be due to changes in structured water or some as yet unidentified process. The highly cross-linked components of cell walls would not be expected to change conformation abruptly at such high temperatures and produce the observed breaks in the Arrhenius plots. This is supported by the observation that a dead root system with an intact cell wall shows no break in response to temperature.

The shifts in Arrhenius break points with growth temperature, such as those shown in Figure 4, have been related to changes in lipid composition in bacteria (20), leaves (14), and roots (3), and strongly suggest a membrane-mediated process. In rye roots a 3-day exposure to 8 C increased the total lipid carbon-carbon double bonds by 58% over the 20 C control plants (3). If the water flow were limited by the structured water along the interface, one would not expect the break to shift with growth temperature.

The fact that there is no apparent hysteresis in the temperature curve (Fig. 1) is consistent with membrane phase changes. Although chilling has been reported to weaken membranes, resulting in leakage and damage, this is not the immediate result of chilling, but rather a second order effect (17). One would expect that if the time at the low temperature were kept at a minimum, no damage would occur, and the critical membranes should have the same transition temperature, regardless of the direction of the changing temperature. When the roots were held at temperatures below the phase transition temperature for an extended period of time (12 h), as was the case in the 10 C pressure series, the exudate potential increased (Fig. 9). The resulting increase in solute concentration could be due to damaged cortical cells or xylem parenchyma resulting in release of ions into the water stream.

In several experiments to be reported elsewhere, root systems were treated with chemicals (valinomycin, gramicidin D, and ABA) known to interact with membranes. Changes in the hydraulic conductance and the reflection coefficient of the root systems were observed. Although no single experiment cited above rules out all alternatives, the explanation that best accounts for all of the data is that a lipid-containing membrane, most likely the

endodermal plasmalemma, limits the flow of water through roots.

Unlike soybean, broccoli, which is a cool season crop, showed no break in the Arrhenius plot of water flow *versus* temperature, probably because the phase transition of its membrane occurs below temperatures used in these experiments. When broccoli was grown at lower temperatures the activation energy was lowered from 19.7 to 9.3 kcal/mol. Possibly changes of a different nature occurred in broccoli than in soybean. The incorporation of sterols into membranes has been shown to alter the energy of activation of associated processes and give added strength to the membrane (1, 16). It is reasonable to suggest that the sterol content of the broccoli membranes changes with growth temperature.

Another interesting difference between soybean and broccoli was the ion concentration of the xylem exudate and flow rate at low pressures. Broccoli root systems grown at either high or low temperatures produced exudate that was two to three times as concentrated as the exudate from soybean. Furthermore, both broccoli and soybeans grown at low temperatures produced a more concentrated exudate than plants grown at the higher temperature. The exudate flow rate from root systems subjected to pressures below 1.0 bar at 25 C was also greater for root systems grown at the lower temperature. This increase in exudate potential and flow rate at low pressures indicates that the active ion pump in the roots increases in activity when the plants are grown at lower temperatures. Clarkson (2) came to similar conclusions for barley and rye root systems.

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